ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

1960

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

ANNUAL REPORT OF PROGRAM ACTIVITIES

U.S. National Institute of Allergy and Infectious Diseases

January - December, 1960

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Laboratory of Biology of Viruses

Laboratory of Tropical Virology
Middle America Research Unit
Arthropod-Borne Virus Section

Laboratory of Parasitic Diseases

Laboratory of Parasite Chemotherapy

DIRECTOR - NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASE

Annual Report of Program Activities

National Institute of Allergy and Infectious Diseases

January - December 1960

OFFICE OF THE DIRECTOR

1. The calendar year 1960 witnessed significant extension of both intramural and extramural activities carried on or supported by the National Institute of Allergy and Infectious Diseases. These are detailed in succeeding sections.

2. Of special significance were the steps taken during the year toward the development of International Centers for Medical Research and Training. These are to provide tangible implementation, in addition to the research presently supported overseas by the various Institutes, for Senator Hill's "International Health Research Act of 1960" (Public Law 86-610).

These Centers have evolved from proposals submitted originally to this Institute for research on diseases in the tropics. This area of concern has now been expanded to encompass most of the research and research training interests supported by the National Institutes of Health. Each Center represents a collaborative undertaking between a sponsoring medical segment of an American university and a host institution of medical learning overseas. Within this setting, it is expected that faculty members, post-doctoral trainees, graduate students, and other specially qualified individuals from this country may work together with foreign scientists and their staffs in solving problems of universal medical interest, in extending scientific knowledge, and in creating and fostering international fellowship and good will.

While the initial emphasis is on the medical and supporting sciences, it is anticipated and hoped that these Centers may provide overseas opportunities of interest to the socio-technical and related research interests supported by foundations or institutions outside the National Institutes of Health.

Four Centers* have been recommended for approval. Administrative and operational responsibilities are assigned, for the present, to the National Institute of Allergy and Infectious Diseases. The Councils of the other

* University of California	---	University of Malaya, at Singapore University of Malaya, at Kuala Lumpur
Tulane University	---	Universidad del Valle, Cali, Colombia
Johns Hopkins University	---	All-India Institute of Hygiene, Calcutta, India
University of Maryland	---	Field Unit, Rawalpindi, Pakistan

Institutes and the Division of General Medical Sciences have agreed to make available to the National Advisory Allergy and Infectious Diseases Council funds appropriated for international health research in Fiscal Year 1960, with the understanding that 1) regular progress and status reports will be made to the other National Advisory Councils, and 2) that incorporation of the lists of grants awarded in the formal minutes of the other Councils will constitute formal endorsement of these Councils.

A standing committee has been established to advise the Director, National Institutes of Health, with reference to the future conduct of the Centers, and to evaluate and advise regarding applications for additional Centers.

3. Staffing, space, and funds for the support of the National Institute of Allergy and Infectious Diseases have been reasonably satisfactory for current needs this last year, with one exception. The death of Dr. Jules Freund, since the last Annual Report was written, has left a unique void in competent leadership in immunology and allergy that is proving difficult to fill. Some eight outstanding scientists and leaders in this field have been seriously considered, brought to the Institute to see and to be seen, and certain of them have been invited to take over this responsibility. This activity has become of special importance since 1955, when the title of the Institute was amended to include the word "Allergy." For various reasons, mainly satisfaction with their current situations, it has not been possible to persuade any of these candidates to accept the position as Chief of the Laboratory of Immunology.

While the existing staff is operating admirably in the discharge of its responsibilities, the lack of expert leadership is recognized. Every effort will be made in the forthcoming year to attract a competent individual to this important position.

EXTRAMURAL PROGRAM

EXTRAMURAL PROGRAM

Annual Report of Program Activities

National Institute of Allergy and Infectious Diseases
January-December, 1960

Extramural Programs

In spite of the commonly held misconception that modern antibiotics have relegated the infectious diseases to a position of relative unimportance in human health, recent statistics from the U. S. National Health Survey indicate that infection and allergy cause more than two-thirds of the acute illness in the United States, and furthermore are responsible for almost one-fifth of the chronic disease in the Nation. Fully cognizant of these facts, the Institute has continued to build a balanced program of research and training emphasizing the fundamental nature of pathogenic organisms and allergens, the mechanisms by which they reach and damage their hosts, and the means which may be developed to combat them.

Research Grants

The distribution of research funds in support of the Institute's objectives is shown in Tables I and II. Data regarding the review and approval of research grants are given in Table III.

The Council continued to emphasize and support areas of special research need. Under the Committee on Standardization of Allergens an important program is progressing along three fronts: chemical isolation of the allergenic fractions of ragweed pollen; immunological comparison and standardization of the active fractions; and clinical testing to determine which fractions are important in human hay fever. Information exchanged last April during an informal conference of the scientists working on these three aspects indicated that the substance known as trifidin apparently is the purest component yet isolated, but that at least eight different ragweed pollen fractions seem to be active allergens in laboratory studies.

To maintain continued stimulation of influenza research between epidemics, the Institute established a program of "Special Grants" which the Council authorized under the Subcommittee of Investigators of the Committee on Influenza Research. Selected scientists work toward specified objectives under protocols developed in collaboration with the Subcommittee. For this purpose the Council recommended a reserve of $175,000 from each of two fiscal years, with unused funds reverting to the regular research grant program. At the close of the

year 10 such projects for $115,713 are being supported. In February the Institute sponsored a symposium on the 1957-58 Asian influenza pandemic. This conference emphasized deficiencies in present knowledge and thus suggested areas for intensive research stimulation. As a further service to research workers, funds were provided for the preparation of an extensive annotated bibliography of influenza research, which has been distributed to all medical libraries in the United States and to the principal medical libraries of the world. In addition, the Subcommittee of Investigators and the Committee on Influenza Research promulgated a series of recommendations for dosage and criteria for vaccination against influenza in the civilian population.

Inability of the antibiotics to control resistant strains of staphylococci, especially in hospital epidemics, created a critical and alarming situation which prompted Congress to include in the 1959 appropriation for this Institute a one million dollar increase over 1958 levels specifically for research in this area. With the impetus of these additional funds the Institute so accelerated its grants program that it now supports 102 staphylococcal research projects totalling $1,922,283. More than one-third of these studies are directly related to hospital problems, especially epidemic outbreaks in nurseries or post-operative infection of surgical wounds. One such project concerns the design of hospital nurseries with relation to the spread of organisms; another is a large-scale coordinated study by five medical school hospitals and the National Research Council to evaluate ultraviolet irradiation of operating rooms in the prevention of wound infections. Approximately one-third of the Institute's program in this area deals with basic studies on the fundamental nature of the staphylococcus organisms and their relationships to the host. Other grant-supported areas of investigation include clinical aspects of staphylococcal disease, new drugs, and the role of these organisms in food poisoning.

The Council pointed up special needs in three other areas through partial sponsorship of research conferences, namely (1) the "Second Conference on Medical Mycology," (2) a "Symposium on Immunochemical Approaches to Problems in Microbiology, " and (3) a "Symposium on Airborne Infection." Moreover, consonant with the Institute's ongoing interest in tropical medicine and related fields the Council supported the "Fourth Conference on Research Needs in Tropical Medicine." This meeting brought together a group of selected authorities to delineate the specific aspects which should be emphasized by future research and training in the broad fields of tropical medicine and environmental health.

As a consequence of the Council's interest and positive action based on a recommendation of the Virology and Rickettsiology Study Section, an Adenovirus Committee was established early in the year to provide needed leadership for research on the adenovirus group of filtrable agents by providing a generally-accepted set of standards on which identification of these viruses can be based. The most urgent of the Committee's objectives has been the production of standardized

reagents which would permit research workers to identify adenovirus strains. Through a technical subcommittee specifications have been developed for the production of type-specific antisera for the first 26 adenovirus types. Proposals have been solicited from prospective producers and it is expected that contracts can be negotiated soon so that the needed reagents will be available for testing and certification by the end of 1961. The Adenovirus Committee now plans to move against other problems in the standardization and characterization of this group of viruses.

Recognizing that significant research of high quality is being conducted at many institutions outside the United States, the Council has felt it both justified and desirable to support carefully selected projects wherever they may be located. Thus research grants have been awarded to scientists at foreign institutions whenever a competent investigator proposed a project which for geographical or other reasons could best be done abroad, or when the investigator was so outstanding that his contribution would have general scientific significance. On this basis some 68 grants for $925,829 have been supported during the year.

The current appropriation for this and several other Institutes includes funds for the planning, negotiating, and initial support of clinical research units spanning the interests of the several supporting institutes. Such centers are being established in large medical research institutions throughout the United States. Central review and administration of this program is the responsibility of the National Advisory Health Council and the Division of General Medical Sciences. In addition, $1,454,000 from the current appropriation of this Institute has been reserved to establish the Institutional Grants Program.

To provide more expeditious payment of continuation grants when the appropriation is delayed beyond the beginning of the fiscal year, all research grants having anniversary dates in the first two quarters are being forward financed to provide for renewal on December 1 or later. This process will be completed before the end of the current fiscal year.

In conformity with the criterion regarding scientific excellence which the President set forth in approving the 1959 appropriation bill, the Council has continued to give special consideration to all applications falling within the lowest ten per cent of the priority list for each study section.

Training Grants

Following a policy initiated last year, the Institute has continued to develop its training grants program along lines designed to emphasize disease-oriented interests in allergy and infectious disorders.

To insure highly competent professional review of applications, two panels of consultants were established to cover the Institute's broad training areas. These are the Allergy and Immunology Training Grant Committee, and the Infectious Diseases and Tropical Medicine Training Grant Committee.

Table IV indicates the distribution of training grant funds in support of the Institute's objectives. Table V gives data regarding review and approval of applications during the calendar year.

The Institute now has completed the forward financing of all training grants so that continuation years will begin on July 1. Under this arrangement it now is possible to assure grantees of continuation support several months in advance of their needs, thus obviating the uncertainty and inconvenience formerly caused by delays in the appropriation of funds.

Certain foreign institutions have opportunities for providing outstanding training in special research areas to American students as well as their own nationals. Recognizing this fact the Institute currently is supporting one training grant at a Canadian institution, with the proviso that stipends from the grant be awarded to American citizens only. Two other training grant applications from foreign institutions are pending review. The Council has continued to insist that, after the first year, at least half the funds awarded through all training grants be devoted to stipends and other direct trainee support such as tuition, travel expenses, and consumable supplies.

As the culmination of more than two years of planning by the Council and the Institute's staff, a program of tropical medicine training centers was established. Four of the six centers originally included in this series subsequently have been broadened under authority of Pl 86-610 (see below). The two remaining centers will provide opportunities for field experience in tropical medicine at cooperating research establishments in various overseas locations. Support for five years was recommended, with re-evaluation after three years. In recognition of the high cost of this type of training only 30 percent of the yearly budgets after the first year must be used for stipends and direct trainee benefits.

International Centers for Medical Research and Training

Under the International Health Research Act of 1960 (PL 86-610) the Congress made funds available to the NIH for the establishment of International Centers for Medical Research and Training. The Council of this Institute accepted responsibility for the review of applications for grants to support multidisciplinary projects under this new program, to be financed from funds made available under PL 86-610 to the Division of General Medical Sciences and various Institutes of NIH. Four of the programs already activated as Tropical Medicine Training Centers (see above) were appropriately expanded into research and training centers

encompassing the categorical interests of the several Institutes and the Division of General Medical Sciences. In making its recommendations the Council had the technical advice of the Committee on International Centers for Medical Research and Training. It is anticipated that additional universities will be considered as potential program participants early in the coming year.

Research Fellowships

Table VI shows the distribution of awards during the year, by areas of study and level of support. The data regarding review and approval of applications are presented in Table VII. The fellowship program of the Institute has followed the pattern set last year, with increasing emphasis on awards at the postdoctoral and special levels to produce increasing numbers of competent independent investigators. Beginning July 1, 1960, the funding as well as the administration of all predoctoral awards was taken over by the Division of General Medical Sciences. The data given in Tables VI and VII for predoctoral fellowships apply to those awarded during FY 1960 and funded by this Institute.

Three additional Fellowships Programs, designed to support faculty positions at different levels, are being activated by the Institute and will be in operation during the third and fourth quarters of FY 1961. The Career Research Professor Grant Program has been established to support individuals of demonstrated capacity to pursue with distinction a professorial career in independent research and teaching. The Senior Fellowship Grant Program, formerly limited to preclinical science departments and administered only by the Division of General Medical Sciences, has been expanded to include clinical departments as well as certain departments in university graduate schools and other appropriate institutions. These awards are for the support of individuals with at least five years of relevant research experience beyond the doctorate who have demonstrated high potential for a research or academic career. The Special Fellowship Grant Program will support the promising young investigator not yet eligible for an award at a higher level but who the applicant institution feels would be an important adjunct to its teaching and research staff. This program is in addition to the present Special Fellowship awarded directly by the Public Health Service to individuals for advanced or special training.

TABLE I -- RESEARCH GRANTS PROGRAM (NIAID) 12/15/60
(FISCAL YEARS)

Area of Support	'58 Appropriation* $10,824,000		'59 Appropriation* $14,880,000		'60 Appropriation*** $21,593,000		'61 Appropriation**** $28,951,000	
	No.	Amount	No.	Amount	No.	Amount	No. (Estimated)	Amount
Allergy	92	$1,339,584	107	$1,550,824	134	$2,184,636	149	$2,651,480
Immunology	65	776,143	77	1,033,864	116	1,910,816	128	2,001,958
Bacteriology	213	2,594,836	280	3,791,288	378	5,613,529	422	6,692,509
Virology	146	2,701,257	173	3,426,721	233	4,794,512	265	5,660,070
All Others (Antibiotics, Arthropods, Biochemistry, Epidemiology, Genetics, Mycology, Parasitology, etc.)	407	3,752,420	492	5,085,583	550	6,410,413	638	7,944,160
Grand Total	923	$11,164,240**	1,129	$14,888,280**	1,411	$20,913,906**	1,602	$24,950,177**

* The '58 and '59 appropriations include $150,000 for Gorgas Memorial Laboratory and $18,000 and $39,000 respectively for Russian Translation Service. In addition, $442,000 was deducted from the '59 appropriation to cover NIAID salary increases.

** Excess listed over appropriation represents extensions without additional funds and grants, paid from previous year fiscal funds, which terminate during the indicated fiscal year.

*** The '60 appropriation included $500,000 for metabolic (clinical) centers, $150,000 for Gorgas Memorial Laboratory, $44,000 for Russian Translation Service, $1,000 for the CDC Diagnostic Reagents Program, and $41,200 for the Children's Bureau project on cystic fibrosis.

**** The '61 appropriation includes $750,000 for Gorgas Memorial Laboratory, $500,000 for Clinical Research Units, $500,000 for International Medical Research, $1,454,000 for the Institutional Grants Program, and $581,000 unprogrammed reserve. In addition, the following reserves have been established: Adenovirus Reagent Program, $250,000; Children's Bureau project on cystic fibrosis, $30,310; Senior Fellowship, Special Fellowship, and Career Research Professor Programs, $140,000.

TABLE II -- RESEARCH GRANTS PROGRAM (NIAID) SPECIAL AREAS 1/

Area of Support	Fiscal Year 1958		Fiscal Year 1959		Fiscal Year 1960		Fiscal Year 1961	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount (Estimated)
1. Acute Respiratory Disease 2/	58	$1,153,109	71	$1,428,586	119	$2,290,187	123	$2,638,234
2. Cystic Fibrosis	4	36,511	23	382,539	19 4/	336,835	9	231,825
3. Tropical Medicine and Parasitology								
Total	281	2,539,916	331	3,229,304	420	5,105,550	483	6,264,885
Parasitology:								
Helminths	67	592,196	78	696,375	101	1,010,601	118	1,311,655
Protozoa	64	566,072	76	762,524	82	945,966	96	1,242,222
Arthropods	60	496,189	75	653,187	86	890,011	105	1,158,992
Arthropod-borne Viral Diseases:								
Dengue	2	37,732	4	69,339	4	89,383	4	79,352
Encephalitis	5	99,955	7	135,046	13	316,625	19	430,647
Yellow Fever	1	32,545	1	27,500	1	25,000	1	25,000
Non-Parasitic Tropical Diseases of Importance:								
Cholera	1	7,360	2	19,165	4	54,259	4	75,924
Leprosy	7	78,709	7	106,297	7	116,681	11	231,477
Salmonellosis	6	65,225	8	96,586	14	171,236	12	167,949
Shigellosis	2	20,126	3	70,726	2	85,250	4	110,090
Fungal Diseases	66	543,807	70	592,559	78	868,312	80	893,022
4. Staphylococci:	19	189,573	59	1,002,566	84	1,541,342	102	1,922,283
5. Tuberculosis and related areas:	44	694,979	73	995,618	94	1,519,821	106	1,715,652
6. Enteroviruses 3/ (excluding poliomyelitis)			9	166,730	11	245,045	13	233,373
7. Urinary Tract Infections (Pyelonephitis) 3/			5	108,578	12	191,000	19	343,784

1/ Included in areas listed in Table I.

2/ Includes Airborne Allergy, Adenoviruses, Influenza, Ornithosis, Pneumococci, Q Fever and Viral Pneumonia.

3/ Not separately listed until Fiscal Year 1959.

4/ Includes $41,200 for Children's Bureau project.

TABLE III

RESEARCH GRANTS REVIEWED DURING CALENDAR YEAR 1960

	Received		Approved	
March	No.	Amount	No.	Amount
New	203	$3,379,967	130	$2,008,527
Continuations	124	1,991,573	105	1,630,318
	327	$5,371,540	235	$3,638,845
June				
New	222	$4,328,276	134	$2,306,799
Continuations*	1286	$19,534,707	1261	$ 18,929,517
	1508	$23,862,983	1,395	$ 21,236,316

* Includes continuation grants for which previously recommended support was reaffirmed. (1186 for $17,447,083).

November				
New	204	$3,955,063	133	$2,367,569
Continuations	110	1,681,293	94	1,341,294
	314	$5,636,356	227	$3,708,863

12/15/60

TABLE IV -- TRAINING GRANT PROGRAM (NIAID)
(FISCAL YEARS)

	'58 Appropriation $650,000 1/		'59 Appropriation $1,787,000		'60 Appropriation $3,621,000		'61 Appropriation $4,790,000	
Discipline	No.	Amount	No.	Amount	No.	Amount	No.	Amount (Estimated)
Allergy and Immunology	12	$254,145	19	$525,203	30	$1,293,010	34	$1,200,085
Infectious Diseases 2/	7	159,377	23	793,830	34	1,449,651	48	1,893,202
Tropical Medicine and Parasitology	8	151,392	18	439,668	22	807,593	29	1,503,572 4/
	27	$564,914 3/	60	$1,758,701 3/	86	$3,550,254 3/	111	$4,596,859 3/

1/ Includes $70,000 sub-allocated to Division of Research Grants for General Research Training Grant Program.

2/ Infectious Diseases included only mycology and rickettsiology in Fiscal Year 1958.

3/ Chairman's grants are not included in these figures.

4/ Includes $478,566 for the first year's operation of four International Centers for Medical Research and Training, established under the International Health Research Act of 1960 (PL 86-610). This figure does not include $399,776 to forward finance the second year's operation of these Centers nor supplemental awards totalling $992,241 for these Centers, since these amounts will be derived from funds available under PL 86-610.

12/15/60

TABLE V

TRAINING GRANTS REVIEWED DURING CALENDAR YEAR 1960 1/

	Received		Approved	
March	No.	Amount	No.	Amount
New	31	$852,681	18	$527,304
Continuations	0	--	0	--
	31	$852,681	18	$527,304
June				
New	25	$1,791,122	15	$1,436,275
Continuations*	83	2,633,605	83	2,633,605
	108	$4,424,727	98	$4,069,880
November				
New	13	$408,090**	9	$277,730**
Continuations	0	--	0	--
	13	$408,090	9	$277,730

* (Includes continuation grants for which previously recommended support was reaffirmed).

** (Includes $32,349 to forward finance one request).

1/ Amounts do not include $50,000 approved as a Chairman's grant, nor $383,162 needed to forward finance 17 grants. Neither do they include awards for four International Centers for Medical Research and Training at a total of $878,342 (1st year $478,566; 2nd year $399,776).

12/15/60

TABLE VI

RESEARCH FELLOWSHIPS PROGRAM (NIAID)
(CALENDAR YEAR 1960)

	PREDOCTORAL				POSTDOCTORAL				SPECIAL			
	NEW		CONTINUATION		NEW		CONTINUATION		NEW		CONTINUATION	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Allergy	-	$------	-	$------	7	$41,876	4	$28,600	1	$12,982	-	$------
Antibiotics	-	------	2	5,864	2	15,023	-	------	-	------	-	------
Bacteriology	5	15,384	1	4,500	5	30,349	3	21,000	3	33,533	-	------
Biochemistry	5	16,480	4	12,048	7	40,803	11	67,169	1	2,736	-	------
Chemotherapy	-	------	1	1,510	-	------	-	------	-	------	-	------
Entomology	2	6,475	2	7,472	1	6,500	-	------	1	4,902	-	------
Epidemiology	-	------	-	------	1	9,135	-	------	-	------	-	------
Genetics	1	3,000	1	2,208	1	6,115	2	11,000	-	------	-	------
Helminthology	-	------	-	------	2	12,793	4	24,500	-	------	-	------
Immunology	3	10,849	-	------	7	44,886	3	19,275	2	15,913	-	------
Mycology	-	------	2	4,070	-	------	-	------	1	10,240	-	------
Physiology	-	------	1	1,400	-	------	-	------	-	------	-	------
Protozoology	2	6,663	1	3,836	1	4,541	-	------	-	------	-	------
Public Health & Sanitation	-	------	-	------	-	------	-	------	-	------	-	------
Rapid Identification	-	------	-	------	1	6,650	-	------	-	------	-	------
Rickettsiology	-	------	-	------	-	------	1	7,000	1	6,252	-	------
Spirochetology	-	------	-	------	-	------	1	8,360	-	------	-	------
Taxonomy	-	------	1	2,335	-	------	-	------	-	------	-	------
Virology	1	2,836	3	9,190	15	89,981	8	57,016	2	18,569	-	------
	19	$ 61,687	19	$54,433	50	$308,652	37	$243,920	12	$105,127	-	------

TABLE VII

RESEARCH FELLOWSHIPS REVIEWED DURING CALENDAR YEAR 1960

	Predoctoral		Postdoctoral		Special	
	No.	Amount	No.	Amount	No.	Amount
Received (New)	77	$269,500	99	$643,500	24	$192,000
(Continuations)	68	238,000	43	279,500	-	-------
	145	$507,500	142	$923,000	24	$192,000
Recommended for Approval (New)	39	$131,687	53	$328,152	13	$113,127
Inactivated (New)	20	$ 70,000	3	$ 19,500	1	$ 8,000
Awarded (New)	19	$ 61,687	50	$308,652	12	$105,127
(Continuations)	19	54,433	37	243,920	-	-------
	38	$116,120	87	$552,572	12	$105,127

12/15/60

ACCOMPLISHMENT HIGHLIGHTS

Important research accomplishments continue to be reported by the grantees of this Institute in increasing numbers as the extramural programs develop and mature. Results of grant-supported research are seen not only in the problems related to diagnosis and treatment of specific diseases, but also in the broader area of basic processes which govern the transmission and production of disease, and the reaction of the host to invading substances or pathogenic agents. Such fundamental studies are essential to a full understanding of the disease process and form the basis on which significant future research efforts can be based. The examples given below represent in part the broad range of interests and approaches within the extramural programs of the Institute.

Important achievements in the related fields of allergy and immunology have been reported during the year. Grantees at the New York Hospital-Cornell Medical Center carried out a study which casts doubt on the commonly held belief that human infants do not begin to form adequate circulating antibodies to injected antigens until the second month of postnatal life. While the scientists feel that their data are not sufficiently extensive to warrant sweeping conclusions, one general fact emerged from the study: newborn and premature infants naturally infected with various types of ECHO viruses and adenoviruses usually respond promptly with antibody formation. Whether or not detectable antibodies develop apparently is closely related to the inherent antigenicity of the agent -- that is, to the particular virus involved -- rather than to the patient's maturity.

A study of chronic asthmatics, conducted with NIAID grant support at the National Jewish Hospital in Denver, was undertaken to test the therapeutic potential of negative and positive air-borne ions. Studies by several scientists on the curative value of artificially ionized air have been under way since before World War II, and a report in 1946 of beneficial effects from air-borne negative ions on paranasal sinusitis and vasomotor rhinitis prompted inquiry by later investigators into the influence of ionization in hay fever. Prompt amelioration of symptoms appeared to be the outcome. Under the present series of experiments, however, no improvement of asthmatic symptoms was noted during exposure to ionized air under randomized test conditions. The investigators' clear cut findings incline them to discard ionization as therapy for patients with asthma.

A recently completed clinical trial by grantees at Northwestern University affirmed the usefulness of the drug SA 97 (homochlorcyclizine) in ameliorating certain allergic symptoms. In general, SA 97 (supplied by Abbott Laboratories) was employed against various allergic manifestations in patients who failed to respond adequately to the usual drugs. Among the asthma group about 75 percent obtained satisfactory results; most of these cases, however, were not acute attacks. About two-thirds of the patients with perennial allergic rhinitis obtained satisfactory relief from protracted nasal blocking. Most of the sea-

sonal hay fever patients had the ragweed type of hay fever, and the 55 percent who improved were mainly those failing to respond to antihistamines. In the urticaria and angiodema group, three-fourths of the patients showed improvement ranging from relief of itching and diminished swelling to complete regression of the lesions. The majority of patients with dermatitis, including atopic, contact, and unknown types, obtained effective relief of itching. The investigators conclude that SA 97 is an effective adjunct to drugs already used in the treatment of certain allergic symptoms.

Studies on infection and parasitism form a major portion of the Institute's grants program. In the field of tropical medicine and parasitology, as in other areas of infectious disease, achievements have come from a wide variety of projects ranging from basic studies on pathogenic organisms and the host-parasite relationship to practical applications of research results in the diagnosis, treatment or prevention of diseases.

Grantees at Tulane University School of Medicine have demonstrated that infection with Clonorchis sinensis can be detected by the indirect tanned-cell hemagglutination test, thus adding a new diagnostic tool against clonorchiasis, a human liver fluke disease highly endemic in the Far East. The new procedure was found to be relatively more sensitive than complement fixation in the detection of Clonorchis infection in man and rabbits.

Other Tulane University grantees studying the biological characterization of venom from the tropical fire ant have demonstrated an antifungal effect possessed by one component of the insect's venom. The crystalline hemolytic component inhibited the activity of 15 fungi, mostly human pathogens. The tropical fire ant, introduced into the United States at Mobile, Alabama, about 1920, is not only a growing agricultural problem in the southeastern United States but a menace to human beings. The sting of this insect is extremely painful and can cause severe allergic reactions, including anaphylactic shock. The investigators caution that their report is not to be interpreted as a study on the antifungal effects of this material for future clinical use but rather an attempt to broaden methods for characterization of the biological activity of the crystalline material obtained from fire ant venom.

Important advances likewise have been reported for the bacterial diseases. In a series of experiments jointly supported by the University of Chicago, the Atomic Energy Commission and this Institute, scientists at the University of Chicago have clarified important details surrounding the known fact of increased mammalian susceptibility to infection following whole body irradiation to moderate doses of radiation. In mice experimentally challenged intravenously or intraperitoneally with Pseudomonas aeruginosa after irradiation, it was found that those inoculated intravenously displayed a higher susceptibility to infection. The difference was due to the regular appearance of a small focus of infection at the site of injection as a result of leakage of inoculum into the surrounding tissue. Bacterial multiplication occurred in

those locations in irradiated mice, but not in unirradiated mice, nor at the site of intraperitoneal inoculation even in irradiated mice.

In studies conducted at Harvard University, one of our grantees has shown that urinary tract infection, one of the commonest causes of acute renal failure, can regularly be averted by early recognition and treatment of asymptomatic bacteriuria. Development of a simple but reliable test for the detection of early cases of pyelonephritis was prompted by the finding at autopsy of 10 to 20 percent incidence of that disease, which is often unsuspected or misdiagnosed. Three-fourths of infected patients respond to treatment with sulfonamides. Alternative therapy on the basis of sensitivity studies is used for patients resistant to the sulfonamides. The investigator observes that pyelonephritis of pregnancy occurs almost exclusively in patients who have bacteriuria at the time of the first prenatal visit. The way is now clear, with this test, for the virtual elimination of pyelonephritis among pregnant women.

One of our grantees at Washington University reported the development of a modified urine hemolytic test for diagnosing bovine leptospirosis without some of the limitations inherent in the currently used methods of direct darkfield examination, cultural procedures or animal inoculations. The new serodiagnostic modification becomes positive within three weeks after infection and, in the later stages of the disease, continues to indicate the presence of leptospiral antigen for several weeks after it is no longer demonstrable by laboratory animal inoculations.

Expecially significant findings have been reported in the field of staphylococcal disease and the special problems presented by antibiotic-resistant strains of staphylococci. Recent reports by one of our grantees at the Hospital for Joint Diseases, New York City, suggest that the emergence of drug resistance actually represents a selection of pre-existing resistant strains rather than the development of new ones. In studying 194 staphylococcal strains isolated between 1927 and 1947, before antibiotics were widely used, this investigator found that 22 per cent belonged to the "phage type 80/81" which has been particularly dangerous in hospital infections during recent years. This observation dispels the apparently common belief that strains of "type 80/81" are "new" staphylococci of recent origin. Another grantee, at the University of Texas Southwestern Medical School, has shown that the genetic factor responsible for resistance to streptomycin can be transferred from resistant to drug-sensitive strains of staphylococci by the viruses (phages) which parasitize them.

At Baylor University Medical Center a grant-supported project has shown that normal human white blood cells, which usually destroy bacteria by ingesting them, actually provide protection against antibiotics for pathogenic staphylococci ingested by the cells. In these studies, 10-100 fold increases in the concentration of penicillin and other antibiotics failed to kill staphylococci which had been ingested by the white blood cells.

Two groups have reported interesting findings on their studies of staphylococcal infections in hospital nurseries. Working in a hospital that permitted intermittent "rooming-in" of infants with their mothers, investigators at the University of California at Los Angeles found that both the infants and the air of the nursery had a very low incidence of staphylococci while the mothers had a relatively high incidence of bacteria which they did not transmit to their rooming-in infants. Another research team, at the New York Hospital-Cornell Medical Center, found that most newborn infants who are infected with staphylococci have a relatively low index of infectivity, while a small minority are highly infectious. Because these latter infants literally are surrounded by clouds of bacteria they are designated "cloud babies." Follow-up studies showed that their explosive role in hospital epidemics continues, in the family unit, after they leave the hospital. The explosiveness of the "cloud baby" outbreaks, the authors say, makes it imperative to design nursery units in such a way as to prevent airborne dissemination of infection.

A great deal of research progress has been reported by our grantees in the field of virology. A research team at Harvard Medical School revealed poliovirus variants which resisted temperatures generally believed to be rapidly destructive of all three types of virus. Whereas previous studies have shown that poliovirus is rapidly destroyed at temperatures slightly exceeding 60 degrees centigrade, the present series of experiments indicated that suspensions of the virus grown in monkey kidney cells were not completely inactivated following exposure for one hour to temperatures of 60 degrees and 65 degrees centigrade. In one instance infectious virus was demonstrated in Type II poliovirus after heating at 75 degrees centigrade for one hour. Progeny of virus surviving at this temperature showed increased thermoresistance. These results suggested that pasteurization of milk and other food products as now carried out cannot assure the complete inactivation of these agents.

Grantees at the University of Pittsburgh found that previously recognized dengue and chikungunya viruses, as well as two new dengue-like agents, apparently have an etiologic role in a new and frequently fatal type of human hemorrhagic fever occurring in the Philippine Islands and Thailand. Aedes aegypti mosquitoes were incriminated as the important vector in two epidemics. This work included the first reported instance of the isolation of a dengue virus from wild-caught mosquitoes.

Among young adults at the University of Wisconsin, the measure of acute respiratory disease which can be identified etiologically has been raised to over 28 per cent by one of our grantees. In previous work this investigator had identified about 20 percent of these illnesses as specifically due to streptococci, influenza virus, adenovirus, or bacteria other than streptococci. The present studies on 227 students hospitalized with respiratory illness over a two year period showed

that the hemadsorption viruses are responsible for another 8 to 10 percent of the cases and thus are the most frequent cause of sporadic respiratory infection yet found in the student body. A clear definition of the relationship between specific viruses and clinical symptomatology in terms of age group, frequency of attack and types of population involved will facilitate the creation of practical measures for the prevention of respiratory disease.

Impressive evidence for the effectiveness of an attenuated virus vaccine for measles has been recently presented in a series of reports by 23 investigators in 11 institutions working on a coordinated program under grant support from seven organizations, including this Institute. The investigators agree that any proposed vaccine for measles must be justified as both desirable and acceptable. In measles, because of the significant mortality, frequency of bacterial complications, occasional involvement of the central nervous system, widespread morbidity of the uncomplicated disease, heightened virulence of outbreaks in isolated populations and unfavorable effects on certain other pre-existing illnesses, a preparation conferring immunity comparable to natural measles seems desirable. The present experimental vaccine may be acceptable because of the following properties delineated in the various reports: ease of administration; lack of local reactions; absence of communicability; high index of serological response; elimination of bacterial complications; and demonstrated prophylactic efficacy. The work specifically supported by this Institute and carried out by grantees at Yale University and Western Reserve University, respectively, compared (1) the effectiveness of the subcutaneous route of injection with others that might be involved in natural transmission of measles and (2) the clinical, antigenic and prophylactic effects of the vaccine in institutionalized and home-dwelling children.

ASSOCIATE DIRECTOR IN CHARGE OF RESEARCH

INTRAMURAL RESEARCH PROGRAM

Summary ....

ASSOCIATE DIRECTOR IN CHARGE OF RESEARCH

INTRAMURAL RESEARCH PROGRAM

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
ANNUAL REPORT OF THE ASSOCIATE DIRECTOR IN CHARGE OF RESEARCH

INTRAMURAL RESEARCH PROGRAM
CALENDAR YEAR 1960

The year 1960 has seen both a widening of the scope of research activities and an intensification of the pursuit of promising lines of investigation in the National Institute of Allergy and Infectious Diseases. The coming year will see even more effective utilization of scientific resources, as new space becomes available and programs initiated this year begin to achieve their objectives.

Clinical investigations of the Institute have advanced in several ways. Of great significance for present and future programs has been the increased utilization of prisoner volunteers for clinical studies, through cooperation with the Federal Bureau of Prisons. Volunteers have been hospitalized in the Clinical Center, exposed to specific respiratory viruses, observed for clinical manifestations, and examined by precise laboratory techniques for evidence of infection. In a short time, these human volunteer studies have made available a vastly greater amount of detailed information concerning the respiratory syncytial virus and the Eaton agent than would have been possible by the usual clinical and epidemiological observations in the general population.

In connection with investigations on simian malaria, prisoner inmates have been inoculated with various strains of the parasite in the Atlanta Penitentiary. Some of them were transferred to the Clinical Center for precise clinical observations. This study also has permitted the extension of clinical and laboratory observations not possible in any other way. Other clinical studies underway for some years such as treatment of fungus infections, bacterial complications of cystic fibrosis, and drug resistance in staphylococcal infection have continued to add to specific knowledge of these diseases.

During the year an observation of great potential importance to public health was made by Institute malaria investigators and quickly substantiated by two other laboratories. Two scientists working with a strain of monkey malaria were accidentally infected, probably by a mosquito bite. This was the first clear-cut evidence that certain strains at least, of simian malaria, are pathogenic for man. An intensive investigation using all available resources quickly showed that man could be repeatedly infected by this strain through mosquito bite, that it could be transferred from man to man by blood, and that monkeys could be infected by mosquitoes fed on human cases. These observations formed the basis of a greatly expanded program on the pathology and biology of malaria, including the establishment of a field party in Malaya, the origin of the monkey strain infectious for man.

The Middle America Research Unit in Panama, established three years ago under the sponsorship of this Institute and the Walter Reed Army Institute of Research, has intensified its studies of the arthropod-borne viruses in

the tropics. Laboratory procedures have been developed and diagnostic reagents prepared for working with a large proportion of the more than 125 arthropod-borne viruses. The Middle America Research Unit also has extended its studies to include special epidemiologic observations of poliomyelitis in Panama, and investigations of the role of mites in Central American virus infections. In conjunction with Gorgas Memorial Laboratory workers, Institute scientists recovered two strains of vesicular stomatitis virus from Phlebotomus flies. This is the first time this group of insects has been definitely incriminated as a possible vector of this important virus.

Increasing interest in immunologic phenomena manifests itself in nearly all aspects of Institute research activities. The development of new immunologic techniques, such as immunoelectrophoresis and immunofluorescence, has stimulated new approaches to new and old questions and attracted investigators into the field. The result has been a noticeable resurgence of clinical and scientific interest in allergic diseases. The Institute initiated a new program in clinical immunology during the year which will investigate such autoimmune diseases as lupus erythematosus, and thyroiditis, as well as certain clinical aspects of hypersensitivity and mechanisms of resistance.

The very important studies on respiratory infections which have been underway for some years continue. It is now clear that the Eaton agent is, as long suspected, an important cause of primary atypical pneumonia. Development of more precise laboratory techniques for isolation of viruses from animals, demonstrated that many laboratory mouse stocks are grossly contaminated with normally occurring latent viruses. At least five different viruses are now known to infect apparently normal mice. This has complicated studies on the relation of viruses to cancer, and confused much of the work in this field conducted over the last few years. It seems unavoidable that the development of pathogen-free animal stocks, particularly mice, will be essential for future investigations in cancer and in other diseases as well. The development of some means to eliminate or control these contaminating viruses from experimental animals poses a major and immediate problem to this Institute and to investigators elsewhere.

The year has witnessed also the planned development of research experience for junior investigators and of opportunities for permanent staff members to work in research centers outside the National Institutes of Health. The key to productive and significant research is, as has been pointed out repeatedly, the selection of imaginative, energetic and well trained investigators and the development of a stimulating environment in which these investigators have the freedom and resources to pursue their scientific ideas. One approach to this goal is to recruit competent interested young men soon after completion of their doctoral training. During the last year three such young men joined this Institute as a part of the NIH Research Associate Program and seven as part of the Clinical Associate Program. Six others were recruited to specialized research activities making a total of 16 new scientific staff members at the junior level. From these no doubt will come a number of our future permanent staff and all will become indoctrinated with research experience of great value to their eventual scientific development. Thus, the intramural activities perform an important role for training in research methods and goals to the future benefit of scientific endeavor, wherever these men may work- in universities, hospitals, or in the practice of medicine.

The Institute has pursued vigorously a policy of encouraging work assignments of its permanent staff in other well known research centers. One scientist is working at the Karolinska Institutet, Stockholm, Sweden; two are at the Pasteur Institute, Paris, France; one at the Max-Planck Institute for Virus-forshung, Tubingen, Germany; one in collaboration with the University of California and Institut de Recherches Medicales de la Polynesie Francaise, Papeete, Tahiti; one at the Wallaceville Animal Research Station, Wellington, New Zealand; and one with the Virus Laboratory, California State Health Department. Others have spent shorter periods of time in Malaya, Africa, and Brazil, pursuing specific research problems. A number of the staff have served on WHO Expert Committees and on special assignments, particularly in the areas of tropical medicine and parasitology. In this field, this Institute has probably the largest group of investigators of any other center in this country.

Research in tropical diseases will be expanded still further under the provisions of P.L. 480 which permits the use of foreign currencies to support specific intramural research projects in certain countries. Projects have been formulated and implementation of them during the coming year will permit the selected expansion of investigations important on a worldwide scale. These include problems in such diseases as malaria, schistosomiasis, fungus infections, arthropod-borne viruses, and filariasis. These international activities emphasize the expanding scope of our research responsibilities.

The Board of Scientific Counselors met twice during the year. The first meeting considered Institute investigations underway on respiratory diseases of viral etiology. The second meeting was held at the South Carolina State Hospital, Columbia, South Carolina where the Epidemiology Section of the Laboratory of Parasite Chemotherapy is located. Institute staff members reviewed studies on simian malaria, chemotherapy of malaria, and intestinal parasite infections.

This report arranged by laboratory activities will summarize the major scientific accomplishments and advances achieved during the year in the direct intramural program.

LABORATORY OF CLINICAL INVESTIGATION

Clinical research activity has expanded during the past year largely as a response to enlarging the professional staff and cooperation with other Institute research units. A further period of growth will be needed to staff the clinical service in a manner consistent with optimum research productivity.

INFECTION OF VOLUNTEERS WITH RESPIRATORY VIRUSES

An extensive clinical study of acute viral respiratory disease was begun this year in association with staff members of the Laboratory of Infectious Diseases. The initiation of this project required much work but with the unstinting assistance of the Clinical Center, very satisfactory arrangements have been made to transfer to the Clinical Center, Federal prisoner-volunteers for study. This has been done with the permission and considerable assistance of Mr. James Bennett, Director, and Dr. Harold Janney, Chief Physician of the Bureau of Prisons, Department of Justice. A team of custodial officers has also been assigned to the Clinical Center by the Bureau of Prisons to oversee the volunteers. The volunteers have uniformly cooperated with the program despite some extended periods of room isolation, frequent blood-letting, and other inconveniences. Many administrative arrangements have been developed so that the program has worked increasingly smoothly.

The results so far justify the investment in money and effort. It has been possible to produce in human volunteers a rather uniform "cold" with the respiratory syncytial virus. It may occur without respect to preinfection immunity, but the subsequent rise in complement-fixing antibody appears to correlate with severity of illness. Forty-six men have so far participated in this study.

Approximately 24 other volunteers have participated in studies with para-influenza 4 virus or Eaton (primary atypical pneumonia) agent. Future studies are planned with human influenza virus and with the recently defined group of REO viruses.

NEW ANTI-FUNGAL DRUG

Beginning about four years ago, the Mycology Section of the Laboratory of Infectious Diseases began studies on an antifungal drug produced by Hoffman La Roche, designated as RO-2. This agent, an antimicrobial, was found to be the most active material ever tested *in vitro* and in animals against several of the pathogenic fungi, notably histoplasmosis and blastomycosis.

In the intervening years, 30 patients have been treated in the Clinical Center. Extremely favorable results have been observed in several patients severely ill with these diseases. From the standpoint of therapeutic activity, it appears that this drug is the best available for blastomycosis and histoplasmosis.

During the studies it was noted that the agent produced unusual hepatic changes. It was found that the dye, bromsulphalein, normally rapidly transferred from the blood to the bowel by the liver, was retained in high concentration in the blood in patients treated with RO-2. This effect appeared in a day or two after start of treatment, before tissue changes would likely occur, suggesting competition of the new drug for the liver excretory mechanism for bromsulphalein. After treatment was stopped, dye excretion promptly returned to normal or nearly normal. Liver biopsy has revealed changes indicative of minor hepatic damage in some cases. Because of the great importance of having a drug in addition to the relatively toxic agent, amphotericin B, for the treatment of fungal diseases, this new agent continues under investigation.

VOLUNTEERS INFECTED WITH SIMIAN MALARIA

Following the demonstration of the infectivity of Plasmodium cynomolgi bastianellii for man, several inmates at the Federal prison in Atlanta volunteered for exposure to this agent. The need for careful clinical characterization of this disease in man led to the transfer of two infected volunteers to the Clinical Center. Both men developed acute malaria which was carefully studied throughout its course. Significant alterations in urinary steroid excretion were detected, an unusual elevation of serum cholesterol occurred in one, and liver lesions not previously described, resembling but not identical with exo-erythrocytic phase parasites, were demonstrated in both patients.

PENICILLINS AND PENICILLINASE

Previous work here had defined nutritional requirements for penicillinase production by staphylococci and many parameters of its interaction with benzyl penicillin (penicillin G). During the year English investigators, working with the stripped molecule of penicillin, 6-amino-penicillanic acid, produced a new compound largely resistant to destruction by penicillinase. Working with this and several other penicillin derivatives, it was found that resistance to penicillinase is greatly influenced by steric positions of ethoxy groups on the side chain and that failure to be destroyed by penicillinase is associated with greater penicillinase inducing-capacity. Perhaps most significantly, this work has added to the now substantial indirect evidence that the major reason for present resistance of staphylococci to penicillin is the capacity of these micro-organisms to produce penicillinase upon contact with even low doses of penicillin.

Clinical studies with the penicillinase-resistant penicillin, dimethoxy-phenyl-penicillin, have revealed that it possesses resistance to penicillinase in vivo, and that it is a powerful and effective anti-staphylococcal drug. After treatment of some patients for periods of three to five months, no penicillin-resistant staphylococci have been isolated. It has long been considered that chronic staphylococcal infection resembles tuberculosis and for the first time it appears that an agent is available which can be employed for extended periods of treatment without loss of effect, such as isonicotinic acid hydrazide in tuberculosis. If long-term therapy can thus be regularly given an enormous benefit will accrue to thousands ill with chronic staphylococcal disease.

ASCITES IN MICE BY INJECTION OF ADJUVANTS

In the past year a staff member has continued to study ascites induced in mice by the injection of adjuvant mixtures. This procedure has provided a much needed laboratory method for producing antibodies of a wide variety and a means of evaluating antigens. Recently, interest has been focused on the pathology and abnormal physiology of the lesion. It has been found that strain differences are associated with differences in susceptibility to ascites. Since ascites was found associated with a local plasma cell reaction, which in some strains of mice went on to plasma cell tumors, many implications toward problems in neoplasia have also been raised. These studies have become the basis for biochemical and pathologic studies with other Institutes.

HYPOGAMMAGLOBULINEMIA

Staff members have shown that patients with hypogammaglobulinemia possess low, but definitely measurable, levels of antibody to the enteric viruses. The implication of this finding, in view of the normal resistance of these patients to viral infections, is that extremely low levels of specific antibody may provide adequate resistance against viral diseases. Subsequent, unpublished studies have indicated that these patients will develop circulating antibodies to Salk polio vaccine.

BENTONITE FLOCCULATION TEST

The modification of the bentonite flocculation test for the detection of gamma globulin promises to provide the practicing physician with an accurate, convenient laboratory aid in the diagnosis of hypogammaglobulinemia. In contrast to the electrophoretic method, the results with the bentonite test can be known to the physician within a few minutes of arrival of the specimen to the laboratory. Other modifications of this technique will also give the levels of albumin and other protein constituents of blood and other fluids without resort to the more cumbersome method of electrophoresis.

The DNA-bentonite test for systemic lupus erythematosus has also been developed in the past year. This test measures the antibody in lupus serum which is directed against nuclear material. The specificity of this test is greater than any previously described test for lupus. This test, however, is positive primarily in those patients with active disease and only rarely is positive when the disease is in remission. A modification of this test, the nucleoprotein-bentonite test, has retained all the attributes of the DNA test in regard to specificity, while achieving a much higher level of sensitivity for cases in remission. These promise to become important standard tests in the diagnosis of systemic lupus erythematosus.

CYSTIC FIBROSIS OF THE PANCREAS

Despite the commonly accepted point of view that antibiotics are helpful in this disease, observations of nasopharyngeal cultures failed to reveal any appreciable effect of antimicrobial treatment on Staphylococcus aureus. This does not negate a possible clinical benefit but does appear to minimize its value. It was coincidentally observed that Escherichia coli was not present in the nasopharyngeal flora of any child over the age of eight.

ROCKY MOUNTAIN LABORATORY

At the Rocky Mountain Laboratory research has continued directed toward both basic laboratory investigations and field studies of insect- and animal-borne diseases. In the first category are those projects concerned with the chemistry and surface properties of viruses, the highly intriguing relations of hypersensitivity and humoral immunity, and the basic relation of structural elements of microorganisms to the activity of the agents, both in vivo and in vitro. Field work is the foundation of projects related to studies of Q fever, tularemia, Colorado tick fever, and the arbo viruses. In addition, the combined efforts of the staff are directed to many other areas of research. Such projects include Q fever, tuberculosis, influenza, poliomyelitis, and cryptococcosis.

HYPERSENSITIVITY Studies of hypersensitivity at the Rocky Mountain Laboratory have been directed primarily toward clarification of the relations existing between delayed hypersensitivity and circulating antibodies and determination of the factors responsible for induction of contact hypersensitivity. It has been demonstrated previously that delayed hypersensitivity precedes circulating antibodies. This delayed hypersensitivity is directed toward protein. When circulating antibody appears, as occurs with relatively large amounts of conjugated protein, or when booster doses are administered, the specificity of the antibody response becomes oriented toward smaller configurations on the antigen molecule. Studies of immunity in neonatal animals have revealed that these animals, when injected with an antigen 12 hours after birth, develop circulating antibodies but fail to develop delayed hypersensitivity. Additional experiments suggest that this inability of neonatal animals to express such reactions is due to a deficiency in a skin reactive factor rather than an inability to respond to a primary injection of antigen.

It was shown that contact hypersensitivity is directed toward a hapten and that simple compounds rather than protein conjugates of these compounds produce contact hypersensitivity when administered to experimental animals. It is considered, however, that the production of this phenomenon is related to the specific proteins present in the host tissues because a conjugate containing soluble guinea-pig skin proteins and a hapten produces in guinea pigs delayed reactions and Arthus reactions to the conjugate and contact hypersensitivity to the hapten.

POLIOVIRUS Continued studies of poliovirus have yielded considerable fundamental data. In cooperation with the group at the University of Minnesota, it was shown that agents such as octyl alcohol-chloroform and neutral hydroxylamine do not materially change the physical properties of purified infectious RNA of poliovirus but destroy over 99% of the infectivity of this material. The destruction of infectivity by uncoupling of a single link in a large particle (probably an acyl link of an amino acid with a phosphate group at the end of an RNA chain) suggests a possible approach to virus chemotherapy. Other studies have revealed that only 0.1% of RNA infectivity could be accounted for by residual protein,

thus strengthening the concept that RNA does indeed constitute the infectious portion of the poliovirus moiety. By the use of chromatographic methods, it was also demonstrated that only certain of the avirulent strains of poliovirus can be differentiated from the virulent strains from which they are derived. This is in direct contrast to work reported by others. In studies of the infectivity of RNA it was found that the bulk of virus particles react with susceptible cells and that the relatively poor correlation between virus particles and PFU is due to the poor efficiency of RNA at entering sites where it can influence virus production. A precipitation test for detection of antibodies against poliovirus has been developed which is more sensitive than the neutralization test presently employed. The antigen used is radioactive virus.

ENDOTOXINS IN BACTERIAL FRACTIONS

Research on endotoxins derived from Gram-negative organisms has continued. As is so frequently the case, a fresh outlook on an old problem yields results of great value. The ideas held by Dr. Westphal, which attributed the activity of endotoxins to the presence of a firmly bound lipid ("lipid A"), were apparently generally accepted until presentation of the work done at RML. The finding that lipid A was not active and that deproteinized and "delipified" endotoxin was active stirred considerable controversy. In fact, the controversy was so intense that efforts to develop a vaccine against Salmonella infections have been diverted to settling this issue. Recent studies of the kinetics of inactivation of toxin by hydrolysis with hot acid have given data which should end this discussion. The old concept of "purified endotoxins" must be abandoned since there have been no previous toxins as good as those obtained at RML, and these are to be still further purified.

The purification of Vi antigen by curtain electrophoresis is a major advance in the study of this most important antigen. The demonstration that certain labile acetyl groups are responsible for the activity of Vi antigen resulted in production of material which was ten times more active than purified preparations prepared by mild acid hydrolysis. Emphasis should be placed upon the new chemical, physical, and biologic methods that have been devised to solve these problems.

TUBERCULOSIS

The problem of immunity in tuberculosis has been studied intensively. Significant findings include the fact that mice may be satisfactorily immunized to subsequent pulmonary infection with virulent organisms by administration of small doses of avirulent organisms by the aerosol method. The demonstration that resistance is not due to interference strengthens the case for the value of immunization with living attenuated organisms for the prevention of tuberculosis. Since it has been shown that the delayed reactions elicited by protoplasm of various acid-fast organisms are specific in nature, it seems practicable to apply these findings to certain diagnostic problems in man. Use of fractions of tubercle bacilli in producing isoallergic encephalitis in guinea pigs shows that the adjuvant effect lies in the cell walls and in a water-soluble protein prepared from the walls. This latter finding is important since it will allow the study of the adjuvant phenomenon from a molecular level.

Q FEVER It has been demonstrated that the number of dairy cattle infected with Coxiella burnetii is large and is increasing, yet it is extremely difficult to detect cases of clinical disease in man. In Idaho and Montana we have shown that a greater number of individuals residing on infected premises have antibodies against this organism than do those living on noninfected premises, yet no difference can be detected in the number of individuals who have symptoms compatible with clinical disease. The fact that organisms isolated from cattle have been uniformly of low virulence for experimental animals may account for the inability to find clinical cases of disease in those exposed only to cattle.

By the use of skin tests to eliminate allergic individuals from the study group, 190 inmates of the Montana State Prison were safely immunized without producing such reactions as have been previously reported. It is evident from these results, as well as from those previously obtained in laboratory personnel, that human beings can be safely vaccinated against Q fever if the precaution is taken to eliminate reactors by previous administration of specific skin-test antigen.

Methods developed for growing rickettsiae on modified Zinsser tissue cultures yielded relatively large volumes of organisms. These studies led to others involving purification of C. burnetii by sucrose gradients and by continuous-flow centrifugation in molar salt solution. These methods likewise made it possible to obtain certain chemical and physical fractions of these organisms. It was found that dimethyl sulfoxide could extract from Phase II C. burnetii a material which acted only as a hapten, but from Phase I organisms the extract obtained acted as a complete antigen. Lauryl sulfate also extracts complete antigen from Phase I organisms. Physically, the cell walls of these organisms can be separated from the protoplasm, and it has been noted that the cell walls are about 25 times more active in producing immunity than is protoplasm.

These studies on Q fever are of considerable significance. The laboratory and related studies have yielded information of both scientific and applied interest. It is apparent now that we can safely use our present vaccines for immunization of man and that it is feasible to produce large numbers of organisms which can be purified and used as vaccine or manipulated to give physical or chemical fractions which may be less toxic. The failure to find clinical cases of Q fever in man in the face of a rising incidence of infection in dairy cattle is highly interesting even if disappointing. The lack of virulence of strains of C. burnetii for laboratory animals probably is responsible for this finding.

OTHER RICKETTSIOSES Studies of rickettsiae other than C. burnetii have been continued. By combining the methods presently used for fluorescent microscopy, a technique for sectioning arthropods has been developed which should be of interest to entomologists working in the field of embryology and anatomy and to medical entomologists, since thin sections in which the organs are not displaced can be obtained routinely. By applying the technique to the study of ticks infected with R. rickettsii it was found that the infection rate in local ticks varies

from 15% to 28%. Not all of the ticks found infected by this method are infective for laboratory animals. The value of this type of study has yet to be fully appreciated.

The use of specific toxins has resulted in clarification of many of the problems related to the taxonomy of rickettsiae and has proved to be useful in ecological and epidemiological studies of this complex group of diseases. In further studies, potent immunogenic extracts have been obtained from certain of the rickettsiae. Their value as diagnostic and prophylactic agents is presently under consideration.

BACTERIAL VACCINES Studies have been continued on vaccines for certain bacterial diseases. It has been found that while live Russian tularemia vaccine is capable of protecting mice more effectively than does ether-extracted vaccine derived from cell walls of P. tularensis, the protection produced by live organisms was not effective for long periods of time. Continued studies have emphasized the value of cell walls in producing immunity to infections with Brucella abortus in laboratory animals. It has also been found that live cells suspended in phosphate buffer and shaken with an excess of ether are killed but not disrupted. These cells constitute an excellent protective antigen which is less toxic (LD_{50} 7.5 mg.) than aqueous ether extracts obtained by conventional methods (LD_{50} 0.9 to 2.0 mg.).

AR-BO VIRUSES Studies of ar-bo viruses have yielded results of interest and suggest that emphasis on field studies would greatly increase the production of useful data. The California strain, described by Reeves and Hammon, has been isolated from a snowshoe hare in Montana, and serologic studies of hares obtained from Michigan indicate that the majority possess antibodies against this virus. In California it has been demonstrated that, although most infections in man with this agent are of the inapparent type, some infections result in serious disease. A virus closely related to Powassan virus was recovered from ticks from Colorado and is of importance since viruses of this group produce serious illness in man. Studies to date indicate that ticks probably are not the natural vector, but the relation to Powassan virus suggests that mosquitoes would most likely be the vector in nature. In studies of the complex relation of WEE virus with snakes and mosquitoes it has been possible to demonstrate that the virus can be readily overwintered in garter snakes and that mosquitoes can be infected by feeding on such snakes. While we have not been successful in isolating virus from snakes collected in the field, the laboratory data suggest that these or similar animals could constitute a host suitable for overwintering of WEE virus. In Idaho and Oregon, WEE virus was isolated with considerable frequency during the summer season, while in North Dakota the virus did not appear to be active. Isolations of a considerable number of strains of trivittatus and inornata viruses were made.

Considerable research was performed to determine the level of viremia attained in wild and domestic birds infected with ar-bo viruses. After infection with WEE or St. Louis viruses, turkeys, ducks, chickens, and pheasants display levels of viremia which should cause infection in mosquitoes feeding on them. It is of interest, however, that in spite of considerable effort

we have been unable to isolate ar-bo viruses from the bloods of vertebrates. Negative results were obtained in examination of 1,074 specimens collected in Montana, North Dakota, Oregon, and Minnesota during the spring of 1960. These studies fail to add weight to the contention that latent infections of birds are a factor in overwintering or of introduction of virus into endemic areas.

COLORADO TICK FEVER

Colorado tick fever continues to be a problem in the western United States. Without stimulation of physicians a large number of specimens for examination were received this year and virus was isolated from 49 of them. Our interest in the spectrum of symptoms has continued and we still see severe cases of illness due either to encephalitis or bleeding tendencies. It was found that the complement-fixation reaction developed at the Rocky Mountain Laboratory is the simplest method for diagnosis of Colorado tick fever. Vaccine has been prepared and has been shown to be efficacious in mice. This type of vaccine has been used repeatedly in man without ill effects, indicating that a vaccine prepared from suckling mouse brain is harmless to man when repeated doses are given.

Ticks collected in Estes Park, Colorado, were examined for the presence of Colorado tick fever virus. The incidence of infection was found to vary from 5% to 21%. This high incidence of infection in ticks accounts for the large number of cases of CTF reported in Colorado annually.

LABORATORY OF TROPICAL VIROLOGY

The activities of this laboratory are conducted at the Middle America Research Unit in the Panama Canal Zone and at Bethesda. The Middle America Research Unit is a joint research effort of the National Institute of Allergy and Infectious Diseases which has cognizance for studies on virus diseases and the Walter Reed Army Institute of Research which has cognizance for studies on fungus diseases.

VIRUS ISOLATES FROM PANAMANIAN MOSQUITOES AND SANDFLIES

During the first 12 months of a 3-year project on the ecology of arthropod-borne viruses in the tropical rain forest, conducted by the Gorgas Memorial Laboratory with the collaboration of MARU, major emphasis has been on virus isolation in suckling mice and hamster kidney cell cultures. Fourteen virus strains were isolated at MARU from 412 pools and 63,000 specimens provided by GML. Virus isolation rates were for Phlebotomus, 1:700 and for mosquitoes, 1:7000, although the rates varied greatly with species. Of the five Phlebotomus isolates, two of broad host range (including cell culture) and short incubation period are serologically identical. These viruses have now been identified as the Indiana type of vesicular stomatitis virus. The other three Phlebotomus and nine mosquito viruses are being related to each other, to known virus groups and to human and/or animal infection and disease.

EASTERN EQUINE ENCEPHALOMYELITIS VIRUS INFECTION IN PANAMA

The prevalence of EEE antibodies in horses and man in two areas of suggested EEE virus endemicity has been determined, allowing an evaluation of the relative usefulness of several serological methods applicable to studies of this type. It was found that the incidence of EEE antibodies in 460 humans tested increased with advancing age (0.8% under 10 years with progressive increase to 9% in the 41-50 year group). Complement fixation results on the same sera indicated the probable presence of other group A viruses.

Lizards of species common to this part of Panama were examined as a possible virus reservoir. Specific EEE virus hemagglutination-inhibitors were found in some of their sera. The occurrence of viremia and HI antibody response following virus inoculation were experimentally confirmed by inoculation of lizards.

ENCEPHALOMYOCARDITIS VIRUS INFECTION

Previously this laboratory described an outbreak of a fatal disease of swine caused by the EMC virus. The outstanding lesion in pigs dying during the outbreak was acute myocarditis. Since epidemiological observations suggested that natural infection resulted from ingestion of contaminated food, experiments were undertaken to reproduce the disease by feeding virus to young pigs. Viremia and virus excretion from the gastrointestinal tract were found to occur following the administration of brain from EMC inoculated mice. Infected pigs developed high titers of HI and neutralizing antibody during convalescence and had myocardial fibrosis at autopsy. Other studies included demonstration of EMC antibodies in a small number of city rats and rats caught on the affected farm, although wild rodents were found to be negative. Human sera were examined with interesting differences in the results depending on the donors' age: while a substantial proportion of the Panamanian population has been infected with EMC virus, the antibodies were found to be more common in persons of younger age.

ENTEROVIRUS FLORA IN CHILDREN OF CENTRAL AMERICA

For a period of 12 months the enterovirus flora of infants at an outpatient clinic in Panama City was systematically explored, establishing a base line of enterovirus fluctuation. The majority of viruses isolated belonged to the ECHO group, although in late 1959 and early 1960 poliovirus type 2 had become very prevalent. This was reflected in an uncommon occurrence of a small outbreak of paralytic disease due to type 2 poliovirus.

Other enterovirus studies have included 1) surveillance for the presence of type 1 poliovirus in Panama in late 1960 as a check on dissemination and threatened spread of this commonly epidemic type, 2) studies on a major epidemic of ECHO-9 virus which swept through the Republic of Panama and the Canal Zone and 3) initiation of a collaborative project on possible relation of enterovirus flora of Guatemalan children to their dietary status.

MYCOTIC DISEASES IN PANAMA

The research program on mycotic diseases has markedly increased local awareness of histoplasmosis in all of its clinical forms, as evidenced by recognition of three disseminated cases, two fatal and one successfully treated, within a period of 18 months. Until then only one fatal case had been described since Darling's original cases in 1906. Ecological and epidemiological studies led to isolation of H. capsulatum from eight additional soil samples, bringing up to 16 the total number of recent isolations from Panamanian soil. The fungus has been repeatedly recovered from the organs of trapped ground mammals, confirming its wide dissemination in nature.

Histoplasmin skin test continues to be a major tool for the study of epidemiology of histoplasmosis. Data on 9,200 children between six and 19 years of age have been obtained indicating, as expected, that the percentage of reactors increases progressively with age. The rate of histoplasmin sensitivity varies from 13% to 58% among six-year olds and from 68% to 92% among 19 year olds, depending on location of their residence. A survey of 631 pre-school children (six months to six years) in the Canal Zone demonstrated an increase in hypersensitivity beginning with three years of age. A continuing similar study of Panamanian children in a city hospital is now in progress with information on over 800 already available.

Projects on other mycotic diseases have included diagnostic study and therapy of moniliasis, found to be a major superficial mycosis among both indigenous and transient population in the tropics.

ARBOR VIRUS STUDIES AT BETHESDA

At Bethesda new projects involved an interesting application of the technique of antiserum pool combinations to typing of arthropod-borne viruses, a wealth of data evaluating experimentally produced EEE virus infection in horses and a promising attempt to develop an inactivated EEE virus vaccine for human use. The infected horses yielded specific antiserum which is being processed for prophylactic use in cases of human exposure under laboratory or natural conditions.

Accidental laboratory infection of a staff member with an arthropod-borne group C(Apeu) virus led to the first clinical-virological study of a syndrome produced by this important and common group of viruses of the western hemisphere.

LABORATORY OF BACTERIAL DISEASES

The research program of the Laboratory of Bacterial Diseases has continued in the same general areas as last year.

INTRACELLULAR PARASITISM

These studies deal with possible changes in characteristics of infected and immune cells as the result of parasitism, and the effect of intracellular growth on the parasite. One such notable change of course is the production of specific antibodies by certain cells of immune animals. Effort has been directed toward the study of antibody production by cells in vitro and the macrophage was

selected as a multipotential cell for such study. Macrophages obtained from the peritoneal cavity of guinea pigs immunized with egg albumin have been found to release antibody in vitro for a period of several days. This provides a system for further study of the nutritional or other requirements for continued antibody production in vitro, or even in serial cultures. Cells derived from macrophages have been carried in serial tissue culture for several months, retaining their phagocytic ability.

BRUCELLOSIS Studies on brucellosis are conducted at a reduced tempo. There is continuing need to collaborate with other brucellosis research centers throughout the world to try and settle problems of classification and epidemiology of the Brucella. Currently we are doing some laboratory testing of brucellosis vaccine for human use prepared in Russia. There is present interest in this vaccine by the World Health Organization for its possible use in occupational and otherwise continually exposed groups.

Studies on the Staphylococcus are directed toward determining the factors responsible for pathogenicity, and toward development and standardization of tests for measuring relative pathogenicity of strains.

LABORATORY OF CELL BIOLOGY

The activities of the Laboratory of Cell Biology during the calendar year 1960 have been along three major lines: (A) The continued exploration of the metabolism of normal cultured cells, and an approach to the problem of metabolic controls; (B) the mechanism of viral synthesis; and (C) the study of cell cultures deriving from patients with hereditary metabolic disease.

METABOLISM OF NORMAL CULTURED CELLS A number of significant observations have been made with respect to the amino acid metabolism of cell cultures. There has been no further elucidation of the pathway of serine synthesis; but the mechanism of cystine synthesis has been clarified, in that all the cell lines so far studied have been shown to use the classical pathway involving the demethylation of methionine to homocysteine, the condensation of the latter with serine to form cystathionine, and the cleavage of the latter to cysteine and homoserine. A dual pathway for proline synthesis has been indicated, one involving glutamine as the source of the carbon skeleton, and the other involving arginine by way of ornithine.

An intriguing recent observation has been the finding that a number of factors which are rigorously required by the cells for survival and growth can in fact be synthesized. Their nutritional requirement reflects the fact that they are lost from the cellular pool to the medium at rates which exceed the biosynthetic capacity of the cell; and with a sufficiently high cell population density, when the loss to the medium per cell is sufficiently reduced, the supposedly essential growth factors are in fact not required for survival.

In these cell cultures, unlike bacteria, the biosynthesis of amino acids is apparently not inhibited by the product of the reaction; and this mechanism of growth control is apparently not operative. Studies are in progress as to whether enzyme repression or feedback inhibition are effective controls in the biosynthesis of pyrimidines. A quite different control mechanism is perhaps indicated by the demonstration of a growth inhibitor in the supernatant medium of heavy cultures. The chemical nature of that inhibitor is under continuing study.

Studies on the mechanism of resistance to 2-deoxyglucose (2DG) have shown the presence in the resistant variants of compounds which inhibit the phosphorylation of 2DG to the metabolically active inhibitor, 2DG-phosphate. The relationship of that inhibitor to the observed resistance is under continuing study.

MECHANISMS OF VIRAL SYNTHESIS

A number of important new observations have been made with respect to the mechanisms of viral synthesis. The puzzling wide disparity between the number of physical particles in viral suspensions, and the number of plaque-forming units, i.e. particles capable of initiating infection in susceptible cells, has been partially resolved with the demonstration that after the viral particle has been absorbed by the cell, it may undergo several alternative fates. A large proportion are rapidly eluted into the medium, essentially intact but no longer infectious, presumably reflecting a minor alteration in the protein coat. Some particles remain unchanged within the cell. Others are degraded intracellularly, in that the nucleic acid is exposed and becomes susceptible to intracellular ribonuclease. Only a small fraction of the absorbed viral particles are stripped of their protein and initiate infection.

In the case of poliovirus in the HeLa cell, although the viral protein and RNA are synthesized concomitantly, a partial dissociation has been achieved with appropriate inhibitors of protein synthesis, which completely block the formation of mature virus, but not of infectious RNA. This is of particular importance in relation to the supposedly obligatory relationship between protein and RNA synthesis in growing cells. Of interest also is the fact that metabolic inhibitors which effectively block the synthesis of cellular DNA and of DNA viruses have no effect on the formation of poliovirus. It would therefore appear that poliovirus RNA may be used directly as a template for the formation of virus, without the necessity for intervening DNA synthesis.

In contrast to the situation with poliovirus, in the case of vaccinia, there was a marked lag between the formation of the viral nucleic acid (DNA) and that of the mature virus.

CELL CULTURES FROM PATIENTS WITH GALACTOSEMIA

An exciting new development has been the successful cultivation from patients with a hereditary metabolic disease (galactosemia) of cells which in culture demonstrate the metabolic defect characteristic of the disease. This suggests an entirely new experimental approach to problems of human genetics.

GERMFREE ANIMAL STUDIES

A series of observations has been made on the behavior of Entamoeba histolytica in the germfree host. It is to be recalled that, in earlier studies with standardized techniques, amoebic lesions were not produced in the germfree animal following inoculation. In fact, the parasite failed to live in the intestine beyond five days. Recent changes have been made in the manner of rearing and handling the amoebae in vitro prior to inoculation which seemed to result in more vigorous organisms. The latter have produced lesions in the absence of bacteria, although the type and severity are still not typical of those encountered with a bacterial associate. Thus, it would appear that the latter is not the only determinant of the course and the pathogenesis of the infection.

Studies have shown that the intestinal mouse parasite, Nematospiroides dubius, does not require a flora to develop from an infective larva to the adult form in the host. However, it apparently does require a flora or its products, to develop from the egg to infective larva. These studies are preparatory to those to be undertaken in an analysis of the nature of the nutritional effects observed in certain parasitisms. One of the interesting observations has been the finding that the sex of the host, which has been noted by several workers to affect the outcome of the infection in conventional (contaminated) animals, has not appeared to be an influence in the germfree host. If these findings continue to hold up, a hitherto unrecognized role (either direct or indirect) of the flora in certain observed sex effects may unfold.

BIOLOGY OF GERMFREE GUINEA PIGS

In studies on the growth and biology of germfree guinea pigs, a staff investigator has obtained several advanced pregnancies in animals maintained on irradiated diets, although no fetus was carried to term. It is to be recalled that germfree guinea pigs have not yet been bred with success. The importance of the intestinal flora to this species was pointed up by the finding that conventional (contaminated) guinea pigs reproduced normally on this same irradiated diet.

In a collaborative project with an investigator at the University of Pennsylvania, it has also been shown that the use of large dosages of a cathartic, or the application of tourniquet shock, increased the number of red cells of germfree chickens coated with human B-like antigens following monoinfection with Escherichia coli O_{86}. These studies are providing information on the manner in which red cells of one type may acquire antigenic characteristics of other cell types, especially B.

GERMFREE MOUSE COLONY

The germfree mouse colony has been undergoing an intensive serologic study including an assay for the presence of certain so-called "natural antibodies" against a variety of bacteria. Such antibodies or antibody-like reactivities for organisms like Staphylococcus, E. coli and Salmonella typhosa have been found to occur in a variety of uninoculated conventional animals and are presumed to originate from encounters with the viable organisms or related

antigens. Animals which have lived for many generations free from contact with live bacteria are almost the sine qua non for establishing finally the validity of these ideas. Studies thus far, with the Communicable Disease Center and investigators in the National Cancer Institute, have shown the germfree animal to be singularly free from antibody-like reactivity toward Staphylococcus and E. coli. Reactivity, however, toward S. typhosa was obtained in several instances, although no evidence of the presence of the latter was found in the germfree colony. Thus, this finding strengthens sporadic reports that non-bacterial substances (perhaps in this case dietary components) can cause "cross" reactions with this organism.

TUMORS IN GERMFREE ANIMALS

The germfree animal colony and a conventional colony derived from the same stock now has existed for for approximately two years. Some of our exbreeders, in spite of the scarcity of germfree unit animal space, are one to two years of age. Whenever a germfree or conventional animal not on an experiment dies, especially if it is six months or more of age, it is examined thoroughly for gross evidence of malformations or tumors. Among approximately 50 such animals so-called spontaneous lung tumors have occurred in some of the germfree as well as the conventional mice. While the numbers of animals are obviously small, the incidence has been markedly higher, thus far, among the conventional animals (those exposed to external contamination) than among the germfree. This seems to be particularly true among animals six to twelve months of age.

LABORATORY OF PARASITE CHEMOTHERAPY

This country's commitment of 38 million dollars in Fiscal Year 1961 toward a program of world-wide malaria eradication and the long-term interest in malaria by most of the senior staff resulted in a research effort, during the past year, largely directed toward problems in that field. Special emphasis was given to the study of simian malaria in man and in monkeys because malaria in simians might be a real deterrent to the eradication program. Clinical facilities for volunteers at the Atlanta Penitentiary were enlarged and the staff increased. A laboratory was established at Kuala Lumpur, Malaya, in cooperation with the Malaya Institute of Medical Research and the United States Medical Research Unit. Studies on several aspects of the simian-human-malaria problem have been in progress there since mid-August.

As a result of the above development, it was decided to move the Section on Cytology, now located at Memphis, to Chamblee, Georgia, early in 1961. This arrangement will bring the simian hosts closer to the human volunteers at the penitentiary, and the insectary maintained by the Section will be geared to accommodate the work at Chamblee and at the prison.

MALARIA - HUMAN

Plasmodium falciparum (McLendon strain): Chloroquine (300 mg, base) and primaquine (45 mg, base) given together beginning three days after mosquito bites and weekly thereafter for a total of eight doses, resulted in suppressive cure in 5/5 subjects. Controls were positive 11 to 15 days after infection. After two days of parasitemia, each control was given the above drug combination which was repeated weekly

for a total of three doses. Parasites were removed promptly and cure was obtained based on no evidence of infection during 227 days of observation.

Primaquine, at daily doses of 0.75 mg, had some sporontocidal effect upon Plasmodium falciparum gametocytes but none against those of P. vivax (one case). Therapeutic doses (1.4 gm in three days) of amodiaquine had no sporontocidal effect against gametocytes of P. falciparum (one case). The effect referred to is against the development of the malaria parasites in the mosquito.

A strain of Plasmodium falciparum from Colombia, South America, was found to be resistant to chloroquine. This finding is of utmost importance in terms of malaria eradication.

Plasmodium vivax (Chesson strain): A drug combination of primaquine (45 mg) and pyrimethamine (50 mg) given weekly beginning seven days after mosquito bite and continuing for a total of four doses, gave suppressive-cure in 4/5 subjects; the other subject developed a patent infection 240 days after infection. Pyrimethamine (50 mg) given alone, as above, produced suppressive-cure in 1/4 subjects; the other three came down on days 82, 83 and 84. Five controls all came down 12 to 13 days after infection.

The Russian 8-aminoquinoline, quinocide, was compared with primaquine and found to be distinctly inferior as a curative drug against early and late primary attacks of Chesson vivax malaria particularly from the standpoint of the occurrence of second and third relapses.

Another 8-aminoquinoline, Win 5037, was studied in five subjects. Toxic effects and failure to cure made further investigation unwarranted.

Plasmodium malariae: The results of a 14-year study of the biology of Plasmodium malariae were drawn together for publication. The highest infectivity for mosquitoes occurred during the eighth to tenth weeks of the primary attack. Although the infection rate of mosquitoes was ordinarily low, the relatively long period during which mosquitoes could be infected may explain the persistence of P. malariae in nature. The ability of the symptom-free malarious patient to infect mosquitoes at a rate similar to that of the symptomatic patient makes eradication difficult.

MALARIA - SIMIAN

Plasmodium cynomolgi bastianellii: In early May, two accidental sporozoite-induced infections with Plasmodium cynomolgi bastianellii occurred at our Memphis Laboratory. This happening was of signal importance because it showed that simian malaria, contrary to the generally held opinion, was infectious to man. In that light, full scale study of human infections was undertaken at our Atlanta Penitentiary installation.

Two infections were induced in inmate volunteers by inoculation of infected blood obtained from one of the accidental sporozoite-induced infections in man. Twenty inmate volunteers were infected by bites of Anopheles quadrimaculatus or Anopheles freeborni which had fed on infected monkeys. The prepatent period ranged from 14 to 29 days and the parasite density ranged

from 5 to 500/cmm. The most constant symptom was headache and the most significant signs were fever, splenomegaly and hepatomegaly. Infections were allowed to run their course, generally without treatment.

Anopheles freeborni were infected from two patients but attempts to infect volunteers by their bites have yielded equivocal results. The finding that P. c. bastianellii will grow consistently and produce clinical illness in man suggested the possibility that malaria is a zoonotic disease, that is, a disease which man can acquire from animals with which he is associated. Whether or not such transfer occurs in nature is not yet determined, but should it occur, it would be of greatest significance to the world-wide malaria eradication program.

Plasmodium cynomolgi cynomolgi: Eleven inmate volunteers were bitten by Anopheles freeborni infected with P. c. cynomolgi on 8 September, and to date (14 December) three have exhibited evidence of infection (i.e., fever). Parasitemia has been demonstrated in only one, on the 58th day after mosquito bites. These results show that this strain infects man far less readily than P. c. bastianellii.

FIELD STUDIES IN MALAYA — Three staff members, Drs. Eyles, Dobrovolny, and Mr. Clinton S. Smith, were detailed to Malaya during the year where they engaged in the study of simian and human malaria in cooperation with the Malayan Institute for Medical Research and the U. S. Army Medical Research Unit at Kuala Lumpur.

The epidemiology of monkey malarias is being studied and the feeding habits of some of the Anopheles determined. By injection of uninfected monkeys with sporozoites from natural infections, it was determined that Anopheles hackeri is a natural vector of Plasmodium knowlesi. This is a most important discovery, especially since the vector of this parasite has been sought for repeatedly during the last 25 years.

Studies of malaria in aborigenes associated with monkeys have been made. Blood passed from aborigenes to monkeys have thus far produced no patent infection in the monkeys.

EE STAGES AND DRUG ACTION — Studies were continued on the direct effect of drugs on the exoerythrocytic stages of primate malaria. When sulfonamides were used with pyrimethamine to exploit the possible synergism of the two drugs, monkeys developed parasitemia 30 to 40 days after inoculation with sporozoites even though all parasites observed in liver biopsies were damaged. The curative efficacy of quinocide, the Russian drug, was compared with primaquine. Even when administered at twice the dosage used with primaquine, quinocide was less effective. Chloroquine had no observable effect upon the liver forms of Plasmodium cynomolgi. Young parasites appeared in the blood in large numbers on the 8th, 16th, and 24th day indicating the existence of secondary exoerythrocytic generations.

INSECT TISSUE CULTURE — Blood cells from caterpillers and cells of the ovariole sheath of several species of moth pupae have been cultivated in several different media. The virus of St. Louis encephalitis has been maintained in cultures of hemocytes from larvae of the

catalpa sphinx for ten days. Oöcysts of Plasmodium gallinaceum attached to the midgut of Aedes aegypti have shown growth in vitro and sporozoites have been produced.

BIOCHEMICAL STUDIES It was shown that mosquitoes infected with malaria have higher levels of ribonucleic acid than uninfected mosquitoes. Chromatographically, the acid-hydrolysate of ribonucleic acid from a pyrimethamine-resistant strain of Plasmodium falciparum differs from the acid-hydrolysate of ribonucleic acid from a pyrimethamine-susceptible strain. Bephenium hydroxynaphthoate inhibited glutamic acid transaminase of Nippostrongylus muris. Bephenium chloride and quinacrine reduced the rate of glucose absorption by the tapeworm Hymenolepis diminuta but low concentrations of dithiazanine iodide stimulated glucose absorption by this cestode.

INTESTINAL PARASITES Epidemiological studies on the inmates of a mental institution show a high persistence of Trichuris and hookworm for six years, with an apparent decrease in Strongyloides. To test dithiazanine and tetrachlorethylene, alone and in combination, heavily parasitized mental patients were given the drugs for about one year. A large number of worms were removed but the cure rate was low and transmission was not stopped. Bephenium hydroxynaphthoate and bephenium chloride were used with good results against hookworm, Ascaris and Trichuris.

SCHISTOSOMIASIS The activity of griseofulvin observed in mice infected with Schistosoma mansoni was not well developed in hamsters or monkeys. A series of tetracycline analogues which show an affinity for microfilaria did not combine with schistosomes and were without activity. One of these analogues was significantly more active against microfilariae of Dirofilaria immitis than tetracycline.

In many tests, the efficacy of stibophen (Fuadin) therapy on mature Schistosoma mansoni infections in mice was increased up to 16 times by feeding a balanced semi-synthetic diet. The toxicity of the drug was not similarly increased. The enhancement of curative action by the purified semi-synthetic diet was thought to be due to the absence of, as yet unidentified, inorganic salt(s) that interfere with drug activity. It was found in mice fed on the purified semi-synthetic diet that higher blood levels of the drug were maintained for a longer period than when the same amount of Fuadin was injected into mice fed on the commercial pellet diet, suggesting that the increased cure-rate was due to higher blood drug level. Similar drug advantage was observed in mice given tartar emetic while on the purified diet.

LABORATORY OF PARASITIC DISEASES

This Laboratory continues to emphasize fundamental studies on parasites and parasitic diseases. No important changes in the program were instituted during the year. The program of the laboratory is well diversified considering the size of the staff and the competencies of the various staff members cover a large proportion of the field of parasitology.

Although the emphasis is on basic studies, this does not imply a narrow viewpoint and the laboratory is well aware of the many practical problems parasitic diseases create throughout the world. The laboratory is often called upon for help and advice concerning prevention and control of parasitic infections and so must maintain competence, and a reputation for competence, to deal not only with basic problems of parasitism but also problems of prevention and control of parasitic diseases. Therefore, the laboratory continues to carry on a variety of activities which help it maintain its international reputation and increase its capacity to cope with problems of parasitism. Such activity also returns benefits in the form of ideas for laboratory research and clues which may explain puzzling laboratory findings.

TOXOPLASMOSIS Studies on toxoplasmosis in New Zealand sheep have shown that the prevalence is high. New information has been obtained concerning the distribution of the organisms in the tissues and their persistence there. After inoculation the distribution of the parasite in tissues is erratic and the parasites rapidly clear from tissues other than the muscle and placenta. Since residual infection occurs in muscle, mutton may serve as a source of human infection. Congenital infection with Toxoplasma is an important medical problem, therefore it is of special interest that the sheep studies have indicated that inoculation of sheep 60 days before pregnancy did not result in congenital infection or abortion but inoculation at 30 days pregnancy caused abortion or foetal death with absorption. Infection at 90 days pregnancy was less likely to be dangerous to the foetus.

The status of resistance or immunity to Toxoplasma continues to be puzzling, since living organisms fail to protect completely animals against challenge, especially when the challenge is great, and because low grade parasitemia may persist for months in mice and rabbits in the presence of high serum antibody levels. The observation that cysts of Toxoplasma probably form in tissue cultures provides a new opportunity to study the manner of cyst formation and the factors that lead to cyst formation.

AMOEBIASIS The work on the preservation of living Entamoeba histolytica and other protozoa has practical significance since success would permit retention of strains without continuous sub-culturing. This is a relatively new field and techniques are still evolving. The work so far has shown that this approach is feasible since four species have been frozen and stored for periods ranging from one to four months depending on the species involved. E. histolytica has been kept at -197° C for 24 hours, suggesting that almost indefinite storage at this temperature may eventually be achieved.

Laboratory culture of E. histolytica continues to receive attention since it is so important to learn more concerning its nutritional requirements and its pathogenicity in the absence of other organisms. It is noteworthy that satisfactory axenic culture of this species has been achieved for the first time. The protozoa are cultured in a complex diphasic medium containing no cells but including chick embryo extract.

The substitution of a species of Crithidia for Trypanosoma cruzi in cultures of E. histolytica provides a more economical and rapid way of producing large cultures of the amoeba. Demonstration of the value of the Coulter Counter for the enumeration of protozoa in suspension adds a valuable tool for quantitative work and suggests this method may be applicable for counting other organisms of similar size such as tissue culture cells.

PARASITIC INFECTIONS IN GERMFREE ANIMALS

The use of germfree animals in worm-parasite studies continues to reveal the value of this tool and adds to our knowledge of the peculiar nature of the germfree state. The technique seems to be particularly useful for studying conditions that influence natural resistance and nutritional relationships of parasite and host. For example, it was found that the roundworm, Nematospiroides dubius, develops as well in germfree as in conventional mice but while in conventional mice the worm recovery is much higher from the male animals, the recovery from germfree mice is the same for both host sexes. The cause of the difference is unknown. Also, it has been shown that the feces of germfree mice do not support development of N. dubius larvae and that bacteria in the feces provide important factors for larval development. There was further evidence that the alteration in levels of serum protein components in germfree animals is due to dietary factors.

STERILE CULTURE OF WORMS

Studies on the sterile culture of worms continues to produce fundamental information on the nutritional requirements of the parasites and brings closer the day when we can use the axenic animals for immunologic and therapeutic studies. Survival studies using relatively advanced larvae of Nippostrongylus muris has produced important results. The intent has been to try, by addition of elements to the medium, to induce the larvae to reach the adult stage. Starting with a salt mixture, dextrose was added until the optimal level was reached. Then casein was added and survival time rose to 11 days, but there was not development of the larvae. Addition of a yeast extract to this mixture not only increased survival but permitted growth to the adult stage. Thus, a much more simple medium than used before has been evolved and the achievement of a defined medium for culture of N. muris adults is much closer. A similar approach is being used in attempts to culture microfilariae of Dirofilaria immitis.

NUTRITION AND SCHISTOSOMIASIS IN PUERTO RICO

Although the study of the relation of nutrition to schistosomiasis in Puerto Rico is still incomplete, it appears that enrichment of the diet does not affect the number of eggs passed in the feces. However, it is interesting to note that the enriched diet did cause a loss of hookworms and whipworms from the intestine. This has a bearing on the

problem of the existence of hookworm infection without hookworm disease. In laboratory studies conducted in Bethesda the enhanced efficacy of stibophen in mice receiving a semi-synthetic diet was shown to be due to the absence from this diet of as yet unknown inorganic salts. Higher blood levels of the drug were maintained longer when the semi-synthetic diet was used and this may explain the greater efficacy. Demonstration of the influence of simple salts on the efficacy of stibophen suggests that other drugs may be similarly affected by diet. If the work with the stibophen-salt problem progresses satisfactorily it is hoped that a test of the effect of human diet on the action of the same drug may be tried in Puerto Rico before the study there is concluded.

DUAL VIRUS AND HELMINTH INFECTIONS

Interaction of two pathogenic organisms in the same host has had relatively little attention in spite of some very provocative work done in years past. A study of simultaneous infection with encephalomyocarditis virus and Trichinella spiralis in rats has produced striking and significant results. While the virus alone does not injure adult white rats when given intraperitoneally, in the presence of Trichinella spiralis infection many of the rats are crippled and die. This potentiation of virus pathogenicity is not due to nonspecific stress but seems to be related to the presence of the worms on the muscles. The virus can be recovered from the muscle of T. spiralis-infected rats but not from muscle of rats without T. spiralis. The reason for the influence of the worm infection on the activity of the virus is unknown. The phenomenon offers an opportunity to study some of the fundamental factors in the pathogenesis of both the virus and the worm parasite. It also provokes the question as to what effect this worm infection may have on other virus infections.

AMMONIA TOXICITY IN MICE

The study of liver damage in relation to ammonia toxicity in mice has revealed that low oxygen in breathed air greatly enhances ammonia toxicity. The mechanism of this effect is not clear. Though hepatic coma is usually considered to be related to ammonia toxicity none of the substances which exacerbate hepatic coma in man increases ammonia toxicity in mice. In fact, six of ten decrease it. Ammonia toxicity in mice was greatly reduced by hypothermia and this suggests that the same measure may be useful in treating hepatic coma in man. Finally, mouse liver damage was induced in eight different ways but none caused any change in the animal's response to intravenous ammonia. Thus, though high blood ammonia levels seem to be related to liver damage, the causal relationships are by no means clear.

BIOCHEMICAL STUDIES OF HELMINTHS

Fundamental physiological studies have focused on the calcareous corpuscles of tapeworms and on the phospholipids of tapeworms. The calcareous corpuscles are amorphous but, on heating, dolomite, brucite or apatite may be formed. Electron microscope pictures of corpuscles heated with KOH reveal the presence of well-formed crystals. The glycerol containing phospholipids of Taenia taeniaeformis are about half lecithid and half cephalin. Sphingomyelin is present and more than one cephalin is known to occur in the larvae of this tapeworm. Hexose-containing phospholipids occur in both larvae and adults.

Study of the mechanism of energy metabolism of sub-cellular elements has dealt, among other things, with the mechanism by which mitochondria which are depleted of high-energy phosphate intermediates are stimulated to oxidize substrates when ATP is added. This is a complex, though fundamental, bioenergetic system for which a better understanding is needed. Addition of ATP not only restored succinate oxidation but also caused reduction of intra-mitochondrial DPN. The succinate oxidation involves an energy-requiring reaction and this energy is apparently added at one site in the respiratory chain and used at another for reducing pyridine nucleotide.

LABORATORY OF BIOLOGY OF VIRUSES

The basic objectives of this laboratory continue to be the same as last year. It is obvious from this annual report that four out of five units have projects with the same general objective -- investigation of mechanisms and localization of animal virus synthesis within the infected cell. Each of these units is also interested in the infectious nucleic acid of viruses. In view of the complexity of this problem and the important implications of any information that is obtained, this "duplication" is quite justified. Actually, it is not duplication since different approaches are used and different virus-cell systems are studied.

The electron microscope has been installed and is now used not only by the Biophysical Unit but also by other units of our laboratory and by units of the Laboratory of Infectious Diseases. With studies on the structure of viruses and a project concerned with the genetics of animal viruses added to the biochemical and biological studies, there is now fairly complete coverage of the important facets of basic virus biology.

INTRACELLULAR LOCATION OF POLIOVIRUS

By use of radioautographs and staining with fluorescein tagged antiviral antibody, the intracellular location of poliovirus antigen--presumably viral protein--during the cycle of virus multiplication has been determined. Demonstrable antigen first appeared one hour after infection and was diffusely distributed through the cytoplasm. At three hours, just before the appearance of new virus, it was present throughout the nucleus with a tendency to be concentrated around the periphery of the nucleolus. At five to seven hours, particulate accumulation of antigen in the cytoplasm was noted. Incorporation of radioactive-tagged amino acid into cell protein ceased shortly after the start of infection, whereas incorporation of thymidine into RNA continued until after three hours and tended to localize in the nucleoli.

MUTANTS OF EMC VIRUS

Plaque type mutants of EMC virus have been found, segregated and characterized. The stability of the mutants has been determined and the plaque type shown to be a function of the viral RNA. It has been shown that the difference in the size of the plaques formed by these mutants is brought out by an inhibitor present in the agar overlay used on the plaque plates. This inhibitor resides in the agaropectin fraction of the agar and can be separated from the agarose fraction which then permits both plaque type mutants to form similar sized plaques.

POLYOMA VIRUS By the use of a serum protection test in newborn hamsters, evidence was found that polyoma virus transforms normal cells to tumor cells quickly and directly without extensive virus multiplication being necessary. Furthermore, no evidence could be found to suggest a lysogenic relationship of virus to tumor cell. All attempts to show the presence of infectious or masked virus or of virus antigen in transplantable polyoma-induced tumors have been negative. It appears that once the virus initiates the tumor it is no longer required for tumor growth and maintenance.

TETRACYCLINE FLUORESCENCE LOCALIZED IN MITOCHONDRIA The discovery has been made that when the antibiotic tetracycline stains tissues in such a way that they fluoresce under UV light, this fluorescence is localized in the mitochondria of the cells. This makes a convenient vital stain of these subcellular elements for further studies. There appears to be some similar localization of the antibiotic fluorescence in certain bacteria.

TMV MODEL A complex model of tobacco mosaic virus has been constructed on theoretical grounds, and on checking this model against known biochemical and biophysical properties of the virus a remarkable consistency is found. Certain refinements of electron microscopic technics have produced photographs of this virus which reveal previously not seen fine structure also consistent with the theoretical model.

LABORATORY OF IMMUNOLOGY

Since the activation of the Laboratory of Immunology in 1957, the program has been concerned, principally, with basic research. However, for some time an important need has been felt for the initiation of clinical studies in immunology and allergy. In September 1960 the Clinical Immunology Section was activated and as space permits, will be expanded and will work in close collaboration with the Laboratory of Clinical Investigation on clinical studies involving immunological aspects of such diseases as lupus erythematosus, nephritis, and chronic thyroiditis, in which an auto-immune basis is suspected.

ALLERGIC THYROIDITIS Experimental allergic thyroiditis was produced in Strain 13, inbred, histocompatible guinea pigs by immunization with a single dose of guinea pig thyroid extract in complete Freund's adjuvant. Thyroiditis developed as early as five days after immunization, was present in all animals at 16 days, and by seven weeks was consistently present and generally severe. Delayed skin test hypersensitivity was found as early as five days after immunization in nearly all animals, and was present in all animals with thyroiditis at seven weeks. At seven weeks after immunization, anti-thyroid antibodies were present, and antibody titres correlated with the presence and degree of thyroiditis. This correlation was not found at certain other times after immunization. The presence of delayed hypersensitivity was correlated with experimental allergic thyroiditis, while the presence of circulating antibody did not correlate with thyroiditis. These observations constitute

the earliest production of experimental allergic thyroiditis and the most severe disease at the time intervals studied.

HOUSE DUST ALLERGENS — Studies on the chemical and physical properties of house dust extracts that are used clinically for the diagnosis and treatment of house dust allergy have been studied to identify the components responsible for the specific skin reactions produced in house dust sensitive individuals. It has been found that the house dust extracts consist of a heterogeneous mixture of acidic polysaccharides. The heterogeneity has been demonstrated by electrophoretic and ultracentrifuge sedimentation analysis and also by the multiplicity of cross reactions obtained with antisera to the various pneumococcal polysaccharides. The chemical composition of the various fractions has been shown to be roughly 5-20% polypeptide and 80-95% polysaccharide, containing about equal amounts of uronic acid (probably glucuronic acid), D-glucose, D-galactose, D-mannose with lesser amounts of L-rhamnose and L-arabinose.

GENETICS OF GAMMA GLOBULIN — Agar-gel immunochemical analysis of sera from rabbit litters, with precipitating antibodies prepared in rabbits, has shown that seven antigenic determinants of the gamma globulins are genetically controlled by at least two gene loci with each specificity exhibited when the appropriate allele is present. Since the gamma globulins are soluble proteins which have properties of both an antigen and an antibody, they should be subject to quantitative estimation and cytological localization. This immunogenetic system, therefore, may be uniquely suited for the study of certain basic problems in genetics, embryology, immunology and protein chemistry.

In other studies, antibodies to human serum proteins were prepared in monkeys since this animal, being a closely related species, might be more discriminating for minor antigenic differences than a distantly related species. Three "slow" gamma globulins were found, instead of the one usually detected with horse or rabbit antibodies. Two of these were shown to be related to myeloma proteins. The quantitative estimation of these gamma globulins in serum should be helpful in the early diagnosis and study of diseases, such as multiple myeloma, which involve qualitative and quantitative changes in the gamma globulins.

MECHANISMS OF HYPERSENSITIVITY — The genetically distinct guinea pigs of inbred Strains 2 and 13 have proved to be a very important immunological tool. After studies established the fact of skin compatibility in the two strains, experiments were conducted to transfer cells with a measurable biological activity. Transfers of tuberculin sensitivity were undertaken by the intraperitoneal injection of living lymphoid cells from compatible donors. The almost quantitative transfers between inbred guinea pigs were a reflection of the continued viability of the active cells in the recipients.

Two models are being developed to study the mechanisms of immediate and delayed hypersensitivity in the inbred guinea pigs; protracted anaphylactic shock and, the massive local hemorrhagic reaction, respectively. It has been shown that there are differences in susceptibility to hypersensitivity

reactions. Strain 2 guinea pigs were more resistant to death by bronchospasm and tended toward a protracted syndrome in anaphylactic shock. Both Strain 2 and 13 guinea pigs required more mycobacteria than did random-bred Hartley guinea pigs for inducing "delayed" sensitivity to egg albumin, using Freund's adjuvant.

HUMAN SERUM AUTO-ANTIBODIES

Fractions of human serum separated by anion-exchange cellulose column chromatography were studied by immunoelectrophoresis. The conditions for elution of eighteen immunologically distinguishable human serum proteins from the columns were determined. Gamma globulin obtained under the appropriate conditions by this method was found to be pure; rabbits immunized with this fraction made antibodies to none of the other serum proteins. By the use of anion-exchange cellulose columns, it has been found possible to separate the 7S from the 18-19S antibody activities in sera of patients with thyroiditis and lupus erythematosus. Initial results indicate that the addition of immunoelectrophoretic characterization of these and other sera will be extremely helpful in our aim of characterizing the antibody activities found in human serum.

FLUORESCENT ANTIBODY STAINING OF MALARIA PARASITES

The fluorescent antibody staining of the human malaria parasite, Plasmodium vivax, has been recorded for the first time. A globulin fraction of convalescent serum from a patient having a long-standing infection with P. vivax was labeled and the fluorescent antibody applied to thin blood films containing the parasite. The organism was visible by virtue of its specific immuno-fluorescence. Fluorescent antibody studies were conducted on P. cynomolgi bastianellii, the monkey malaria parasite which, recently, has been shown transmissible to man. Considerable morphological detail was observed at fluorescence. Preliminary studies on the serological relationships, as based on degrees of fluorescence, indicate that P. vivax and P. cynomolgi bastianellii parasites may have common antigens and that the two species may be closely related.

LABORATORY OF INFECTIOUS DISEASES

In 1960 the Virus and Rickettsial and Epidemiology Sections of this laboratory continued integrated and comprehensive efforts to define the importance of virus infections in disease. Field investigations of human and animal virus infections were made possible through collaboration with a number of other organizations, including the Bureau of Medicine, USN; the District of Columbia Children's Hospital Research Foundation; the District of Columbia Welfare Department; the New York City Health Department; the National Cancer Institute; the National Institute of Allergy and Infectious Diseases; the Laboratory of Clinical Investigation, NIAID; and in Paris, France the Laboratoire des Virus, Hopital Saint-Vincent-de-Paul; and Le Centre Claude-Bernard de l'Hopital Saint Louis.

Natural events and opportunities afforded by our collaborators shaped the course of most field studies. Technical breakthroughs in the laboratory made it possible to take fuller advantage of these opportunities to study natural disease and thus acquire not only new information about specific virus infections, but also to move nearer our ultimate goal, namely, a clear view of the numerous viral causes of human diseases sufficiently comprehensive to make concerted efforts to control them appear feasible and worthwhile.

NEW CAUSES OF VIRUS PNEUMONIA

Pneumonia and other lower respiratory tract infections continue to represent major causes of death and a large segment, presumed to be viral in origin, is still uncontrolled. Until recently it was wholly undefined. During 1958 and 1959 our studies at Children's Hospital and Junior Village helped define the relative importance of adenoviruses, para-influenza viruses, and influenza viruses in causing lower respiratory illnesses of childhood. The data suggested that as much as 40 percent of croup bronchiolitis and pneumonia were explained by these viruses. In 1960, using more sensitive methods, we were able to explain a much larger percentage of such illnesses, chiefly because we were now able to assess the very significant contributions of respiratory syncytial virus (RS) to the respiratory disease problem. Early in the year large outbreaks of RS virus were intensively studied both at Children's Hospital and Junior Village. Over 80 strains of RS virus were isolated from children with pneumonia and 60 percent with bronchiolitis yielded RS virus, whereas virus was recovered from less than one percent of comparable control patients without respiratory illness.

Retrospective analysis of serologic surveys of respiratory illnesses in Children's Hospital since 1957 suggested that perhaps 20 percent of all lower respiratory illnesses observed during the last three years was due to RS virus. Thus, considering the contributions of adenoviruses, para-influenza viruses, influenza viruses, and "PAP" virus it now appears that 50 to 60 percent of the more severe respiratory illnesses of young children can now be explained and, hopefully, controlled. Except for influenza virus (which contributed probably less than 5 percent of the total), the LID respiratory virus unit personnel played key roles in the discovery of the first representatives of each of the other virus groups - adenovirus, para-influenza, and RS. Delineation of still undefined viral causes of the respiratory disease syndrome represents the major challenge to respiratory disease investigators for 1961.

During 1960 several experimental but commercially prepared killed vaccines containing various combinations of adenoviruses (6 types), para-influenza viruses (3 types), and Coxsackie B viruses (5 types) were tested in Junior Village. The evidence suggests that while modestly antigenic, the vaccines had insufficient potency to be regarded as satisfactory for larger scale studies.

PRIMARY ATYPICAL PNEUMONIA

The etiologic role of PAP (Eaton's virus) in primary atypical pneumonia suggested earlier by Eaton and Liu, was finally fully established in 1960. In cooperation with the Bureau of Medicine, USN, the continuing "epidemic" of virus pneumonia in Marine recruits at Parris Island was studied in several ways. Serological

studies showed that 51 percent of 530 pneumonia cases had antibody rises to PAP virus; only six percent revealed contemporary infection with adenoviruses. Serologic studies of infection showed PAP virus to be much more common than disease; approximately 30 recruits were infected for each case of pneumonia, information vitally important to fuller comprehension of the natural history of this important virus.

In 1959 treatment of Parris Island pneumonia cases with broad spectrum antibiotics (tetracyclines) appeared to reduce the severity and the duration of the Eaton pneumonias. In 1960 the efficacy of a new tetracycline drug, demethylchlortetracycline, was tested in a well-controlled double blind study including 290 pneumonia patients. The drug greatly reduced the severity and duration of pneumonitis and fever in those shown to have serologic responses to PAP virus. These findings, based on accurate laboratory diagnosis, fully confirm earlier but controversial reports of the efficacy of tetracyclines in atypical pneumonias. It also adds further support to the importance of the Eaton virus as a cause of virus pneumonia.

An additional link in the chain of evidence establishing the PAP virus as an important cause of pneumonia was achieved recently in collaborative studies with the Laboratory of Clinical Investigation, NIAID. Volunteers inoculated intranasally with PAP virus grown in tissue cultures reacted with a wide gamut of respiratory signs and symptoms, including pneumonitis characteristic of PAP.

COMMON COLDS AND VIRUSES

Recent studies have served to clarify and enlarge existing concepts of the etiology of common mild respiratory illnesses in adults. It is now quite clear that instead of a few specific closely related viruses, numerous viruses belonging to different groups each contribute in part to the syndrome called the "common cold." Thus the newer viruses (adenoviruses, para-influenza viruses, respiratory syncytial virus and others), together with older agents (influenza viruses and certain bacteria), each contribute only a small proportion of the milder respiratory ailments of adults. They contribute a larger segment of more serious diseases, particularly in children. Very recent reports of common cold viruses from England, together with the prior reports of agents with somewhat similar properties in this country, served to focus our attention on these viruses in 1960. Together with investigators elsewhere, it was found that most, if not all, of these agents - the British HGP and FEB, the American 2060, JH, Coe and PETT viruses which grow selectively and rather "fussily" in human epithelial cell lines, really represent "fastidious" enterovirus strains which have (as do almost all Coxsackie A's and some ECHO viruses) special growth requirements. These viruses, as do a number of still unclassified agents found in Junior Village during the past several years, have properties very similar to the Coxsackie A viruses; indeed, several have been shown, on the basis of serologic markers and/or by suckling mouse pathogenicity, to be indistinguishable from Coxsackie A viruses.

NEW SEROLOGICAL TEST PROCEDURES

The laboratory section of the Epidemiology Section concentrated on the development, application and evaluation of <u>in vitro</u> test procedures for the identification of new viruses as well as for detecting virus infection as expressed

in antibody responses. Thus, using conventional complement fixation (CF) and newly developed hemagglutination inhibition (HI) procedures it has been possible for our group to type thousands of virus isolates belonging to the adenovirus, myxovirus, enterovirus, and reovirus groups. As was true during the past several years, LID in 1960 again described and characterized more new representatives of these viruses than all other virus laboratories in the world combined. This was made possible during 1960 because each of our various virus research units contributed new diagnostic techniques. One group developed additional specific HI procedures for identifying adenoviruses and adenovirus infections; and for reoviruses and enteroviruses as well. Similarly, another group developed tissue culture procedures for isolating Eaton's PAP virus, while others not only discovered several "new" mouse viruses in tumor virus study systems, but developed serological procedures for recognizing their presence.

SEROLOGIC REAGENTS

But the availability of simplified procedures are of very little use unless the necessary reagents are also available. Although many virus research laboratories could do the tests, few laboratories are able to produce the necessary reagents. The magnitude and cost of producing and certifying them promises to continue to exceed any possible resources available. This fact has had a very depressing effect on research efforts aimed at the study of viruses as causes of disease, and serves as yet another deterrent to early delineation of the common virus diseases as public health problems. Consequently, with the help of NINDB and Microbiological Associates, LID in 1959 and 1960 accepted responsibility to develop and evaluate more than a hundred commercially produced virus antigens. LID, of course, has been active in the certification of virus prototypes and furnishes many to the Virus Registry of the American Type Culture Collection. It is also collaborating with the Enterovirus and Adenovirus national committees in setting up standards for large scale production of certified antiserums for serotyping and classification of viruses, perhaps the highest priority need of all virus laboratories concerned with human infection and disease.

UNOFFICIAL WORLD REFERENCE LABORATORY FOR VIRUSES

Wholly through the operation of circumstances, the Virus Section of LID has become virtually the chief (in many instances only) reference laboratory for many of the newer viruses, including adenoviruses (about 30 human and several animal serotypes), myxoviruses (five new parainfluenzas occurring in three species), reoviruses (three serotypes in four species), many of the newer and some older enteroviruses (5 - 10), salivary gland viruses (from four species), and new mouse viruses (six), the latter frequently found in tumor virus study systems.

Until virus reagents desperately needed for many extremely common viruses are made available either commercially, through government agencies, or both, LID as the sole custodian of many of these agents cannot avoid responsibility for assisting other excellent virus laboratories to identify their viruses, and on a pro-tem basis at least for keeping order in the general virus field. Unfortunately it has no specific commitment to provide such services and even worse, no specific budget to cover them, so that the involuntary, constantly growing and unavoidable service functions must be done at the expense of research missions.

However, it must be admitted that the simpler virus diagnostic techniques and the availability of a complete supply of viral reagents in the laboratory (developed out of necessity) facilitate not only epidemiologic studies of naturally occurring virus infection but also enable it to evaluate the significance of the data furnished by other laboratories who come for technical assistance.

PROBLEMS OF CANCER VIRUSES

Studies of cancer viruses can be subdivided into several categories: (a) Laboratory studies of the properties of cancer viruses and development of laboratory tools for detecting and working with them; (b) field studies of the behavior in nature of those tumor viruses for which suitable detection tests are available; (c) studies of extraneous viruses ("background noise") now preventing high caliber virologic practice in the study of animal tumor viruses and obscuring interpretation of nearly all current observations on them; and (d) the study of general virus experiences in relation to human cancer - the "background noise" in the human cancer problem - which must be done eventually if the role of viruses in human cancer is to be defined.

The approach to these various interdependent studies is based on the following beliefs: 1) That the conventional methods of standard virology must be applied to cancer virus research if significant progress is to be made; 2) the study of cancer viruses obviously cannot be separated from general virology; and 3) that the "biologic point of view" rather than attitudes fostered by preoccupation with categorical disease, represents the best approach to a real understanding of the natural history of cancer viruses just as it does to other viruses.

MOUSE POLYOMA CANCER VIRUS

New in vitro survey tools developed during 1959 (CF, HI, and MAP) were evaluated and applied in 1960 in studies of polyoma virus growth and excretion, its experimental epidemiology, and its natural history. This interesting and versatile cancer virus causes tumors not only in all strains of Mus musculus, but also in hamsters, rats, rabbits, and guinea pigs (Stewart and Eddy). Of equal interest is the fact that it can be studied and surveyed with the same facility as ordinary viruses, such as influenza and polioviruses. Virus isolation and serologic procedures, combined with epizootiologic studies have produced the following interesting observations:

Polyoma virus was found to be widely disseminated in mouse colonies nearly everywhere. Infection was found to be more commonly present than absent in laboratory strains raised in experimental or commercial laboratories and in wild strains found in city tenements. However, the basic ecology or natural cycle appears to exist in rural areas - on farms and in feed mills in small towns.

A full year's surveillance of Mus musculus infestation and polyoma infection of crowded tenements in Harlem revealed that virus infections persisted without exception in numerous separate foci. Three epidemiologic factors seem most important, namely, large mouse populations capable of furnishing adequate supplies of young susceptible mice, the extensive con-

tamination of the tenement environment (virus was demonstrated in sweepings from areas showing signs of mouse activity), and finally the overcrowding which insures the continuous and extensive use of communal nesting areas (also demonstrated to be contaminated by virus). Apartment houses having smaller and less dense mouse infestation were generally free of infection and remained so during the study.

Systematic studies of polyoma in rural environments were undertaken during the last quarter of 1960. However it appears from preliminary data that here may be found the basic natural cycle of mouse polyoma. Mus musculus infestation and polyoma infection of Mus was found to be most intense in feed granaries on the farm and in cereal grain storage elevators in mills. As many as 30 per cent of several hundred mice trapped in these environs showed persistent evidence of polyoma infection, many of them apparently excreting virus in their urine. The virus has been found on cereal grains in the vicinity of mouse nesting areas, which appear to be very numerous in the granaries so far examined. The actual extent of cereal grain contamination by mouse excreta containing polyoma and no doubt other microbes must still be evaluated; however, present evidence suggests that it probably is very extensive, if not appalling.

Since natural infection of wild mice is not limited to polyoma virus, but includes a number of other viruses known or suspected to infect man and domestic animals, the extension of these preliminary findings will likely prove very interesting.

EXTRANEOUS VIRUSES IN CANCER VIRUS STUDIES

In 1960 the "background noise" problem in cancer virus research grew to almost "deafening" proportions and, in the opinion of LID virologists, constitutes the number one obstacle to intelligent and truly effective research on cancer viruses.

Nearly every animal tumor virus system currently under study was shown to be contaminated with extraneous agents and several viruses widely proclaimed as "tumor" viruses turned out to be fellow traveling ordinary viruses. To list a few examples: Friend leukemia was found contaminated with polyoma and mouse adenovirus; Gross leukemia by polyoma, K virus and mouse adenovirus; Schwartz leukemia with polyoma, K virus and mouse adenovirus; Moloney leukemia with mouse hepatitis and mouse reovirus; the polyoma itself became contaminated with mouse adenovirus, hepatitis and salivary gland viruses.

LID virologists showed that the "seeds" of the "background noise" viruses are commonly present in the animals used for the induction of tumors, and of course in the subsequent passage materials as mentioned above. The extraneous viruses most commonly encountered in cancer systems were the newer ones, such as polyoma, K virus, mouse reovirus and adenovirus; but this in part may be due to newly developed easily applied survey tools for these agents. Other viruses encountered less often (perhaps because of comparatively less sensitive tools) were mouse hepatitis, mouse salivary gland virus, the newly discovered "thymic agent" (TA). Except in newborns, most of these viruses occur subclinically and latently.

MEDICAL MYCOLOGY

Investigations on pathogenic fungi have included broad fields of research and although definitive goals have been reached in most of them, all will be continued in order to further exploit productive lines of investigation. In most cases new or additional species of pathogenic fungi will be used in investigations, or techniques will be altered to permit further development of experimental studies.

The antibiotic X-5079C was found to be fungistatic but not fungicidal and its apparent low degree of in vitro activity due to its decay in culture medium. The yeast form of Histoplasma capsulatum is much more sensitive to X-5079C than the mycelial form and an assay method, sensitive to 1 ug/ml using H. capsulatum, was developed. X-5079C has low toxicity for HeLa cells and is active against H. capsulatum grown in HeLa cells.

A second strain of Coccidioides immitis has been converted to serial culture in the spherule form. Quantitative measurements show the ability of various carbon and nitrogen sources to support growth of spherule and mycelial forms of strain M-11 of C. immitis. Only mannose is utilized as readily as glucose by spherules. Mannose and fructose support growth of the mycelial form as well as does glucose. A substrate which preferentially supports growth of the spherule form was not found in this study.

Spherules were utilized to immunize mice. An increased survivor rate in the immunized mice was noted after challenge with a lethal infecting dose and an earlier clearance of organs (negative cultures) in the immunized mice was observed after challenge with a sublethal dose.

CRYPTOCOCCUS NEOFORMANS

By titrating and plating out organs of experimentally infected mice, it was found that several minutes after Cryptococcus neoformans was injected either intravenously or intracerebrally into mice, the largest numbers of yeast cells had been retained in the lungs. The fungus population in the lung then decreases and 2-3 days after infection multiplication in the brain is apparent. Although the interval from infection to death of infected mice varies with the strain of C. neoformans, the numbers of yeast cells per gram of brain tissue are approximately the same regardless of strain.

Studies of the saprophytic occurrence in natural habitats of fungi which cause mycoses have continued. Cryptococcus neoformans has been isolated from many additional collections of pigeon guano. When this material is collected from old pigeon nests and from roosting sites in hay mows of barns and upper floors of buildings, Histoplasma has never been found. There is increasing circumstantial evidence that a presently unstudied pneumonic form of cryptococcosis has occurred in men heavily exposed to such material and that such epidemics have been erroneously diagnosed histoplasmosis.

EMMONSIA CRESCENS

In collaboration with an investigator at the Rocky Mountain Laboratory a new species, Emmonsia crescens, was described. This fungus differs from the first species of Emmonsia (E. parva) in vivo and in vitro at 37° C by its multinucleate condition (instead of uninucleate), its ability to produce the in vivo form in vitro at 37° C, and its greater size. E. parva conidia when inhaled or incubated

at 37° C increase in diameter from 2 - 4 u to 400 - 480 u. This 10^6-fold increase in volume of a single cell is very unusual in the fungi.

STAPHYLOCOCCUS STUDIES — It has been established that staphylococcal penicillinase is associated with particulate material in the cell and thus an explanation has been given for the refractoriness in preparation of this enzyme by conventional methods. New and more potent inhibition of Staph. penicillinase have been uncovered and the hope remains and is heightened for the ultimate finding of a chemically useful inhibitor. Further, sea water has been found to possess strong inhibitory activity against both penicillin-sensitive and penicillin-resistant staphylococci (phage type 80/81).

Real progress has been reported in the understanding of iron metabolism in the staphylococci. As a direct result of continuing work dealing with mechanisms of the development of non-specific immunity and in particular the function of the iron-transporting protein of plasma, siderophilin, fundamental observations on the effects of iron deficiency on the growth and metabolism of S. aureus have been reported. Work on the biology of the staphylococci so long neglected during the "antibiotic era" is cardinal to effective new therapy of staphylococcal infections.

STREPTOCOCCAL M PROTEIN — Progress has also been made on the search for better methods of isolation and purification of M protein of streptococci. These results are of obvious importance in the understanding of Group A streptococcal virulence. Further, highly interesting observations have been reported dealing with the mechanism and significance of the long-chain test for determination of anti-streptococcal immunity.

BACTERIAL METABOLISM — Real understanding of the intimate mechanisms of energy metabolism in Hydrogenomonas in particular and other bacteria and higher forms in general is closer as a result of work performed in this section this year. In an enormously complicated field, progress has occurred in the definitions of the essential reactions.

Detoxification studies on potentially useful chemotherapeutic agents have continued and new and promising leads have been uncovered for agents active against bacteria, fungi, parasites and, it should be added, against cancer as well. Several of the aforementioned detoxified compounds have passed preliminary screening processes performed by the Cancer Chemotherapy Center.

Pinpointing of the enzymic locus of discrimination among hydrogen isotopes by Pseudomonas has been reported this year. The area lies in formic acid metabolism.

LITERATURE OF CLINICAL

LABORATORY OF CLINICAL INVESTIGATION

Summary of Research Progress
Calendar Year 1960
Laboratory of Clinical Investigations

This year has seen some widening of the scope of our research, largely as a response to enlarging the professional staff. We believe that a further period of growth will be needed to staff the clinical service in a manner consistent with optimum research productivity.

In the past the wards have operated at an occupancy rate of about 65 percent. From the service point of view, a rate of 85 percent would be most acceptable i.e. 44 instead of 34 patients. It is estimated that the unit could quite easily provide material for at least seven research sections (at present there are four). We hope to have additional sections functioning as soon as qualified investigators can be obtained. Upon the removal of the administration offices to the new building, additional space will become available for a pediatric section, presently viewed as our most important deficiency in professional staffing.

In connection with staffing, it is worth noting that it is difficult to obtain qualified investigators to head sections. I believe the evidence will show that civil service stipends for professional personnel are appreciably less that those offered by the best - paying medical schools, but better than the poorer-paying ones. Medical schools have a double standard of employment, however, and our salaries are most competitive with the lower paying, non-clinical appointments. I believe one can employ without great difficulty biochemists, microbiologists, etc., but because of the exceedingly high returns from the practice of medicine, the medical schools have developed private practice and consultation arrangements for the men of their clinical faculties which reduce the deficiency between their base pay and the return obtainable from practice. This mechanism is not available to us.

I believe the salary issue alone would not be a crucial factor in securing competent investigators, however, there is a feeling of reluctance among many young investigators to accept government employment. The attitude seems to have developed from apprehension concerning the large size of the operation, the comparative anonymity of the individual, the division of authority among departmental heads, the civil service organization and the several administrative branches above the operating unit. Promotion is usually slow and the salary increments are not large. Good men can advance much more rapidly out of government. I don't believe the government is adequately authorized to recognize talented young investigators who in university life often achieve high faculty rank. It should also be recorded that decisions concerning the salaries which can be offered are also very slowly reached.

It is my view that the clinical sections of the National Institutes of Health will best achieve their goals by attracting the kind of men who serve on medical school faculties. I think we should train many who take those positions. I believe our senior staff should also interchange with academic medicine. Mechanisms to protect retirement and to permit such exchanges without loss of security should be made. Such a thing as membership in the

Teachers Insurance Annuity Association as an alternative to the civil service annuity program would be worthy of consideration.

Professional Staff

We are completing the year with 13 clinical associates and 9 senior staff members. In July, Dr. Donald Kayhoe left the staff and in October Dr. Howard Goodman began a joint appointment with a Section divided between this laboratory and the Laboratory of Immunology. Mr. John Bozicevich also joined this staff in July, by transfer from the Laboratory of Immunology. (We regret to announce the anticipated retirement of Mr. Bozicevich early in 1961 after 30 years of service).

Research Program

Infection of volunteers with respiratory viruses. An extensive clinical study of acute viral respiratory disease was begun this year in association with staff members of the Laboratory of Infectious Diseases. The initiation of this project required much work but with the unstinting assistance of Dr. Clifton Himmelsbach, Associate Director, the Clinical Center, very satisfactory arrangements have been made to transfer to the Clinical Center, Federal prisoner-volunteers for study. This has been done with the permission and considerable assistance of Mr. James Bennett, Director, and Dr. Harold Janney, Chief Physician of the Bureau of Prisons, Dept. of Justice. A team of custodial officers has also been assigned to the Clinical Center by the Bureau of Prisons to oversee the volunteers. The volunteers have uniformly cooperated with the program despite some extended periods of room isolation to prevent spread of infection, frequent blood-letting, and other inconveniences. Many administrative arrangements have been developed so that the program has worked increasingly smoothly.

The results so far justify the investment in money and effort. It has been possible to produce in human volunteers a rather uniform "cold" with respiratory syncytial virus. It may occur without respect to preinfection immunity, but the subsequent rise in complement-fixing antibody appears to correlate with severity of illness. Forty-six men have so far participated in this study.

Approximately 24 other volunteers have participated in studies with para-influenza 4 virus or Eaton (primary atypical pneumonia) virus. Future studies are planned with human influenza virus and with the recently defined group of REO viruses. We believe that the promise of this program is great and that it may continue for some years.

New antifungal drug. Beginning about 4 years ago, Dr. Chester Emmons began studies on an antifungal drug produced by Hoffman La Roche, designated as RO-2. This agent, an antimicrobial, was found to be the most active material Dr. Emmons had ever tested in vitro and in animals against several of the pathogenic fungi, notably histoplasmosis and blastomycosis.

In the intervening years, the agent has been studied clinically by Dr. Utz and his staff. Now 30 patients have been treated. Extremely favorable results have been observed in several patients very severely ill with these diseases. From the standpoint of therapeutic activity, it is the judgment that this agent is the best available for blastomycosis and histoplasmosis.

During the studies it was noted that the agent produced unusual hepatic changes. It was found that the dye, bromsulfhalein, normally rapidly transferred from the blood to the bowel by the liver, was retained in high concentration in the blood in patients treated with RO-2. This effect appeared in a day or two after start of treatment, before tissue changes would likely occur, suggesting competition of the new drug for the liver excretory mechanism for bromsulfhalein. After treatment was stopped dye excretion promptly returned to normal or nearly normal. Liver biopsy has revealed changes indicative of minor hepatic damage in some cases. Because of the great importance of having a drug in addition to the relatively toxic agent, amphotericin B, for the treatment of fungal diseases, this new agent continues under investigation.

Studies on volunteers infected with simian malaria. Following the discovery by Dr. Eyeles and Dr. Coatney of the infectivity of P. cynomolgi bastianellii for man, several inmates at the Federal prison in Atlanta volunteered for exposure to this agent with the establishment of infection in several. The need for careful clinical characterization of this disease in man led to the transfer of two infected volunteers to the Clinical Center. Both men developed acute malaria which was carefully studied throughout its course. All results are not yet available, but significant alterations in urinary steroid excretion were detected, an unusual elevation of serum cholesterol occurred in another, and liver lesions not previously described, resembling but not identical with exoerythrocytic phase parasites, were demonstrated in both patients. This interesting program will be continued.

Studies on penicillins and penicillinase. Previous work by Dr. Steinman had defined nutritional requirements for penicillase production by staphylococci and many parameters of its interaction with benzyl penicillin (penicillin G). During the year English investigators, working with the stripped molecule of penicillin, 6-amino-penicillanic acid, produced a new compound largely resistant to destruction by penicillinase. Working with this and several other penicillin derivatives Dr Steinman has found that resistance to penicillinase is greatly influenced by steric positions of ethoxy groups on the side chain and that failure to be destroyed by penicillinase is associated with greater penicillinase inducing-capacity. Perhaps, most significantly, Dr. Steinman's work has added to the now substantial indirect evidence, that the major reason for present resistance of staphylococci to penicillin is the enormous capacity of these micro-organism to produce penicillinase upon contact with even low doses of penicillin.

Clinical studies with the penicillinase-resistant penicillin, dimethoxyphenyl-penicillin, have revealed it to possess resistance to penicillinase in vivo, and that it is a powerful and effective anti-staphylococcal drug. After treatment of some patients for periods of 3 to 5 months no penicillin-resistant staphylococci have been isolated. It has long been considered that chronic staphylococcal infection resembles tuberculosis and for the first time it appears that an agent is available which can be employed for extended periods of treatment without loss of effect such as isonicotinic acid hydrazide in tuberculosis. If long term therapy can thus be regularly given an enormous benefit will accrue to thousands ill with chronic staphylococcal disease.

Ascites induced in mice by injection of adjuvant mixtures. In the past year Miss Lieberman has continued to study the ascites induced in mice by the injection of adjuvant mixtures. This discovery provided a much needed laboratory procedure for producing antibodies of a wide variety and a means of evaluating antigens. Recently, her interest has been focused on the pathology and abnormal physiology of the lesion. It has been found that strain differences are associated with differences in susceptibility to ascites. Since ascites was found associated with a local plasma cell reaction, which in some strains of mice went on to plasma cell tumors, many implications toward problems in neoplasis have also been raised. These studies have become the basis for biochemical and pathologic studies with other Institutes.

Allergy and Immunology. Dr. Nasou and Mr. Bozicevich have shown that patients with hypogammaglobulinemia possess low, but definitely measurable, levels of antibody to the enteric viruses. The implication of this finding, in view of the normal resistance of these patients to viral infections, is that extremely low levels of specific antibody may provide adequate resistance against viral diseases. Subsequent, unpublished studies have indicated that these patients will develop circulating antibodies to Salk polio vaccine.

The modification of the bentonite flocculation test for the detection of gamma globulin promises to provide the practicing physician with an accurate, convenient laboratory aid in the diagnosis of hypogammaglobulinemia. In contrast to the electrophoretic method, the results with the bentonite test can be known to the physician within a few minutes of arrival of the specimen to the laboratory. Other modifications of this technique will also give the levels of albumin and other protein constituents of blood and other fluids without resort to the more cumbersome method of electrophoresis.

The DNA-bentonite test for systemic lupus erythematosus has also been developed in the past year. This test measures the antibody in lupus serum which is directed against nuclear material. The specificity of this test is greater than any previously described test for lupus. This test, however, is positive primarily in those patients with active disease and only rarely is positive when the disease is in remission. A modification of this test, the nucleoprotein-bentonite test, has retained all the attributes of the DNA test in regard to specificity, while achieving a much higher level of sensitivity for cases in remission. These promise to become important standard tests in the diagnosis of systemic lupus erythematosus.

Cystic Fibrosis of the Pancreas. Despite the commonly accepted point of view that antibiotics are helpful in this disease, observations of nasopharyngeal cultures failed to reveal any appreciable effect of antimicrobial treatment on Staphylococcus aureus. This does not negate a possible clinical benefit but does appear to minimize its value. It was coincidentally observed that Escherichia coli was not present in the nasopharyngeal flora of any child over the age of eight. This interesting observation will receive further study.

Serial No. NIAID - 10 (c)
1. LCI
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Office of the Chief

Principal Investigator: Dr. Vernon Knight

Other Investigators: None

Man Years (calendar year 1960);

Total:	7.0
Professional:	1.0
Other:	6.5

Project Description:

Objectives:

To develop, direct and coordinate the program of the Laboratory, as defined in the individual research projects; where necessary, to re-direct individual projects to meet current needs and advances in the field; to align clinical projects with patient availability; to provide an unexcelled standard of patient care for patients utilized in research.

Methods Employed:

Organize available staff and recruit qualified personnel, both professional and sub-professional, to develop the program and carry out its aims; close liaison with area medical societies, institutions and individuals for referral of patients whose diagnoses fall within the active or proposed disease research categories; the highest standards of patient care are maintained by selection of qualified physicians; the development of necessary policies and continued close and direct supervision of patient care activities; professional consultant services to other institutes in the area of infectious diseases; continued guidance of research projects undertaken by younger investigators; maintenance of staff morale.

Part B included Yes / / No /X/

Serial No. NIAID - 12(c)
1. LCI
2. Infectious and Pediatric Disease Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Respiratory Viral Diseases

Principal Investigators: Dr. John P. Utz
Dr. Vernon Knight

Other Investigators: Dr. Howard Kravetz
Dr. David Rifkind
Dr. Anderson Spickard (from 7/1/60)
Dr. Robert Carpenter (from 7/1/60)
Dr. Hugh Evans (from 7/1/60)
Clarence F. Szwed
Margret A. Huber

Cooperating Units: Dr. Robert Channock, LID, NIAID
Dr. Karl Johnson, LID, NIAID

Man Years (calender year 1960):
Total: 5.5
Professional: 3.5
Other: 2.0

Project Description:

Objectives:

1. To define clinical entities in relation to newly isolated respiratory viruses.

2. Further to define diagnostic criteria, pathogenesis, immune response, persistence, sites and effects on tissues of virus in certain infections of respiratory passages and mouth.

3. To study host-parasite relationships in reference to susceptibility to chronic or recurrent respiratory diseases.

4. To improve clinical laboratory techniques in the laboratory confirmation of respiratory viral disease diagnosis.

5. To characterize in man the clinical course and associated virological and immunological phenomena of infection with selected respiratory viral agents.

Methods Employed:

1. Results of clinical observations and procedures are correlated with bacterial, mycologic and viral isolations employing both animals and tissue culture techniques.

2. Clinical observations are made under carefully controlled isolation following intranasal infection with certain viral agents. Virus isolation and immunologic studies performed by recognized procedures.

Patient Material and Major Findings:

1. The Virology Unit of the Infectious Disease Service examined a total of 1245 specimens from patients under study. Virus was isolated in 73 of these specimens, achieving etiologic confirmation of the diagnosis in a total of 35 patients.

2. The status of treatment of viral pneumonias has been critically evaluated and reported.

3. Inmates selected from volunteers from several Federal correctional institutions were infected or used as controls in studies with the following viral agents: Para-influenza 4, 12 volunteers; respiratory syncytial virus infection, 46 volunteers, Eaton (primary atypical pneumonia) virus, 13 volunteers.

4. Clinical and virological infection occurred in a high proportion of patients given RS virus. Infection was less frequent with the other agents, apparently as a result of low dosage. This matter is receiving further study.

Significance to Microbiological Research:

Studies in the laboratory and the clinic of patients naturally infected with respiratory viruses will hopefully provide information on pathogenesis, distinctive clinical findings, effects of giving or withholding antimicrobic treatment as related to the specific, known virus involved. This approach also provides the only opportunity of discovering and defining new viruses.

Studies in human volunteers may provide information on the effect of dose, route of administration, and kind of virus on the induction of clinical infection. They may also indicate the role of immunity and other protective mechanisms as a defense against infection. Such studies

would logically precede the development of effective vaccines against these diseases. There are approximately 100 different respiratory viral agents whose role in human infection is not adequately defined. Moreover, increasing evidence suggests that viruses may participate with bacteria in the cause of infection. This may be studied by the present methods.

Proposed Course of Project:

Studies will continue as outlined under "Objectives" in patients referred to the Clinical Center and in other hospital or community surveys when indicated. This project should be expanded because of the great importance of careful clinical studies on patients with respiratory illnesses in order to link these illnesses with viral agents that are now being isolated in a number of laboratories.

Further studies with the Eaton, RS, and Para-influenza viruses in human volunteers are planned. Human influenza and REO virus are also being considered for this program.

Part B included: Yes /X/ No / /

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Utz, J. P.: Pneumonias, Viral. Current Therapy, 1960 ed.

2. The studies with RS virus were part of a Clinical Center Symposium, October 20, 1960, which will be published in Annals of Internal Medicine.

Serial No. NIAID - 13 (c)
1. LCI
2. Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Antimicrobial Drug Therapy

Principal Investigator: Dr. Vernon Knight

Other Investigators: Dr. David Rifkind
Margret Huber

Cooperating Units: None

Man Years (calendar year 1960):

Total:	2.4
Professional:	1.7
Other:	0.7

Project Description:

Objectives:

a) To evaluate an antimicrobic drug.
   1. Effectiveness in treatment of penicillin-resistant staphylococcal infections.
   2. Effectiveness in treatment of other coccal infections.
   3. Appropriate dosages and routes of administration.
   4. Toxic and allergic side effects.
   5. Effectiveness in treatment of the nasal carrier state of staphylococci.

Methods Employed:

Hospitalized patients with staphylococcal and streptococcal infections were evaluated by clinical and laboratory methods for response to antimicrobics. The laboratory methods included:

a) Isolation and identification of the etiological agent.
b) Determination of the in vitro sensitivity of the organism to drug.
c) Assay of serum and urine for drug concentrations during the course of treatment.
d) Determination of the inhibitory effect of serum against the offending microorganism.

e) Bacteriologic culture of lesions and blood during the course of treatment.

Patient Material and Major Findings:

1. A total of 22 hospitalized patients were treated or are under treatment with dimethoxyphenyl penicillin; 2 with staphylococcal septicemia; 1 with staphylococcal subacute bacterial endocarditis; 1 with staphylococcal arthritis and pneumonia; 2 with chronic staphylococcal osteomyelitis; 8 with staphylococcal abscesses; 6 with cystic fibrosis of the pancreas; 1 with Group M streptococcal endocarditis and 1 with Group D streptococcal meningitis.

2. All patients treated with dimethoxyphenyl penicillin showed clinical evidence of improvement or cure. There were no deaths. Therapy was particularly successful in staphylococcal septicemia, abscesses, arthritis, pneumonia and subacute bacterial endocarditis. Therapy has to date been of insufficient duration to evaluate in the case of patients with cystic fibrosis of the pancreas. The results with regard to clearing of staphylococci from the sputum appear promising.

3. One patient with staphylococcal osteomyelitis relapsed after one month of therapy. Staphylococcal infections whether due to penicillin-G-sensitive or penicillin-G-resistant organisms responded equally well to the experimental penicillin. Of the 20 patients with staphylococcal infections treated, 14 or 70% had penicillin-G-resistant infection.

4. Daily doses of 8-10 grams I.M. and 10-15 grams I.V. were well tolerated and gave good clinical results. One patient has been treated with 780 grams of the drug over a 130 day period.

5. Probenecid elevated and prolonged blood levels of the experimental penicillin and was used in all patients where it was tolerated.

6. All staphylococci were found to be sensitive to 2-4 mcg/ml of the experimental penicillin; this being true for both penicillin-G-sensitive and resistant strains. This drug was found to be actively bactericidal in vitro. Streptococci were also found to be sensitive, however, enterococci were relatively resistant.

7. The sensitivity of penicillin-G-resistant staphylococci in vitro to penicillin-G decreased with large inocula; however, the sensitivity to the experimental penicillin remained constant under these conditions. This phenomenon is presumably due to the production of penicillinase by the staphylococci which destroys penicillin G but does not affect dimethoxyphenyl penicillin.

8. Blood levels were found to vary from 19.7 to 97.5 mcg/ml at varying times following administration.

9. In 3 cases of maculopapular rash resulted from experimental penicillin treatment. In 1 case the rash was severe enough to require discontinuance of the medication.

Significance to Biomedical Research:

1. Dimethoxyphenyl penicillin is of importance, both clinically and theoretically because of its almost total resistance to penicillinase: Its effectiveness in the treatment of penicillin-G-resistant staphylococcal infections is of significance and utility in itself. On a more basic level, it helps clarify the role of penicillinase in microbial resistance to penicillin.

2. This drug holds promise for the long term treatment of chronic staphylococcal disease such as osteomyelitis, recurrent furunculosis, cystic fibrosis of the pancreas and other diseases due to penicillin resistant staphylococci.

3. Dimethoxyphenyl penicillin may prove to be a useful agent in the study of penicillin allergy.

4. Dimethoxyphenyl penicillin, because of its rather narrow microbial spectrum should provide information on the role of microorganisms in the chronic pulmonary disease associated with cystic fibrosis of the pancreas.

Proposed Course of Project:

1. Continue present clinical studies with particular emphasis on chronic staphylococcal infections and on cystic fibrosis of the pancreas.

2. Extend preliminary observations of allergic reactions to this new penicillin and other drugs and define the extent of cross reactivity with penicillin G.

Part B included Yes / / No /X/

Serial No. NIAID - 14(c)
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Staphylococcal Disease

Principal Investigator: Miss Rose Lieberman

Other Investigators: Mr. John Douglas
Mr. William Humphrey

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.7
Professional: 0.5
Other: 1.2

Project Description:

Objectives:

Basic studies of the pathogenesis and immunology of staphylococcal disease which include:

1. The investigation of the antigenicity and the types of antibody produced to staphylococcus cellular components obtained by chemical and physical treatment of the intact organisms.

2. Development of techniques to study the alteration and changes in antibody produced by various components of staphylococcus administered to individual animals.

Methods Employed:

Staphylococcus is fractionated by chemical extraction, disintegration and sonication. The various components thus obtained are investigated for their antigenicity and for the types of antibodies produced to them. Induction of ascites in mice by staphylococcus-adjuvant mixtures is employed to provide a continuous source of large amounts of high titered antibody in individual animals. This method has the advantage of being able to study the effect on the types of antibody produced by administration of two or more different antigenic components of staphylococcus at different times in an individual mouse over long periods of time. The antibodies thus produced are studied by various means including passive cutaneous

anaphylaxis, immunoelectrophoresis, gel diffusion, hemagglutination, quantitative precipitation and passive protection tests.

Major Findings:

High titers of staphylococcal antibody are present in the ascitic fluid of immunized mice. These antibodies are not all alike and appear to reach optimal levels at different times after primary immunization. Different fractions of staphylococcus vary in their antigenicity and on immunoelectrophoresis appear in different areas of the gamma globulin range.

Significance to Microbiological Research:

Increase of both primary and hospital infections with antibiotic resistant strains of staphylococcus has become a serious public health problem.

Research into the pathogenesis, immunology and etiology of staphylococcal diseases is especially important because of the lack of any effective antimicrobiols.

Proposed Course of Project:

To continue as outlined under objectives.

Part B Included: Yes /X/ No / /

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Lieberman, R., Douglas, J. O. A. and Mantel, N.: Production in mice of ascitic fluid containing antibodies induced by Staphylococcus or Salmonella-adjuvant mixtures. J. Immuno., 84:514-529, May, 1960.

Serial No. NIAID - 15(c)
1. LCI
2. Infectious and Pediatric Disease Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Hepatitis and Mononucleosis

This project has been temporarily inactivated due to lack of patient material. If patients become available, the project will be reactivated.

Serial No. NIAID - 16(c)
1. LCI
2. Infectious and Pediatric Disease Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Aseptic Meningitis

Principal Investigator: Dr. John P. Utz

Other Investigators: Clarence F. Szwed

Cooperating Units: Children's Hospital, Washington, D. C.

Man Years (calendar year 1960):
Total: 0.4
Professional: 0.4
Other: 0.0

Project Description:

Objectives:

1. To develop methods and use newly available methods to diagnose the large group of non-bacterial meningo-encephalitides.

2. To correlate etiological findings with detailed historical physical and clinical laboratory observations.

Methods Employed:

Hospitalization of patients for clinical observation and laboratory studies. Laboratory procedures include: (a) tissue culture and suckling mouse inoculation of material for virus isolation; (b) appropriate serological procedures with acute and convalescent serum for the known viral meningo-encephalitides. A number of patients selected from other hospitals or surveyed groups will be studied similarly.

Patient Material and Major Findings:

1. A remarkably small number of cases of aseptic meningitis occurred in 1960 in the Washington area and only a few of these were studied. No single viral agent predominated.

2. A patient with lymphocytic choriomeningitis was intensively studied in cooperation with the National Naval Medical Center. Note-

worthy clinical aspects of his disease included biphasic course, absence of direct contact with mice, encephalitis, parotitis and, especially, a severe orchitis. Virus was isolated from cerebrospinal fluid in this laboratory.

Significance to Microbiological Research:

Non-bacterial meningitis and meningo-encephalitis is a common and often serious disease of whose etiology and pathogenesis there is insufficient knowledge. It often results in brain damage and behavior disturbances. Increased knowledge may also clarify the pathogenesis of the encephalitic complications of the otherwise minor diseases of children.

Proposed Course of Project:

Continue above studies.

Part B included Yes / / No /X/

Serial No. NIAID - 17(c)
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Allergy-Immunology

Sub-project I: Diseases of Immune Etiology

Principal Investigator: Dr. John P. Nasou
Dr. Donald E. Kayhoe (from Jan. 1 to June 30, 1960)

Other Investigators: Mr. John Bozicevich (from July 1, 1960)
Dr. Edward Eyerman (to June 30, 1960)
Miss Margret Huber
Mr. Robert Bowser
Mr. Stanley Ward

Cooperating Units: Laboratory of Infectious Diseases, NIAID;
The Laboratory of Immunology, NIAID;
Division of Biological Standards;
Laboratory of Viral Products:
National Heart Institute, Laboratory of Cellular Physiology and Metabolism, ICPM;
Georgetown University Medical Center Clinical Laboratories; and George Washington University Hospital Rheumatology Group.

Man Years (calendar year 1960):

Total:	4.95
Professional	2.65
Other	2.3

Project Description:

Objectives:

1. To evaluate the role of immune serum globulin in host response to infectious diseases.

2. To evaluate the role of bacterial, fungal, or viral allergies in recurrent or chronic respiratory disease.

3. To determine the rate of metabolism of individual antibodies compared to that of gamma globulin, and to investigate the mechanism whereby the reticulo-endothelial system differentiates between otherwise chemically homogenous specific antibodies.

4. To measure, quantitatively, the rate of synthesis of specific antibody in the human during the primary and secondary immune responses.

5. To measure quantitative differences in the specific antibody response of acute leukemics, normal human volunteers, hypo and hyper-gammaglobulinemias.

6. To determine whether, in the presence of a specific antigenic challenge, the specific antibodies are selectively removed from the total exogenous gamma globulin injected into agammaglobulinemia patients.

7. To determine the relationship of hormones to the agammablobulinemic state.

8. To determine the pathogenic significance of the abnormal gamma globulin of patients with systemic lupus erythematosus.

9. To reproduce lupus lesions in experimental animals.

10. To study the antinuclear, anti-DNA and antinuclear protein factors found in the serum of patients with systemic lupus erythematosus and to evaluate the relationship of the titer of the substances to the clinical status of the patient.

Methods Employed:

Clinical studies supplemented with:

1. Plasma protein analysis by (a) electrophoresis both by paper chromatography and moving boundary technique, (b) chemical determinations, and (c) immunologic assays.

2. Immunization and testing of responses by serologic techniques or by challenge.

3. Antibody and total gamma globulin alteration measurement in agammaglobulinemic patients following hormone administration.

4. Pathologic study of animals following injection into the circulating blood of suspensions of L. E. cells and L. E. bodies.

5. Phase contrast microscopic study of the L. E. cell phenomenon.

Patient Material and Major Findings:

1. In cooperation with Dr. Howard Goodman of the National Heart Institute and Dr. Richard Malmgren of the National Cancer Institute, studies of lupus factor as measured by the nuclear proteins sensitized and red blood cell technique and the fluorescent antibody technique, and its clinical correlation have been conducted. This data is currently being prepared for publication.

2. The clinical application of the procedure devised by Mr. John Bozicevich of the Laboratory of Immunology, NIAID, the DNA-bentonite flocculation test, has been conducted within the framework of this laboratory. This diagnostic method for systemic lupus erythematosus has a great specificity. Further investigations with this procedure (the DNA-bentonite flocculation test) are underway in an attempt to evaluate the status of the lupus factor in these patients over a period of time.

3. Certain water precipitable proteins of the serum of patients with collagen vascular diseases are providing interesting immunologic phenomenon. The fractions obtained in this method of separation of proteins are being used in the DNA-bentonite test. In doing this, we have discovered that this test assumes even greater importance in the diagnosis of collagen vascular diseases.

4. We are continuing clinical studies of the patient with lupus erythematosus and agammaglobulinemia.

5. In cooperation with Dr. Samuel Baron and Dr. Eugene Barnett of the Division of Biologic Standards, the study of agammaglobulinemia patients and the presence of antibodies in their serum to the enteric viruses has been conducted by use of very sensitive techniques to detect these antibodies. It has been found that all of these patients have significant titers. Further studies are being conducted in this vein in the attempt to elicit antibody formation by means of polio vaccine in those agammaglobulinemics not showing a significant titer. These studies are now completed and are being prepared for publication.

6. A variation of the bentonite test using nucleoprotein, instead of DNA, has been devised by Mr. Bozicevich. This is a much more sensitive test and is capable of detecting systemic lupus erythematosus regardless of the state of activity of the disease. The clinical evaluation of this test is almost completed.

7. A bentonite procedure for the detection of gamma globulin has been devised by Mr. Bozicevich. This test will make an excellent screening procedure for agammaglobulinemia since it may be done in quantity and with accuracy at a minimun cost. Clinical evaluation is underway.

8. Preliminary studies are underway with bentonite flocculation procedures to detect albumin, beta-lipoprotein, and siderophilin.

9. Mr. Bozicevich has been immunizing rabbits with DNA and nucleoprotein in an attempt to produce antinuclear antibody. To date there is no evidence that such antibody can be formed, though additional studies are underway.

Significance to Microbiological Research:

Agammaglobulinemia provides us with a biological system relatively free of humoral defense in which we may study infectious diseases. Systemic lupus, with its attendant hyperglobulinemia, is an ideal disease for the study of hypersensitivity and autoimmunity.

Proposed Course of Project:

Studies will be continued in a wide variety of clinical disorders as to the pathogen-host factors involved in infectious disease.

Study of the present methods of clinical management of agammaglobulinemia patients especially as to the required frequency and dosage of gamma globulin injection and their management with antibiotics will be continued.

The immune mechanism of agammaglobulinemic patients will be studied further, especially as to their response to vaccine and other antigenic stimulation. Non-antibody factors in resistance to disease will also be studied.

Of a special importance is the continuation of the long term followup of patients with systemic lupus erythematosus. This group of patients has been tediously followed in the clinic and with frequent inpatient admission and represents an outstanding group in regard to long term followup. During the course of the coming year it is anticipated that the relationship of the clinical course to the level of lupus factor as detected by the bentonite flocculation test will be a major endeavor.

The pathologic significance of the L. E. cell, especially when found in diseases other than lupus, will be studied.

Part B Included: Yes / / No / /

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Antibodies to Enteroviruses in Hypogammaglobulinemic Patients. Barnett, E. V., J. P. Nasou, J. P. Utz, and S. Baron. New England Journal of Medicine 262:563-565, March 17, 1960.

2. Desoxyribonucleic Acid (DNA)-Bentonite Flocculation Test for Lupus Erythematosus. Bozicevich, J., J. P. Nasou and D. E. Kayhoe. Proceedings of the Society for Experimental Biology and Medicine 103:636-640, 1960.

3. Clinical Evaluation of the DNA Bentonite Flocculation Test for Systemic Lupus Erythematosus. Kayhoe, D. E., J. P. Nasou and J. Bozicevich. New England Journal of Medicine 263:5-10, July 7, 1960.

4. Naming the Bentonite Procedure (proposals relative to the nomenclature of the bentonite flocculation tests). Nasou, J. P., J. Bozicevich and D. E. Kayhoe. Journal of the American Medical Association 174(10):1348, 1960.

Sub-project II: Ascites in Mice

Principal Investigator: Miss Rose Lieberman

Other Investigators: Mr. William Humphrey
Mr. John Douglas
Mrs. Jocelyn Blakely

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.4
Professional 0.5
Other 0.9

Project Description:

Objectives:

1. To determine the incidence of ascites in 12 pure inbred and 3 hybrid strains of mice employing staphylococcal-adjuvant mixtures.

2. To observe the frequency of the development of plasma cell tumors in these various strains of mice.

3. To investigate the role of the plasma cells in the production of specific antibody.

Methods Employed:

Ascites inducing doses of staphylococcal-adjuvant mixtures are administered to 15 different strains of mice immunized with ovalalbumin or horse serum. The incidence of ascites, reactions to immunizing agents, amount of ascitic fluid produced, titers of antibody in the ascitic fluid, effect of single versus multiple immunizing doses on titer, appearance of persistence of antibody, and the effect of "boosters" on antibody titer are observed in individual mice over a long period of time for each strain studied.

In collaboration with the Cancer Institute, the incidence of plasma cell tumors appearing in the different strains of mice are removed and transplanted into unimmunized mice for studies on appearance and production of antibodies.

Major Findings:

Specific strains of mice show a higher incidence of ascites with persistent production of large amounts of ascitic fluid. Plasma cell tumors appear with some consistency in about 18% of the Balb/c mice treated with the staphylococcal-adjuvant mixtures. Several of these tumors have been successfully transplanted through 4-5 generations of mice. Indications are that a low incidence of plasma cell tumors may appear in other strains of mice.

Significance to Microbiological Research:

Production of ascites in mice is an excellent tool to obtain a continuous source of large amounts of potent antibody from individual small animals over a long period of time. This method will facilitate the study of different antibodies produced by purification and fractionation of antigens and provide some information on the mode of action and synthesis of antibody.

The possibility of a genetic relationship to the incidence of ascites in mice may be ascertained.

The role of "immune" plasma cells transplanted into normal mice in the production of antibody may be investigated.

Proposed Course of Project:

To continue as outlined under objectives.

Sub-project III: Interactions of Antibodies

Principal Investigator: Miss Rose Lieberman

Other Investigators: None

Project Description:

Objectives:

To study the effect of mixtures of antibodies in conferring passive protection.

Methods Employed:

Mixtures of Salmonella and of Proteus immune rabbit sera are separately investigated to determine the effect on mouse protection of increasing, decreasing or removing specific antibody components present in the antisera combinations.

Major Findings:

Antibodies employed in combination are either simply additive,

enhancing or antagonistic in conferring passive protection in mice.

Significance to Microbiological Research:

Antibodies in combination behave differently from the equivalent amounts of the same antibodies used separately and may be more or less effective in conferring protection.

Proposed Course of Project:

To continue as outlined under objectives.

Part B Included: Yes /__/ No /__/

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Lieberman, R., Douglas, J. O. A. and Mantel, N.: Production in mice of ascitic fluid containing antibodies induced by Staphylococcus or Salmonella-adjuvant mixtures. J. Immunol., 84:514-529, May, 1960.

Serial No. NIAID - 18(c)
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Enteric Diseases

Principal Investigator: Dr. John P. Utz

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.1
Professional: 0.1
Other: 1.0

Project Description:

Objectives:

Basic studies of the etiology, pathogenesis, and immunology of enteric diseases which include:

1. The evaluation of antibiotic immunologic and surgical therapy on the "carrier state" of salmonellosis.

2. Studies of cellular and humoral mechanisms in active Salmonella infections as compared to those present in the "carrier state."

Clinical studies: To investigate the factors that produce persistence of bacteria in some patients to test new antibiotics in such infections.

Methods Employed:

1. Complete bacteriologic and immunologic survey of enteric disease study patients.

(a) In vitro determinations of synergistic, or additive effects of combinations of antibiotics on organisms isolated from "carriers."

(b) Determination of gamma globulin, electrophoretic pattern, and agglutinating antibodies in acute and chronic enteric disease states.

(c) Cholecystectomy or abscess irradication as indicated.

Patient Material and Major Findings:

1. Studies of the Salmonella carrier state in humans have been continued in 2 additional patients. Gall stones removed from these patients have been implanted, as described in last year's report, in rabbit gallbladders. This brings to a total 6 patients who had stones so implanted in 8 rabbits, 7 of whom became carriers. The results of this study in abstract form were published.

2. Studies described in last year's report on the patient with Fusobacterium septicemia were published.

Significance to Microbiological Research:

Outbreaks of salmonellosis represent an increasingly serious public health problem. The typhoid and other Salmonella "carrier states" are responsible for spread of the more virulent Salmonella to man, and pose a serious problem. There is no standardized therapy for the "carrier state."

Proposed Course of Project:

To continue as outlined under "Objectives."

Part B included Yes /X/ No / /

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Tynes, B. S., and Utz, J. P.: Fusobacterium septicemia. Am. J. Med. 29: 879-887, 1960.

Serial No. NIAID - 19(c)
1. Laboratory of Clinical Investigation
2. Parasitic Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
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Part A.

Project Title: Clinical Investigations on Helminthic Diseases

Principal Investigators: Henry K. Beye, G. Robert Coatney, Leon Jacobs, John E. Tobie, John Bozicevich

Other Investigators: Steven Schenker (since July 1959), Donald Kayhoe (until 7/60), Sanford Kuvin (since 7/60), and Elizabeth Guinn

Cooperating Units: Parasitic Disease Service, LCI, NIAID
Laboratory of Parasitic Diseases, NIAID
Laboratory of Parasite Chemotherapy, NIAID
Laboratory of Immunology, NIAID
Medical Division, Department of State, Washington, D. C.
British West Indian Labour Organization
National Cancer Institute

Man Years (calendar year 1960)
Total: 3.5
Professional: 2.0
Other: 1.5

Project Description:

Objectives:

The objectives of this group of related projects which include: (I) Filariasis, (II) Nematode Infections, (III) Trematode Infections, (IV) Cestode Infections, are to: (1) develop improved diagnostic techniques, (2) better understand host-parasite relationships, (3) more accurately describe diseases associated with these parasites and (4) elucidate the pathogenesis of the disease process.

Methods Employed:

Methods for patient procurement utilized in 1960 consist of physician referrals, a cooperative arrangement with the Medical Division, Department of State, whereby returning State Department employees are screened for the presence of intestinal parasites, arrangements with the

British West Indian Labour Organization utilizing British West Indian agricultural migratory workers, when indicated and a diagnostic serological service for indicated special tests not performed elsewhere. Two new administrative aids have come into existence this year which will be very helpful in future studies. The provision of funds for aiding the transport of research candidates with special problems, and the use of volunteers are notable advances.

The development of a modest museum of specimens coming to the attention of the parasitic screening laboratory is beginning to be fruitful in terms of investigations and training.

I. Filariasis

Patient Material and Major Findings:

Probably the two most important major findings associated with this project in 1960 were (1) the demonstration of the absorption of tetracycline by filarial worms with consequent fluorescence when exposed to ultraviolet light and (2) the demonstration of the viability of microfilaria of Wuchereria bancrofti for at least 18 months when frozen at -10°C. The former studies commenced in 1959 and involved the administration of tetracycline to 2 patients exhibiting cutaneous migrations of Loa loa. These patients, when subsequently examined under ultraviolet light, showed fluorescence of adult filarial worms. During the past year fluorescence has also been demonstrated to some degree in malaria parasites, E. histolytica and tapeworm proglottids. Microfilaria used in the viability studies were from British West Indian Migratory workers, with filarial infections that were reported and studied during 1959 and discussed in last year's annual report. Specimens of heparanized blood from these individuals containing microfilaria were placed in the refrigerator and maintained at -10°C. At approximately 3 month intervals, separate portions were removed and allowed to thaw at room temperature. As of the present date, microfilaria have retained viability.

Attempts were made to develop a bentonite flocculation test for use in the diagnosis of filariasis. Preliminary results on the sera obtained from immunized animals gave encouraging results. This is to be exploited on sera obtained from infected individuals. A paper was presented at the First International Congress on Trichinosis, Warsaw, Poland

Significance to Bio-Medical Research and the Program of the Institute:

The demonstration of the phenomenon of tetracycline concentration in filarial worms as well as in other parasites, has much potential significance. There has been a great interest elicited since the publication of the original article for using this as a diagnostic procedure. At the same time, interest has also been expressed in the utilization of this concentration of tetracycline in the development of more effective antifilarial drugs. The viability studies are very simple as performed in our laboratories and these findings may have interest

on the metabolism of these parasites. In time, such a technique would be extremely useful to medical schools providing viable microfilaria for class demonstration and study.

Proposed Course of Project:

Additional studies will be undertaken on utilizing and understanding the tetracycline fluorescence phenomenon in filarial worms. In pursuing these studies, it is hoped to utilize staff and facilities in British Guiana where a filariasis research and control project is being established, following the recommendations made by the senior investigator when he served as a WHO consultant for that country in filariasis. In addition, it is anticipated to pursue these studies by the use of PL-480 counterpart funds in various countries where filariasis is endemic. The question of the elucidation of the pathogenesis of eye lesions in onchocerciasis is a very important and controversial one. It is hoped that these techniques might be applicable in this filaria infection.

II. Nematode Infections and Disease

Patient Material and Major Findings:

1. Trichostrongylus infected patients: As reported last year, State Department employees continued to come to our attention who were infected with trichostrongylus. Species have not yet been determined because of the difficulty of procuring adult worms. Studies conducted this year indicate that eggs of this parasite obtained from patients treated with tetracycline fluoresce. Attempts have been made to utilize this phenomenon to find adult worms after treatment but so far these attempts have been unsuccessful.

2. Parasites among the British West Indian Migratory Workers: This group of individuals with rather high prevalence of intestinal nematodes is still available to us but during this past year we have been unable to follow through with this group.

3. Visceral Larval Migrans: A very informative case referred to the Clinical Center because of the diagnosis of eosinophilic leukemia has been studied and proven to have visceral larval migrans. In this case the diagnosis was established through recovering larvae of probably Toxocara canis at liver biopsy and the adult worms were recovered from the family dog which died and was posted. The patient has responded well to diethylcarbamazine. In summary, the case provides probably one of the best documented cases of visceral larval migrans which has yet been described. This patient was admitted to the Cancer Service and was studied in conjunction with our laboratory. At the present time observations are still continuing.

Significance to Bio-Medical Research and the Program of the Institute:

Probably the most significant finding in this project during 1960 was the demonstration of this case which had many characteristics of

eosinophilic leukemia but which responded well to diethylcarbamazine therapy. The elucidation of the role of trichostrongylus in returning State Department people from the Near East and further development of the various nematode infections amongst West Indian Migratory Laborers are significant and will be pursued when time and facilities permit.

Proposed Course of the Project:

This project will be intensified, particularly in respect to investigation of trichostrongylus and trichinosis. The further exploration of tetracycline and autofluorescence; the programming for our activation of studies of parasitoses in 1962 in countries where counterpart funds are available, will be pursued. The instrument section is now building a sigmoidoscope, utilizing ultra-violet light which will be of great help in these investigations.

III. Trematode Infections and Disease

1. Schistosomiasis and other diseases due to flukes: Little activity has been carried on in this area during the past year, primarily because of emphasis on simian malaria and tetracycline studies. Dithiazanine iodide reported as being effective against Clonorchis sinensis has been tested in this laboratory. The results of follow-up studies are still in progress.

Significance to Bio-Medical Research and the Program of the Institute:

With the intense migration of Puerto Ricans with high infection rates due to Schistosoma mansoni, increasing number of Americans going to infected areas and the increasing larger areas of the world in which infected snails are found, makes schistosomiasis one of the major world diseases and of increasing importance to the people in the United States. The treatment trials against Clonorchis sinensis with dithiazanine iodide is interesting and significant in that no previous medication has been very effective. The most universally used drug, chloroquine diphosphate, has not been very effective in our hands as well as in other workers.

Proposed Course of the Project:

Inasmuch as facilities and staff allow, continued efforts will be made to evaluate new drugs. The use of PL-480 funds has made it possible to program a clinical study in schistosomiasis in Brazil with possible activation by July 1961. This will help provide a better understanding of the pathogenesis and the manifestations of hepatosplenic schistosomiasis.

IV. Infections due to Cestodes

Patient Material and Major Findings:

A series of patients with Taenia saginata, mentioned in last year's report, has now increased to approximately 26. During 1960 experimental

administration has consisted of tetracycline with follow-up studies on tetracycline fluorescence plus oral atabrine. In cases that are unsuccessful, the treatment of atabrine transduodenally is employed. Dithiazanine has been tried with inconclusive results.

Significance to Bio-Medical Research and the Program of the Institute:

Infections with these parasites are difficult to treat, and the success of our method of using the atabrine through a duodenal tube is a rather significant contribution.

Proposed Course of Project:

It is anticipated that a great deal of emphasis will be made in the next year on Echinococcus disease, using patients from Alaska or in planning studies in Echinococcus in countries of high endemicity, utilizing counterpart PL-480 funds.

Part B included Yes No

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Beye, Henry K., and Gurian, Joan: The Epidemiology and Dynamics of Transmission of Wuchereria bancrofti and Wuchereria malayi. Indian Journal of Malariology, December, 1960.

2. Beye, Henry K.: International Organizations and Filariasis Control (Wuchereria bancrofti and Wuchereria malayi). Indian Journal of Malariology, December, 1960.

3. Tobie, John E. and Beye, Henry K.: Fluorescence of Tetracyclines in Filarial Worms. Proc. Soc. Exper. Biol. and Med., 104:137-140, 1960.

4. Kayhoe, Donald E. and Beye, Henry K.: The treatment of Taenia saginata infections with quinacrine HCL (Atabrine) administered through a duodenal tube. Presented at the American Society of Tropical Medicine and Hygiene meeting, November 2-5, 1960.

5. Beye, Henry K.: Helminthic Diseases and Anthelminthic Drugs. Clinical Proceedings of The Children's Hospital, In Press.

6. Beye, Henry K.: Filariasis. Cyclopedia of Medicine, Surgery and Specialties. In Press.

Serial No. NIAID - 20(c)
1. Laboratory of Clinical Investigation
2. Parasitic Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Clinical Investigations in Protozoan Infections and Disease

Principal Investigators: Henry K. Beye, G. Robert Coatney, Leon Jacobs, John E. Tobie and John Bozicevich

Other Investigators: Donald Kayhoe to July 1960, Morton Getz, Harvey Elder from 7/60, Steven Schenker to 7/60, Sanford Kuvin since 7/60, and Elizabeth Guinn

Cooperating Units: Parasitic Disease Service, LCI, NIAID
Laboratory of Parasite Chemotherapy, NIAID
Laboratory of Parasitic Diseases, NIAID
Laboratory of Immunology, NIAID
Medical Division, Department of State, Washington, D. C.
Federal Bureau of Prisons

Man Years (calendar year 1960)
Total: 3.5
Professional: 2.0
Other: 1.5

Project Description:

Although the activities of interest on specific protozoan infections vary from year to year, fundamental objectives and methods remain essentially the same. Since April 1960, a great deal of the emphasis was on the clinical investigations associated with simian malaria. Activities in relation to amebiasis continued, but at a reduced activity. Little emphasis was given to trichomoniasis, toxoplasmosis or other protozoan diseases.

The development of a modest museum of specimens coming to the attention of the parasitic screening laboratory is beginning to be fruitful in terms of investigations and training.

Objectives:

(1) Improve diagnostic techniques (2) better understand host parasite relations (3) more accurately describe disease manifestations and host response (4) elucidate the pathogenesis of the disease process

in protozoan infections and (5) more accutate evaluation of various treatment regimes.

Methods Employed:

Methods of patient procurement utilized in 1960 consisted of physician referrals, a continuation of the cooperative arrangement with the Medical Division, Department of State, whereby returning State Department employees were screened for the presence of intestinal parasites, the use of inmate volunteers, primarily through the Malaria Project at the Federal Penitentiary in Atlanta, Georgia, a new method started this year of the utilization of selected inmate volunteers at the Clinical Center, and the maintenance of a research serological diagnostic service for protozoan diseases.

Methods for investigation and study of these research candidates remain the same, that is, close communication and liason with the basic laboratories and workers participating in these investigations, direct activities through our own Parasitic Disease diagnostic and investigative laboratory, utilization of the Service activities provided by the Clinical Center and finally, educational and program activities related to patient procurement such as lectures and talks at various universities, exhibits and discussions with organizations which might provide a source of research candidates.

I. Blood and Tissue Protozoan Diseases

A. Simian Malaria in Man

Patient Material and Major Findings:

(1) Two blood induced infections with Plasmodium cynomolgi bastianellii in inmate volunteers in May, (2) twenty sporozoite induced infections with the same parasite in inmate volunteers during May and June 1960, the infective mosquito in this instance being Anopheles quadrimaculatus, (3) six sporozoite induced infections amongst inmate volunteers using the same parasite but using Anopheles freeborni. Two of these patients were transferred to the Clinical Center immediately after being bitten while the remainder stayed for observations at the Unit at the Atlanta Panitentiary, (4) eleven inmate volunteers bitten by Anopheles freeborni heavily infected with Plasmodium cynomolgi. As a result of these experiments, a new clinical entity associated with this previously thought simian parasite has been first described. These results can be briefly summarized as follows: Prepatent period usually under 18 days, onset of symptoms ranging from 14 days to 2 1/2 months, extremely low parasitemias associated with rather severe clinical manifestations in a few patients, other patients having infections but with mild or asymptomatic clinical manifestations. Manifestations seemingly more benign in Negroes than in Caucasians. Changes in certain laboratory tests such as increase in erythrocyte sedimentation rate, increase in cholesterol beginning early after the infection, decrease in platelet counts and neutropenia, are associated with the

infection. Individuals, infected with regular cynomolgi on the other hand, do not seem to develop the infection. A most significant finding is the demonstration that simian malaria parasite is probably a zoonoses, the parasite has been transmitted from monkey to man via the mosquito, and then from man to mosquito. Suggestive evidence indicates the transmission from man to man by the mosquito.

B. Chemotherapeutic studies using inmate volunteers at Atlanta Penitentiary. More than a 100 volunteers participated in various studies associated with the prophylactic and sporozonite properties of primaquine, chloroquine and a Russian preparation, quinacide. The results amongst these studies can be summarized as follows:

1. Suppressive cure effect of a pyrimethamine-primaquine combination: Pyrimethamine 50 mgm plus primaquine 45 mgm given once a week for four weeks, has protected five men exposed to the bites of mosquitoes infected with Chesson strain of vivax malaria for at least 219 days of observation.

2. Suppressive cure effect of chloroquine-primaquine combination: Ten Negro male volunteers were divided into two groups of 5 men each. Each man in Group A received chloroquine 300 mg plus primaquine 45 mg in a single oral dose three days prior to the date of infection, (0-3), and on the same day of each week for a total of four doses. The subjects in Group B received no drugs and served as controls.

Both groups were infected with the McLendon strain of P. falciparum on 1 April 1960, by the bites of ten heavily infected A. quadrimaculatus mosquitoes. In an attempt to simulate field conditions the ten volunteers were subjected to three subsequent bitings at irregular intervals. At each subsequent biting time additional controls were added.

Each of the five men in Group B developed parasitemia on the 11 to 13 experimental day. After two days of demonstrable parasites, each was given chloroquine 300 mg plus primaquine 45 mg. This regimen was repeated on the same day of the week for a total of three doses.

None of the five volunteers in Group A developed parasitemia during 90 days of observation. None of the man in Group B has exhibited any evidence of infection following the chloroquine-primaquine therapy.

One control was added for each of the last 2 biting episodes. Neither control developed malaria and the last two bitings are considered non-infecting even though sporozoites were demonstrated at the post-prandial dissection of the mosquitoes.

3. Curative effect of CN 1115 (WIN 10448, Quinicide): To date, 8 patients have received Quinicide therapy and 10 have received Primaquine. Of those patients who received CN 1115, four exhibited early primary attacks and four delayed primaries. There was a total of 6

recurrences in the early primary group and two in the delayed primary group.

To date, 11 patients have received primaquine therapy and ten have received CN 1115. The dosage of each drug was 15 mg. single dose daily for 14 days. There have been two relapses among those who received primaquine therapy. There have been 13 relapses among those given CN 1115. Of these relapses, one man had four, three had two, and three had one relapse each. Relapse infections were treated according to the original regimen.

Significance to Bio-Medical Research and the Program of the Institute:

The significance of these investigations in terms of the world-wide malaria eradication and in terms of more adequately understanding the pathogenesis of malaria, including the human form, is very great. For the first time, reservoirs of human malaria have been definitely elucidated and perhaps for the first time, a malaria parasite with clinical manifestations similar in monkeys and man has been found. This will provide a very useful tool in other studies on malaria.

Proposed Course of Project:

This project in clinical investigation is one aspect of an intensive program of investigating the role of simian malaria in the epidemiology of human malarias coordinated through the Laboratory of Parasite Chemotherapy. It is anticipated that additional work will be done on the clinical description of the disease involving the use of additional volunteers at the Clinical Center. Studies of cross-immunity of the simian malarias to human malarias, particularly the regular cynomolgi are programed. It is anticipated that some activity may be extended to other countries through the utilization of PL-480 funds.

II. Amebiasis

A. State Department Personnel

Patient Material and Major Findings:

As indicated, patient material has consisted of the more than 6,000 State Department employees who have submitted stool specimens to the Parasitic Disease Service for screening since the inception of this cooperative program with the Medical Division, Department of State. This material has been analyzed, the most interesting features being that those individuals infected with E. histolytica did not exhibit any additional clinical manifestations or did they lose more time from work while overseas than those individuals who were not infected. This re-emphasizes again the beginning appreciation of the benign character of E. histolytica in normal healthy individuals. At the same time, an occasional case was admitted to the Clinical Center with the classical symptoms of acute amebic dysentery and one case of amebic liver abscess from the Washington area was studied. Studies continued

on the development of more definitive and more perfected serological tests for amebiasis including a bentonite flocculation test. For those individuals who were accepted for research study, the use of the anti-amebicide, Humatin (Puramycin) has been studied this past year. This drug, in our present investigations, seems to be the most satisfactory amebicide which has been our experience to study. The definitive analysis, however, of these cases, including follow-up observations, are still in process.

B. Immunology and Serology

Three series of rabbits were immunized to Entamoeba invadens, E. terrapinae and E. histolytica antigens. The antigens were grown in vitro on the appropriate media and the animals immunized with the supernatant fluid contining the metabolic products and the entire organisms. At intervals, the animals were bled and complement fixation tests were conducted with the specific and heterologous antigens. N_o cross reactions were found at any time as elicited by a positive complement fixation test. Additional immunization was given to each group with the specific antigen. Again, no cross reactions were encountered with the heterologous antigens but specific reactions were obtained as elicited by the complement fixation test.

The purpose of the above study was to investigate the possibility of utilizing a closely associated antigen for the complement fixation test for the detection of E. histolytica infection. If this proves to be successful, it would facilitate preparing antigens for the diagnosis of amebiasis.

Significance to Bio-Medical Research and the Program of the Institute:

It is only through the understanding of the pathogenesis of E. histolytica infections and disease that this entity will be placed in proper prospective. Work continues on the elaboration of the pathogenesis of E. histolytica in man in our basic laboratories, particularly the germ-free laboratory. The significance of our observations is rather large in that it provides a great deal of epidemiologic data on benign characters of this infection among foreign service personnel. The presence of the disease, however, in occasional cases only emphasizes the importance of understanding what factors are responsible for these manifestations.

Proposed Course of Project:

With adequate staff and time, it is hoped that this project can, in time, be re-oriented to concentrate on a few individual cases which can be followed with or without treatment for long periods of time, using in association with these studies newer techniques for the study of the upper GI tract. This would make possible the description of the invasiveness and the characteristics of the E. histolytica complex (E. hartmani, small and large races of E. histolytica) along with the bacterial associates in the human GI tract. During this past year,

little work was done on trichomonaisis, toxoplasmosis or other protozoan diseases which have received emphasis in the past. We hope to reactivate these projects in the near future as they are of great biomedical significance. This is particularly true with toxoplasmosis. The infection is wide-spread, the disease manifestations are occasionally very severe, and the spectrum of host response and clinical manifestations is as yet undescribed.

Part B included Yes No

(Attachment I)

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Beye, Henry, Brooks, Charles and Guinn, Elizabeth: Intestinal and Blood Parasitism among British West Indian Agricultural Laborers. Review of Protozoan and Helminthic Infestations among Migratory Agricultural Workers. Accepted for publication by the Journal of American Public Health Association.

2. Kayhoe, Donald E., Beye, Henry, Guinn, Elizabeth and George, George P.: Prevalence of Intestinal Parasites Among U. S. State Department Employees and Their Dependents. Presented at the American Society of Tropical Medicine and Hygiene Meeting, Los Angeles, California, November 2-5, 1960.

3. Coatney, G. Robert and Beye, Henry K.: Simian Malaria Infections in Man. Presented at the American Public Health Association Meeting, San Francisco, California, October 31-November 5, 1960.

4. Beye, Henry K. and Coatney, G. Robert: Preliminary Report on Sporozoite Induced Plasmodium Infections in Man. Presented at World Health Organization Expert Committee on Malaria.

5. Beye, Henry K., Coatney, G. Robert, Getz, Morton and Eyles, Donald: A Vivax-Type Malaria of Simian Origin in Man. Clinical and Parasitological Characteristics. Presented at the American Society of Tropical Medicine and Hygiene Meeting, Los Angeles, California, November 2-5, 1960.

6. Getz, M.E., Elder, H. A. and Todd, Chas. S., Jr.: Clinical Manifestations of Plasmodium cynomolgi bastianellii. Presented at the National Institute of Allergy and Infectious Diseases and Communicable Disease Center Joint Conference, November 17-18, 1960.

Serial No. NIAID - 21(c)
1. LCI
2. Pediatric Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Project Title: Cystic Fibrosis of the Pancreas

Principal Investigator: Dr. George T. Bryan

Other Investigators: Dr. Hugh Evans (from July 1, 1960)
Dr. Philip Fireman (from July 1, 1960)
Dr. Edward Eyerman (to June 30, 1960)
Dr. Lowell Good (to June 30, 1960)
Dr. George Owen (to June 30, 1960)

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2.85
Professional: 2.85

I. Objectives

A. To study the microbial flora in patients with cystic fibrosis of the pancreas.

B. To evaluate the role of staphylococcal immunology.

C. To evaluate the effect of certain antibiotic agents on the bacterial flora of the respiratory tract.

D. To study the immunology of purified staphylococcal penicillinase.

E. To study the relationship of growth failure in these patients to their serum growth hormone levels.

2. Patient Material

Children with proven cystic fibrosis of the pancreas who are being followed regularly in this clinic.

3. Major findings

The major findings are limited to the study of the microbial flora in patients with cystic fibrosis of the pancreas. These would indicate that children with this disease have a relatively stable bacterial flora in spite of continuous therapeutic antibiotic therapy. Investigations are continuing in the study of microbial flora and the role of the staphylococci in cystic fibrosis of the pancreas.

Part B included Yes /_/ No /X/

Serial No. NIAID - 22(c)
1. LCI
2. Biochemistry and Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Project Title: Basic Biochemical Studies

Principal Investigator: Dr. Harry G. Steinman

Other Investigators: Dr. Eskin Huff
Dr. Steven Schenker
Dr. Anderson Spickard
Dr. Sheldon Wolff

Cooperating Units: Hildegard Wilson, Laboratory of Nutrition and Endocrinology, NIAID
Ellsworth R. Buskirk, Metabolic Diseases Branch, NIAID
Ronald H. Thompson, Metabolic Diseases Branch, NIAID
Howard Goodman, Laboratory of Immunology, NIAID
Emanuel S. Hellman, General Medicine Branch, NCI

Man Years (calendar year 1960):
Total: 4.5
Professional: 2.5
Other: 2.0

Project Description:

Objectives:

To study the biochemistry of pathogenic micro-organisms; the nature of penicillin resistance; the mechanism of action of penicillin; the biochemical abnormalities associated with specific allergic and infectious disease processes.

Methods Employed:

1) Biochemistry of pathogenic micro-organisms. The biosynthesis of the polysaccharides of bacterial cell wall material has been investigated with emphasis on the role of certain three-carbon precursors.

2) Nature of penicillin resistance. The role of penicillinase in the resistance of S. aureus to penicillin is being investigated under a variety of conditions.

3) Mechanism of action of penicillin. A number of new penicillins now available are being compared in a number of biological and biochemical systems in order to correlate chemical structure with biologic activity.

4) Biochemical abnormalities in allergic and infectious diseases. The biochemical origins of a rare clinical entity known as etiocholanolone fever have been examined with respect to the metabolic fate of specific steroid substances. The amino acid abnormalities associated with Familial Mediterranean Fever have been studied. The effect of chloroquine on producing porphyria is being investigated in both animals and patients.

Patient Material and Major Findings:

1) Biochemistry of pathogenic micro-organisms. The study on the identification of lactaldehyde as the oxidation product of 1,2-propanediol and the sterespecificity of the enzymatic reactions catalyzed by alcohol dehydrogenases, which was described in a previous Project Report (NIAID - 22(c), 1959) has been summarized in a paper entitled "The Metabolism of 1,2-propanediol" and which is now in press. This publication concludes the work on the metabolism of 1,2-propanediol and its phosphate ester.

2) Nature of penicillin resistance. Although there is a very high degree of coincidence between the presence of penicillinase and the penicillin resistance of pathogenic S. aureus, the direct proof that the enzyme is responsible for the resistance has been lacking. There would appear to be no question as to the role of penicillinase were it not for the fact that penicillin resistance of ordinarily sensitive strains of S. aureus can be readily achieved in the laboratory by a process of selection of natural mutants. This type of natural resistance, which does not appear to be stable, is not dependent on the presence of the destructive enzyme penicillinase. Its true basis remains unknown. A variety of experiments have been performed which support the thesis that penicillinase is indeed the primary factor effecting resistance to penicillin in S. aureus. These will be reported at the 5th International Congress of Biochemistry to be held in Moscow, 1961.

3) Mechanism of action of penicillin. As a corollary of the studies on penicillinase the activities of the reaction with several different penicillins had been made. It became apparent that a penicillin which was insensitive to the action of the enzyme would be of great value to the basic studies as well as of clinical interest. When such a penicillin became available, as the result of a major breakthrough in penicillin technology, our laboratory techniques were able to demonstrate immediately the unique resistance to the destructive action by staphylococcal penicillinase of the new penicillin and to indicate the potential usefulness of the drug in therapy almost before clinical evidence of the effectiveness in the treatment of penicillin G-resistant staphylococcal infections was at hand. These findings were embodied in 2 papers, one presented at a Symposium devoted to the new penicillin held in Syracuse on September 7, 1960 and the other at the Conference on Anti-Microbial Agents held in Washington on October 26-28, 1960. By examining a series of penicillins in a variety of biological and biochemical reaction systems,

it has been possible to ascertain the effect of various chemical substituents in the basic penicillin molecule on a number of specific biologic activities. These studies will be reported at the First International Pharmacological Meeting to be held in Stockholm, 1961.

4) Biochemical abnormalities associated with specific disease processes.

a) Etiocholanolone fever. In colloboration with Dr. H. Wilson, NIAID, a patient with the diagnosis of etiocholanolone fever has been studied with respect to the biochemical pathogenesis of his disease. This disease was characterized by bouts of fever, chills, myalgia and anorexia lasting from a day to several weeks, recurring at irregular intervals and associated with a mild normocytic normochromic anemia and a nonspecific granulomatous infiltration of the liver. At the time of his febrile attacks, the patient was found to have elevated plasma levels of nonconjugated etiocholanolone and dehydroepiandrosterone. The illness responded dramatically to steroid therapy.

In order to elucidate the pathogenesis of this rare disease, metabolic studies have been carried out to determine 1) the ability of the patient to conjugate and excrete exogenously administered etiocholanolone and 2) the capacity of the patient to synthesize etiocholanolone from its precursors. Twenty five milligrams of etiocholanolone were administered intramuscularly to the patient while his own etiocholanolone secretion was suppressed by steroid therapy, and urine was tested for etiocholanolone output. Preliminary results, by the hot acid hydrolysis method, indicate that the patient excretes 40-50% of the administered dose in 24 hours comparable with normal patients. The urine will be studied further for total quantitative excretion of the compound as well as the amount which is conjugated as a glucuronide and sulfate. The patient's glucuronyl transferase system was studied by determining his ability to conjugate menthol and this was found to be normal. Subsequently the patient was given doses of testosterone and hydroxy-androstenedione and his urine checked for conversion of these substances to etiocholanolone. Initial results reveal normal conversion. Should the patient redevelop his symptoms, it is planned to investigate the possible beneficial role of thyroid analogues on the course of his illness as well as to attempt to isolate a possible thermogenic principle from the plasma.

b) Familial Mediterranean Fever. Preliminary investigations are under way dealing with Familial Mediterranean Fever. In collaboration with Drs. E. Buskirk and R. Thompson of NIAMD, we are studying the physiologic response of a patient to cold stress and also during an attack of fever. Biochemical studies of the alpha amino nitrogen excretion and chromatographic analyses of the amino acid content of blood and urine are being done. Some immunologic studies in conjunction with Dr. H. Goodman, NIAID, are planned. Some therapeutic trials are anticipated also, using female hormones.

c) Porphyria. A patient with porphyria is also being studed. Elevations in the patient's excretion of 5-hydroxyindoleacetic acid has been noted and it is planned that this finding be looked for in other patients and some investigation as to why this occurs will be undertaken. Also the effect of chloroquine in this disorder is being studied by measuring urinary porphyrins and delta aminolevulinic acid before and after chloroquine therapy. The latter study and a study of the effect of chloroquine in experimental porphyria (in rats) is being done in collaboration with Dr. E. Hellman of NCI.

Significance to Biomedical Research:

The discovery of the high degree of resistance of the new penicillin, 2,6-dimethoxyphenylpenicillin, to the destructive action of staphylococcal penicillinase supplied the scientific basis for pursuing the clinical trials of the drug. Other laboratory data helped establish proper dosage and treatment schedules. The new antibiotic shows great promise of ameliorating the problem of hospital-borne staphylococcal infections. However, its intrinsic chemical properties prevent it from being used except under hospital conditions because it is not absorbed into the blood stream well enough to be given orally, but must be injected intravenously (or intramuscularly). Our laboratory know-how in this field will enable us to evaluate immediately and exploit any superior penicillin that may appear in the future.

The studies on the biochemical observations associated with such obscure diseases as etiocholanolone fever, Familial Mediterranean Fever, and chloroquine-induced porphyria will be helpful in understanding the pathogenesis of these diseases.

Proposed Course of Project:

These studies are being continued.

Part B included yes [X] no [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

1. Huff, E.: The Metabolism of 1,2-Propanediol. Biochem. et Biophys. Acta. In press.

2. Steinman, H.G.: Factors modifying induced formation of penicillinase in Staphylococcus aureus. J. Bacteriol. In press.

3. Steinman, H.G.: A biochemical comparison of 6-aminopenicillanic acid, benzylpenicillin, and 2,6-dimethoxyphenylpenicillin. Proc. Soc. Exptl. Biol. Med. In press.

4. Steinman, H.G.: Comparative activities of 6-aminopenicillanic acid, benzylpenicillin, and 2,6-dimethoxyphenylpenicillin. Paper delivered at State University of New York, Upstate Medical Center, Syracuse, New York, September 7, 1960; to be published in Bunn, P.A. (ed.): A Symposium on a New Synthetic Penicillin, Syracuse University Press, 1961.

5. Steinman, H.G.: A comparison of the biochemical activities of 6-aminopenicillanic acid, benzylpenicillin, 2,6-dimethoxyphenylpenicillin, 2-phenoxyethylpenicillin, and phenoxymethylpenicillin. Paper delivered before the Society for Industrial Microbiology, Washington, D. C., October 27, 1960; to be published in Transactions of the Conference on Antimicrobial Agents, 1960.

Serial No. NIAID - 23(c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Systemic fungal disease

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. Vincent T. Andriole
Dr. Michael W. Brandriss
Margret A. Huber

Cooperating Units: Laboratory of Infectious Diseases

Man Years (calendar year 1960):
Total: 2.1
Professional: 2.1
Other: 0.0

Project Description:

Objectives:

1. To study diagnosis, pathogenesis and natural course of systemic fungus infections.

2. To study the problem of fungus disease due to overgrowth during antibiotic and steroid treatment.

3. To evaluate new agents in antibiotic and chemotherapeutic treatment of systemic fungus infections.

4. To set up means of measuring blood levels of new antifungal agents and to study acute and long term toxic effects of the drugs.

5. To study various immunologic aspects of this group of diseases.

Methods Employed:

Patients with suspected fungal infections are studied diagnostically using the skin test, serologic, histologic, and cultural methods. Family contacts are similarly investigated and environmental studies undertaken, when indicated. Infecting fungi from such patients are

screened for sensitivity against a considerable number of chemotherapeutic or antibiotic agents and therapy is undertaken after such studies and where clinically advisable.

Drugs are screened for activity against an organism isolated from a patient by in vitro methods. Promising drugs are then tested in animals for acute and long-term toxicity and for therapeutic effectiveness.

New methods are being developed for determining serum drug levels, rates of excretion of drug, and in vitro screening of drugs.

Patient Material and Major Findings:

1. Studies were continued on patients with a variety of fungal diseases. New patients with culturally proved disease this year included cases of cryptococcosis (7), coccidioidomycosis (3), histoplasmosis (5), sporotrichosis (5), nocardiosis (1), blastomycosis (3), candidiasis (1), aspergillosis (2), and mucormycosis (1).

2. Continued observation of a total of patients treated with amphotericin B has permitted more conclusions as to effectiveness. Although clear-cut treatment failures have occurred in 3 cases of cryptococcosis, 7 patients have "apparently recovered" (clinically well with normal cerebrospinal fluid) from their infections 32, 41, 31, 30, 12, and 6 months after cessation of therapy. One patient died from an unrelated disease. An additional 6 patients are improved (clinically well, but with cerebrospinal fluid not yet normal). An analysis of the failures of amphotericin-treated patients was completed and presented at the 2nd Conference on Medical Mycology, New York Academy of Sciences, January 1960.

3. Eleven new patients with various culturally proved fungus diseases, histoplasmosis (3), blastomycosis (2), coccidioidomycosis (1), aspergillosis (1), mucormycosis (1), and sporotrichosis (3), have been treated with the new antifungal drug RO-2-7758, making a total of 26 patients now treated with this drug. RO-2 seems effective in histoplasmosis, blastomycosis, aspergillosis, maduramycosis, mucormycosis, and sporotrichosis.

4. An investigation has been undertaken of the renal toxic effects of amphotericin. Renal plasma flow glomerular filtration rate in addition to concentrating ability in the kidney have been determined in 5 patients treated with this drug.

5. An investigation of the toxic effects of RO-2 on the liver has also been undertaken. Liver biopsy and BSP determinations have been evaluated in patients treated with this drug.

6. An exhibit showing the results of therapy with amphotericin B and RO-2 was prepared and presented at the Annual Meeting of the American Medical Association at Miami Beach in June 1960.

7. An exhibit showing the results of RO-2 was prepared and presented at the Annual Meeting of the American Public Health Association in San Francisco, California, in October 1960, and at the Clinical Meeting of the American Meeical Association in Washington, D. C., in November 1960.

8. A clinical study of Candida endocarditis was undertaken and observations made on 6 patients with this disease.

9. A study is being made of granulomatous disease of undifferentiated cause occurring in man.

Significance to Microbiological Research:

The group of fungus diseases under study is poorly understood; there is no uniformly successful treatment. Apparently, certain of them, such as histoplasmosis, are being recognized as quite prevalent; others such as candidiasis create problems by the very ubiquity of the organism, which may grow more abundantly and cause systemic disease in the presence of most antibiotics and steroids, creating serious and extensive clinical problems.

Proposed Course of Project:

To proceed as outlined under "Objectives" and "Methods."

Part B included: Yes /X/ No / /

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Utz, J. P., and Andriole, V. T.: Analysis of amphotericin treatment failures in systemic fungal disease. Ann. N. Y. Acad. Sci. 89: 277, 1960.

Awards

1. Emmons, C. W., and Utz, J. P.: New chemotherapy of systemic mycosis: Experimental and clinical studies. An exhibit with brochure. Honorable mention, Experimental Medicine & Therapeutic Section of Scientific Exhibits at the Annual Meeting of the AMA, Miami Beach, June 1960.

Serial No. NIAID - 24(c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Sarcoidosis

This project has been temporarily inactivated due to lack of principal investigator. Project to be reactivated at a proper time in future.

Serial No. NIAID - 25(c)
1. LCI
2. Infectious and Pediatric Disease Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Pyelonephritis

This project has been temporarily inactivated due to lack of investigators. Project to be reactivated at a proper time in the future.

Serial No. NIAID - 26(c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Urinary Tract Viruses

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. Hugh Evans (from 7/1/60)
Clarence F. Szwed

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2.8
Professional: 0.8
Other: 2.0

Project Description:

Objectives:

1. To study the urine of all patients with viral infection for the presence of viruses and to study renal function concurrently.

2. To investigate in animals the effect on the kidneys of the inoculation of virus recovered from human urine.

3. To attempt to produce pyelonephritis in animals by virus inoculation alone or accompanied by secondary physiologic trauma or bacterial infection.

Methods Employed:

Clinical studies: Patients with a variety of viral infections will be studied intensively.

Laboratory studies: Tissue culture and animal inoculation techniques will be used.

Patient Material and Major Findings:

1. Two additional viral isolates have been recovered from the urine of patients under study here.

2. In continuing animal studies on the effects on the kidney of viral infections, microscopic lesions, not found in controls, have

been observed in the proximal convoluted tubules of mice infected with Coxsackie B-3 virus. The significance of these lesions is being studied further with fluorescent antibody techniques.

3. A direct comparison has been made of tissue culture (cytopathic effect), hemadsorption, and embryonated hens egg techniques in the isolation of mumps virus from urine of patients. In 20 patients mumps virus was recovered from urine by criteria of cytopathic effect in 14, by egg inoculation in 1 and by both methods in 2. In general hemadsorption occurred when cytopathic effect was seen in tissue culture but occasionally one manifestation of infection was present without the other.

4. Because of the isolated reports of illness resembling mumps in dogs and of viral isolations from dogs, studies were undertaken to define the matter by inoculation of quantitative amounts of virus into puppies. In 4 animals so inoculated no virus has been recovered subsequently and no detectable disease has been produced. Presence or absence of antibody response will be determined, in addition.

Significance to Microbiological Research:

The recovery of a number of viruses from the urine of patients raises the question of the role they play in disease. The relationship of this infection to subsequent pyelonephritis needs to be explored.

Proposed Course of Project:

As outlined in "Objectives" and "Methods."

Part B included: Yes /X/ No / /

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Habel, K. and Utz, J. P.: Mumps, Ped. Clin. N. America. 7(4): 979-988, 1960.

Serial No. NIAID - 27(c)
1. LCI
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biochemical Studies on Staphylococcal Cell Walls.

Principal Investigator: Dr. Eskin Huff

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 0.4
Professional: 0.4

Objectives:

The general objective of this project is an understanding of the chemical mechanisms involved in the synthesis of staphylococcal cell walls and the manner in which penicillin affects this synthesis. It is hoped that a study of differences in the chemical composition and synthesis of cell walls by penicillin sensitive and resistant staphlococci will throw some light on the mechanism of antibiotic resistance in these organisms.

Specifically an attempt is being made to chemically characterize the organic phosphate containing compounds occurring in the cell walls of staphylococcal species.

Methods Employed:

A strain of Staphylococcus aureus (obtained from Miss Rose Lieberman) has been grown in large batches on trypticase-soy-broth and the cells broken by shaking with glass beads in a Nossal Shaker. The cell walls have been harvested and purified by differential centrifugation using a Spinco preparative ultracentrifuge. Lithium bromide solutions have been used to extract organic phosphate esters from the cell wall material and these organic phosphate esters are being studied at present.

No major findings can be reported at present. However, it has been observed that staphylococcal cell walls have a high density (specific gravity 1.5 to 1.6) as compared to most proteins (specific gravity less than 1.4). It has also been noted that a considerable amount of the organic phosphate containing compounds present in these cell walls can be extracted by means of treatment with solutions of lithium bromide.

Significance to Microbiological Research:

Badiley et al have evidence for the presence of polymers of ribitol phosphate and glycerol phosphate in the cell walls of Bacillus subtilis, Lactobacillus casei, and Staphylococcus aureus. These workers have also demonstrated that in some strains of S. aureus, D-ala-nine and N-acetyl-glucosamine are attached to these polymers which they call teichoic acids. However, other than the studies by these workers little is known of the nature of the phosphate containing compounds present in staphylococci. In some of the bacteria studied the phosphate containing compounds make up as much as 60% of the weight of the cell wall material. In Staphylococcus aureus these phosphate containing compounds may account for as much as 30% of the weight of the cell walls. Although it has been demonstrated that penicillin stops the synthesis of cell wall peptides in S. aureus without an effect on the synthesis of intracellular protein (work by Mandelstam and Rogers), it is not known what effect penicillin has on the synthesis of the phosphate containing compounds present in these cell walls.

A study of the biosynthesis and chemistry of the phosphate compounds of Staphylococcal cell walls appears to be a reasonable approach to an understanding of the manner in which cell wall synthesis is affected by penicillin.

Proposed Course of Project:

At present organic phosphate containing compounds have been prepared from a penicillin sensitive strain of S. aureus. A study of the chemical nature of these compounds is planned for the immediate future. In the future, it is also planned to study the molecular weight, mode of attachment to cell wall, and pathway of biosynthesis of these phosphate containing compounds. These questions are being pursued in the penicillin sensitive strain of S. aureus now under study. Eventually the properties of the cell walls of these penicillin sensitive organisms will be compared to the properties of the cell walls of penicillin resistant organisms.

Part B included Yes /__/ No /X/

Serial No. NIAID - 28(c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Pathogenesis of Viral Infections

Principal Investigator: Dr. Julius A. Kasel

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.3
Professional: 1.0
Other: 0.3

Project Description:

Objectives:

1. To investigate the prevalence of antigens separable from infectious virus and their significance in relationship to the pathogenesis of virus infection.

2. To study exfoliated cells in the urine, buccal mucosa and blood cells from patients ill with viral infections for the presence of viral antigen by fluorescent microscopy concurrently with virus isolation procedures.

3. To determine sialic acid levels of serum and urine from patients ill with respiratory viruses.

Methods Employed:

Clinical studies: Material from patients with viral infections and non-viral illnesses will be utilized in the projects under study.

Laboratory studies: Tissue culture, chromatography, fluorescent microscopy, serologic and biologic techniques will be utilized.

Patient Material and Major Findings:

1. Recent adenovirus type 1 and 2 isolates and prototype strains 1,2,4 and 15 were found to possess the property of removing

erythrocyte receptor sites possibly by enzymatic action, from human red blood cells for three of five adenovirus hemagglutinins. The antigen responsible for this property is separable from the infectious virus particle and not related to the antigens causing cell detachment and early cytopathogenicity. Studies indicated that the adenovirus factor was a macromolecular substance, heat stable, resistant to proteolytic and nuclease enzymes and was neutralized by homologous but not heterologous serum.

Virus suspensions devoid of infectious virus, cell-detaching and early cytopathic factors exhibited an "inhibiting property" for certain adenovirus serotypes in tissue culture.

2. Viruses sharing antigenic properties of adenovirus types 9 and 15 were isolated during an investigation of an outbreak of febrile illness among children.

In one patient convalescing from rheumatic pancarditis, the viral infection was associated with signs and symptoms interpreted as consistent with re-activation of the rheumatic process.

3. Significant neuraminidase activity was associated with prototype adenovirus type 15 grown in HeLa tissue cultures. Screening of type 15 suspensions were constantly negative for the presence of myxovirus contaminants. The enzyme activity appeared to be associated with the virus particle since suspensions adsorbed with susceptible erythrocytes demonstrated a marked decrease.

Significance to Microbiological Research:

1. The presence of viral antigens other than infectious viral particles in infectious tissue culture fluids raises the question of their significance in disease and immunity. Their role in viral reproduction and possible effect on the human host needs to be defined.

2. Since prolonged excretion in the urine of mumps virus raises the possibility of virus growth in the kidney; a study of exfoliated cells in the urine for presence of virus needs to be studied.

3. Determination of sialic acid levels in the blood and urine of patients ill with viral infections may warrant this biochemical assay as a clinical tool. The further definition of the enzyme-like factor associated with adenoviruses needs to be further explored for reaction products also, for consequent use as a diagnostic test.

Proposed Course of Project:

As outlined in "Objectives and Methods."

Part B included: Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

1. Kasel, J. A., Rowe, W. P. and Nemes, J. C.: Modification of Erythrocyte Receptors by a Factor in Adenovirus Suspensions. Virology, 10: 389-391, 1960.

2. Cramblett, H. G., Kasel, J. A., Langmack, M. and Wilken, F. D.: Illnesses in Children Infected with an Adenovirus Antigenically Related to Types 9 and 15. Pediatrics, 25: 822-828, 1960.

LABORATORY OF CELL BIOLOGY

LABORATORY OF CELL BIOLOGY

SUMMARY

Research Projects from
The Laboratory of Cell Biology
(Serial Nos. NIAID 50-55)

The activities of the Laboratory of Cell Biology during the calendar year 1960 have been along 3 major lines: (A) The continued exploration of the metabolism of normal cultured cells, and an approach to the problem of metabolic controls; (B) the mechanism of viral synthesis; and (C) the study of cell cultures deriving from patients with hereditary metabolic disease.

A.

A number of significant observations have been made with respect to the amino acid metabolism of cell cultures. There has been no further elucidation of the pathway of serine synthesis since last year's report; but the mechanism of cystine synthesis has been clarified, in that all the cell lines so far studied have been shown to use the classical pathway involving the demethylation of methionine to homocysteine, the condensation of the latter with serine to form cystathionine, and the cleavage of the latter to cysteine and homoserine. A dual pathway for proline synthesis has been indicated, one involving glutamine as the source of the carbon skeleton, and the other involving arginine by way of ornithine.

An intriguing recent observation has been the finding that a number of factors which are rigorously required by the cells for survival and growth can in fact be synthesized. Their nutritional requirement reflects the fact that they are lost from the cellular pool to the medium at rates which exceed the biosynthetic capacity of the cell; and with a sufficiently high cell population density, when the loss to the medium per cell is sufficiently reduced, the supposedly essential growth factors are in fact not required for survival.

In these cell cultures, unlike bacteria, the biosynthesis of amino acids is apparently not inhibited by the product of the reaction; and this mechanism of growth control is apparently not operative. Studies are in progress as to whether enzyme repression or feedback inhibition are effective controls in the biosynthesis of pyrimidines. A quite different control mechanism is perhaps indicated by the demonstration of a growth inhibitor in the supernatant medium of heavy cultures. The chemical nature of that inhibitor is under continuing study.

Studies on the mechanism of resistance to 2-deoxyglucose (2DG) have shown the presence in the resistant variants of compounds which inhibit the phosphorylation of 2DG to the metabolically active inhibitor, 2DG-phosphate. The relationship of that inhibitor to the observed resistance is under continuing study.

B.

A number of important new observations have been made with respect to the mechanisms of viral synthesis. The puzzling wide disparity between the number of physical particles in viral suspensions, and the number of plaque-forming units, i.e. particles capable of initiating infection in susceptible cells, has been partially resolved with the demonstration that after the viral particle has been absorbed by the cell, it may undergo several alternative fates. A large proportion are rapidly eluted into the medium, essentially intact but no longer infectious, presumably reflecting a minor alteration in the protein coat. Some particles remain unchanged within the cell. Others are degraded intracellularly, in that the nucleic acid is exposed and becomes susceptible to intracellular ribonuclease. Only a small fraction of the absorbed viral particles are stripped of their protein and initiate infection.

In the case of poliovirus in the HeLa cell, although the viral protein and RNA are synthesized concomitantly, a partial dissociation has been achieved with appropriate inhibitors of protein synthesis, which completely block the formation of mature virus, but not of infectious RNA. This is of particular importance in relation to the supposedly obligatory relationship between protein and RNA synthesis in growing cells. Of interest also is the fact that metabolic inhibitors which effectively block the synthesis of cellular DNA and of DNA viruses have no effect on the formation of poliovirus. It would therefore appear that poliovirus RNA may be used directly as a template for the formation of virus, without the necessity for intervening DNA synthesis.

In contrast to the situation with poliovirus, in the case of vaccinia, there was a marked lag between the formation of the viral nucleic acid (DNA) and that of the mature virus.

C.

An exciting new development has been the successful cultivation from patients with a hereditary metabolic disease (galactosemia) of cells which in culture demonstrate the metabolic defect characteristic of the disease. This suggests an entirely new experimental approach to problems of human genetics.

NIAID 50
Laboratory of Cell Biology
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Biosynthesis in Mammalian Cell Cultures

Principal Investigator: Dr. Harry Eagle

Other Investigators: Dr. Jacob Maizel, Mr. Ralph Fleischman, Mr. Aaron Freeman, Miss Mina Levy and Mr. Vance I. Oyama

Cooperating Units: NIDR, Dr. Karl A. Piez
New York University, Dr. Bernard Horecker

Man Years (calendar year 1960):
Total: 7 1/2
Professional: 5 1/4
Other: 2 1/4

Project Description:

Objectives: This program involves a broad exploration of the nutritional requirements, metabolic activities and biosynthetic pathways of a variety of human and animal cells, under varying conditions of culture.

Methods Employed: The techniques used in the culture and chemical fractionation of these cells, in the separation and identification of their metabolic products, and in the use of isotopically-labeled precursors, have been described in detail in previous publications.

Major Findings:

1. Amino acid biosynthesis and interconversion

a. Metabolic independence of serine and alanine. The so-called "glucose family" of amino acids (alanine, serine and glycine), all of which derive their carbon solely from glucose when cells are grown in a limiting minimal medium, actually fall into two sharply defined groups, between which there is little or no metabolic inter-conversion. Serine is the immediate precursor of glycine; but there is no significant metabolic relationship between alanine and these two amino acids. When cells are provided with C^{14}-labeled serine or glycine, these two amino acids in the cell are heavily labeled, but

Part B included.

protein.
essentially none of the isotope appears in the alanine residues of the cell/ Further, when cells are grown on a medium which contains ribose and pyruvate in lieu of glucose, the serine and glycine derive almost completely from the ribose, while the alanine derives completely from the pyruvate. This finding provides a direct approach to the as yet unknown mechanisms involved in the biosynthesis of serine.

b. Cystine synthesis. It has now been clearly established that all serially propagated human cell lines so far studied are indeed capable of synthesizing cystine from methionine and glucose by the classical homocysteine-cystathionine pathway. The capacity to synthesize cystine is therefore not limited to liver cells, as had previously been presumed to be the case.

c. Proline synthesis. In a minimal medium, the glutamine family of amino acids (aspartic and glutamic acids, asparagine and proline) devolve primarily from glutamine. In the case of proline there has however been a consistent discrepancy, in that only half of the carbon skeleton derived from glutamine, about 10 per cent additionally from glucose, while the rest was unaccounted for. This has now been resolved with the finding that slightly more than 1/3 of the proline carbon derives from arginine by way of ornithine.

d. Negative feedback. There is now an increasing body of evidence that the biosynthetic control mechanisms operative in bacteria, in which the product of a given reaction serves to limit its synthesis, is not operative in mammalian cells, at least with respect to the amino acids. In the examples so far studied the provision of serine, cystine, and homocystine has not served to curtail their continuing biosynthesis.

2. The anamolous requirement by human cells for metabolites which they can synthesize. The interesting observation has been made that cultured mammalian cells frequently require metabolites which they are actually capable of synthesizing, and in amounts which should suffice for growth. This has been found to be the case for cystine, homocystine, serine, inositol, and in the case of certain cell lines, for pyruvate as well. This paradoxical observation has now been resolved, in that the function of the added metabolite is simply to prevent the loss of the newly synthesized compound from the cellular pool to the medium at a rate which exceeds the biosynthetic capacity of the cell. When the population density is sufficiently increased, the exogenous metabolite is no longer required for cell survival and growth. When the task of "conditioning" the medium is then shared by a sufficiently large number of cells, the biosynthetic capacity of an individual cell is no longer exceeded.

3. Chemostat studies. The development of a chemostat for mammalian cell cultures was described in last year's progress report. It has since been learned that when the rate of growth is greatly reduced by appropriately limiting the rate at which fresh medium is introduced into the culture, the factor which then limits the growth of the cells is not the depletion of

medium, but instead, the elaboration of an active growth inhibitor. This growth inhibitor is not dialyzable and is presumably associated with the proteins of the medium. Attempts at its isolation and characterization are now in progress.

4. Protein growth factor. Most human and animal cells in serial culture have an absolute requirement for serum protein. That protein however is not used to a significant degree for the synthesis of cell protein, but instead has a dual role: it promotes the adhesion of cells to glass surface, and provides essential and as yet undefined growth factors. The active factor has proved to be, not the protein molecule as such, but diffusible compounds of small molecular weight which are formed ~~from it~~ on its proteolytic digestion. It is not yet clear whether those growth factors are peptides, or compounds ~~of small molecular weight~~, initially bound to the protein and released from it as it is digested. In either case, the dialysate of such a digested protein preparation supports the indefinite growth of a wide variety of human cells in suspension culture. Studies on the identification of the active compounds in such dialysates are now in progress.

5. The utilization of inositol phosphates. Dr. S. J. Angyal of the University of New South Wales, Australia, has prepared all the inositol phosphate isomers. All have proved to be equally active in supporting the growth of human cells. It is not yet clear whether the phosphates are taken into the cell directly, or whether they must first be hydrolyzed extracellularly.

Significance to the Program of the Institute: Studies on the metabolic activities of human and animal cells are of obvious basic significance. For NIAID research specifically, these studies are relevant to the problems of antibody production, allergy, phagocytosis, inflammation, virus propagation, and many other problems which are the concern of the microbiologist interested in the study of human disease.

Proposed Course of Program: It is clear from the foregoing that none of these are finished projects. The long-range program envisages a similar broad-scale study on the metabolism of tissue explants. This will have the multiple purpose of pin-pointing possible differences between the metabolism of dispersed and structurally organized cells, exploring the conditions necessary for the preservation of specialized functions in vitro, and following the chromosomal aberrations as the cells multiply under varying conditions of growth.

PHS-NIH
Individual Project Report
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Eagle, H. Nutritional Requirements for Cell Growth and Poliovirus Propagation. Perspectives in Virology 75-87. John Wiley & Sons, Inc., N. Y. 1959.

Eagle, H. Metabolic Studies with Normal and Malignant Human Cells in Culture. The Harvey Lectures, 1958-1959. Academic Press, Inc., N. Y. 156-175, 1960.

Eagle, H. The Sustained Growth of Human and Animal Cells in a Protein-Free Environment. Proc. Natl. Acad. Sci. 46: 427-432, April 1960.

Eagle, H. and Piez, K. A. The Utilization of Proteins by Cultured Human Cells. J. Biol. Chem. 235: 1095-1097, April 1960.

Eagle, H., Oyama, V. I. and Piez, K. A. The Reversible Binding of Half-cystine Residues to Serum Protein, and Its Bearing on the Cystine Requirement of Cultured Mammalian Cells. J. Biol. Chem. 235: 1719-1726, June 1960.

Eagle, H., Agranoff, B. W. and Snell, E. E. The Biosynthesis of meso-Inositol by Cultured Mammalian Cells, and the Parabiotic Growth of Inositol-dependent and Inositol-independent Strains. J. Biol. Chem. 235: 1891-1893, July 1960.

Piez, K. A., Levintow, L., Oyama, V. I. and Eagle, H. Proteolysis in Stored Serum and its Possible Significance in Cell Culture. Nature 188: 59-60, October 1960.

Darnell, J. E., Jr. and Eagle, H. The Biosynthesis of Poliovirus in Cell Cultures. Advances in Virus Research. In press.

Cohen, E. P., Nylen, M. U. and Scott, D. B. Microstructural Changes Induced in Mammalian Cell Cultures by Omission and Replenishment of a Single Essential Amino Acid. Exptl. Cell Research. In press.

Cohen, E. P. and Eagle, H. A Simplified Mammalian Cell Chemostat. J. Exptl. Med. In press.

Levintow, L. and Eagle, H. The Biochemistry of Cultured Mammalian Cells. The Annual Review of Biochemistry. In press.

Eagle, H., Piez, K. A. and Oyama, V. I. The Biosynthesis of Cystine and Mammalian Cell Culture. In preparation.

Honors and Awards relating to this project:

Invited Lectures

January 22-26, 1960: Perspectives in Virology. New York City.

February 9, 1960: NIH Lecture Series. Clinical Center.

March 24, 1960: Bristol Laboratories. Syracuse, New York.

March 25-26, 1960: Symposium on the Phenomena of Tumor Viruses. N.Y.C. Sponsored by The Virology and Rickettsiology Study Section, DRG-NIH.

June 20-22, 1960: Kingston, Rhode Island. University of Rhode Island. Symposium: Newer Chemical and Biological Techniques of the 1960 Medicinal Chemistry Section of the American Chemical Society.

July 5, 1960: Seminar, Research Division of Ethicon. Somerville, N. J.

August 9, 1960: Long Island Biological Labs., Cold Spring Harbor, N. Y.

September 12, 1960: American Chemical Society, New York City.

September 28, 1960: Brandeis University seminar, Waltham, Massachusetts.

November 4, 1960: University of Delaware seminar, Wilmington, Delaware.

November 5, 1960: Eastern Psychiatric Research Assoc. symposium. N. Y. C.

November 13-15, 1960: American Cancer Society Lecturer, Purdue University, Lafayette, Indiana.

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Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Mechanism of Production of Poliovirus in Cultured Mammalian Cells

Principal Investigator: Dr. Leon Levintow

Other Investigators: Dr. James E. Darnell, Jr., Dr. W. K. Joklik, Dr. Jacob V. Maizel, Mrs. Marilyn M. Thoren and Mr. J. Leonard Hooper

Cooperating Units: None

Man Years (Calendar year 1960)
Total: 7 1/4
Professional: 2 1/4
Other: 5

Project Description:

Objectives: To define the events during the adsorption and maturation of poliovirus in biochemical terms, and to investigate the mode of replication of viral protein and RNA.

Methods Employed: The program is based primarily on the following techniques: a) Infection of cells with poliovirus under controlled conditions in a chemically defined medium; b) Purification of the poliovirus so produced; c) Preparation of infectious RNA from infected cultures, and from purified virus; d) Assays of the infectivity of whole virus and infectious RNA by a plaque assay on HeLa cell monolayers.

Additional techniques include paper and column chromatography, preparative and analytical ultrcentrifugation, and, in collaboration with other groups, electron microscopy.

Major Findings: The events early in the infective cycle have been studied with purified poliovirus labeled with P^{32}. Virtually all virus particles are capable of being adsorbed to HeLa cells; once adsorbed, a particle has several alternative fates. It may: 1) be eluted into the medium, essentially intact but no longer infectious; 2) be degraded intracellularly so that the P^{32} label becomes acid-soluble; 3) be retained essentially unchanged within the cell; or 4) be stripped of its protein coat and initiate infection.

Part B included.

These observations provide an explanation for the low ratio of infectious: physical particles characteristic of poliovirus.

Following adsorption of virus to cells, there is a latent period of 2-2 1/2 hours during which there is appreciable synthesis of viral material. The synthesis of viral RNA and protein then begin simultaneously, and mature virus begins to appear shortly thereafter. Unlike the situation in most other viral systems, there is at no time a sizable pool of unassembled viral macromolecules. The synthesis of viral protein and RNA appears to be more closely coordinated than in any viral system heretofore studied. This coupling of RNA and protein synthesis is not obligatory, however; for in the presence of p-fluorophenylalanine, an inhibitor of protein synthesis, the synthesis of viral RNA is initiated at the usual time, but protein and mature virus are not formed. When the inhibition of protein synthesis is reversed by the subsequent addition of phenylalanine, the synthesis of viral protein begins, shortly followed by the appearance of mature virus. These observations imply that the synthesis of viral RNA is not dependent on concurrent protein synthesis.

Significance to the Program of the Institute: Virus replication provides a useful model of a rapidly reproducing unit at the cellular and subcellular level in which protein and nucleic acid synthesis can be studied chemically and biologically. Such studies not only provide insights into the mode of the replication of protein and nucleic acid molecules, but have an obvious and important bearing on the evolution of viral disease at both the cellular and whole animal level.

Proposed Course of the Project:

1. The procedure for the purification of poliovirus is being adapted to provide the larger quantities of pure virus necessary for the physico-chemical characterization of the protein subunit.

2. Further investigation will be made of the fate of the infecting viral particle, a problem complicated by the large number of ineffective virus particles.

3. The reasons for the delay between adsorption and the initiation of virus replication will be investigated.

4. Further studies will be carried out on the chemical, physical and biological properties of the infectious RNA extracted from purified virus.

5. Experiments on the relationships between the synthesis of viral protein and RNA synthesis will be continued, using appropriate metabolic antagonists.

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Levintow, L. and Darnell, J. E., Jr. A Simplified Procedure for Purification of Large Amounts of Poliovirus: Characterization and Amino Acid Analysis of Type 1 Poliovirus. J. Biol. Chem. 235: 70, 1960.

Darnell, J. E., Jr. and Levintow, L. Poliovirus Protein: Source of Amino Acids and Time Course of Synthesis. J. Biol. Chem. 235: 74, 1960.

Darnell, J. E., Jr., Levintow, L., Thoren, M. M. and Hooper, J. L. The Time Course of Synthesis of Poliovirus RNA. Virology. In press.

Joklik, W. K. and Darnell, J. E., Jr. The Adsorption and Early Fate of Purified Poliovirus in HeLa Cells. Virology. In press.

Darnell, J. E., Jr. and Eagle, H. The Biosynthesis of Poliovirus in Cell Cultures. Advances in Virus Research. In press.

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Laboratory of Cell Biology
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Effects of Animal Viruses on the Metabolism of Mammalian Cell Cultures and the Mechanism of Viral Replication

Principle Investigator: Dr. Norman P. Salzman

Other Investigators: Mr. Edwin D. Sebring, Dr. William Munyon and Dr. Chiaki Nishimura

Cooperating Units: None

Man Years (calendar year 1960):
Total: 3 3/4
Professional: 1
Other: 2 3/4

Project Description:

Objectives: To determine the alterations in cell metabolism resulting from infection with poliovirus and vaccinia virus, and to study the primary site of action of the virus; to determine the time course of synthesis of the component parts of vaccinia virus (nucleic acid and protein) as related to the formation of infectious virus, and to study the effect of inhibitors on these synthetic processes.

Methods Employed: The techniques employed involve cell fractionation, isolation of the purified component nucleic acid bases, the use of radioactive precursors, the various techniques required for the viral infection of cells, the plaque assay for measurement of infectious virus, and chemical methods of virus purification.

Major Findings: The findings are in three separate areas.

1. Interrelation between cellular RNA, DNA and protein. Using a specific inhibitor of DNA synthesis, 5-fluorodeoxyuridine, the presence of two distinct classes of RNA and protein have been demonstrated in animal cells. When DNA synthesis is inhibited, nuclear RNA and nuclear protein synthesis are also inhibited; under these conditions however, cytoplasmic protein and RNA synthesis continue at optimal rates. Because of the linkage of nuclear RNA and protein synthesis to DNA synthesis, certain species of protein will be synthesized only in growing cells, for under non-growing conditions, there is no increase in DNA. This may be a fundamental mechanism for the control of the levels of various enzymes in mammalian cells.

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Part B included.

2. Incorporation of nucleic acid analogues into poliovirus. Our previous studies established that the presence of 5-fluorouracil or its deoxyriboside in the medium did not affect the de novo synthesis of poliovirus. Since poliovirus synthesis occurred under conditions where no DNA synthesis is possible, it seems likely that poliovirus RNA is used directly as the template for the synthesis of poliovirus protein. Changes in the virus RNA template might then be expected to produce changes in the virus protein. Thus far an altered template has been produced by the incorporation of 5-fluorouracil into virus RNA. The degree of incorporation of 5-fluorouracil is concentration dependent. Even when 20 per cent of the uracil in viral RNA has been replaced by 5-fluorouracil, the resultant virus is fully infectious. Further studies on the properties of fluorouracil-containing poliovirus are planned.

3. Vaccinia virus formation. The kinetics of formation of vaccinia virus DNA and of infectious virus was previously determined. The synthesis of viral DNA was almost complete prior to the formation of any new infectious virus. It has now been shown that the viral protein is synthesized in the time between DNA formation and the appearance of infectious virus.

Significance to the Program of the Institute: The effect of viruses on animal cells, the manner in which viruses replicate, and the effect of inhibitors on these processes is of obvious relevance to an understanding of viral infection, and may conceivably suggest new areas of exploration in relation to viral chemotherapy.

Proposed Course of Projects

1. The properties of a limited number of enzymes involved in cellular metabolism will be examined to determine if they are associated with the nucleus or the cytoplasm. The importance of these findings as a basic mechanism in growth regulation will be evaluated.

2. By paper electrophoresis of the RNA nucleotides of purified poliovirus, it will be determined if labeled fluorouracil is incorporated as the nucleotide. The percent replacement of uracil by fluorouracil will be measured by the amount of radioactive precursor incorporated and by spectrophotometric determinations of the eluted nucleotides. The chromatographic analysis of the amino acid residues of both fluorouracil-substituted and control viral protein is planned. Other possible effects of fluorouracil incorporation into poliovirus will be studied, such as altered UV inactivation, heat inactivation, specific infectivity, and possible changes in the one step growth curve.

3. Procedures for vaccinia virus purification from cell cultures are being studied. The various protein components of the virus will be purified and used to prepare specific precipitating antisera. These will be used in studies on the kinetics of viral protein formation.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Salzman, N. P. The Rate of Formation of Vaccinia Deoxyribose Nucleic Acid and Vaccinia Virus. Virology, 10: 150 (1960).

Salzman, N. P. and Sebring, E. D. The Source of Poliovirus Ribonucleic Acid. Virology. In press.

Honors and Awards relating to this project: None.

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Laboratory of Cell Biology
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Mechanism of Drug Resistance in Cultured Mammalian Cells

Principal Investigator: Dr. Stanley Barban

Other Investigators: Mr. Henry O. Schulze

Cooperating Units: None

Man Years (calendar year 1960)
Total: 2 3/4
Professional: 1
Other: 1 3/4

Project Description:

Objectives: To study the mechanism of resistance to a glucose analogue, 2-deoxyglucose (2DG), by a resistant strain of human cells in culture.

Methods Employed: By the use of cloning procedures, a strain of HeLa cells has been isolated which can grow in the presence of high concentrations of 2DG. Other techniques have included procedures for the separation and identification of the carbohydrates and their metabolic products, preparation of enzymatic extracts of drug-sensitive and drug-resistant strains, and the use of isotopically labeled precursors. All these procedures have been described in detail in previous publications relating to this project.

Major Findings: A strain of HeLa cells has been developed which can grow in the presence of a molar ratio of 2DG:glucose of 10:1, while the parent strain is completely inhibited at equimolar concentrations of the inhibitor:glucose. The resistant cells grow at approximately one-half the rate of the parent strain, and the addition of pyruvate to the basal medium is essential for its continued propagation.

2DG is rapidly metabolized by HeLa cells to its phosphorylated intermediate, 2-deoxyglucose-6-phosphate. There is a significant difference

Part B included.

in the metabolism of both compounds by the sensitive and resistant strains, in that the rate of 2DG metabolism in the resistant cells was markedly reduced as much as 5-fold. In cell extracts there was a corresponding marked depression of the enzyme which phosphorylates 2DG, as well as the enzyme which phosphorylates glucose, fructose and mannose. The decreased kinase activity for 2DG in cells resistant to this compound may well be the primary basis of their resistance.

It has also been found that an extract of resistant cells inhibits the enzymatic activity of a sensitive extract, suggesting that the resistant extracts contain an active inhibitor of hexose phosphorylation.

Significance to the Program of the Institute: The mechanism of the development of resistance to a glucose analogue in HeLa cells may serve as a model for the development of drug resistance. This is relevant to the general problem of chemotherapy, not only of infectious diseases, but of other disease areas (e.g. cancer, heart disease, etc.).

Proposed Course of Project:

1. Attempts will be made to isolate and characterize the nature of the metabolic inhibitor in resistant extracts.

2. The enzymatic differences between drug-resistant and -resistant strains will be further explored.

3. The nutritional requirements of normal and resistant variants will be explored, with particular reference to the essential role of pyruvate in the growth of resistant cells.

4. Attempts will be made to transform 2DG-sensitive cells to drug resistance, using the genetic material of the 2DG-resistant cells.

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project: None

Honors and Awards relating to this project: Member, Society of Biological Chemists.

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Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Metabolic Control Mechanisms in Cultured Mammalian Cells

Principal Investigator: Dr. Jacob V. Maizel, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objective: To study the intracellular mechanisms for the control of metabolic activities in mammalian cell cultures.

Methods Employed: Enzymatic assays by modifications of techniques employed in other systems.

Major Findings:

1. Phosphatases.

In Escherichia coli alkaline phosphatase is a repressible enzyme, the synthesis of which is affected by the level of orthophosphate in the medium. In mammalian cells also the level of alkaline phosphatase was found to be variable, in that cultures grown as monolayers attached to glass regularly had at least ten times as much alkaline phosphatase activity as suspension cultures. However, the high activity of monolayer culture was not decreased at elevated phosphate concentrations, nor was the low level in suspension cultures increased by growth at low phosphate concentrations. When suspension cultures were planted as monolayers, the alkaline phosphatase levels increased ten-fold in less than two days, independent of the concentrations of phosphate or calcium, or population density. These findings suggest a control mechanism unlike that of bacterial systems.

The phenomenon cannot be explained as the result of a generalized loss of enzymes from cells in suspension, since the levels of acid phosphatase

and aspartate transcarbamylase were the same in suspension and monolayer cultures.

II. Control of pyrimidine synthesis.

Aspartate transcarbamylase is involved in the biosynthesis of pyrimidines in a number of systems, and in Escherichia coli is involved in the control of pyrimidine synthesis by exogenously supplied pyrimidine. The enzyme was found in extracts of HeLa cells and equally in both monolayer and suspension cultures. Again unlike bateria, the enzyme level was unaffected by the addition of pyrimidines to the medium.

Proposed Course of Program:

a. The question as to whether the product controls pyrimidine biosynthesis will be approached by using appropriate radioisotope precursors in the presence and absence of added preformed pyrimidines.

b. The enzymatic steps in pyrimidine synthesis remain to be elucidated.

c. Factors of possible importance in the regulation of alkaline phosphatase activity remain to be investigated.

Knowledge of the control of protein synthesis is of fundamental significance to studies of antibody production and virus propagation.

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PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Application of the Technique of Tissue Culture to Selected Genetics Diseases

Principal Investigator: Dr. Robert S. Krooth

Other Investigators: Miss Mary Jane Madden

Cooperating Units: NIAMD, Dr. Arnold W. Weinberg, and Dr.J. H. Tjio
NINDB, Dr. Paul Altrocci

Man Years (calendar year 1960):
Total: 3 3/4
Professional: 1
Other: 2 3/4

Project Description:

Studies to follow a number of genetic diseases in tissue culture are being continued. Biopsies are performed on affected patients, and cell lines are then developed from the excised tissue. Evidence bearing on the persistence of the defect in cell lines from affected persons (compared with cell lines from controls) is obtained. Ultimately, mutant cell lines from patients with inborn metabolic errors should prove useful in the study of genetic exchange among human cells as well as in other aspects. The system permits one to study mutant human cells in a way which hitherto could be applied only to mutants of bacteria and fungi. However, the number of genetic diseases which are susceptible to this approach is at present quite limited.

Galactosemia has been shown to persist as a defect in culture, even after 6 months of propagation and an increase in cell number known to exceed 30 billion-fold. The defect can be shown by growth studies, the ability of the cells to metabolize galactose-1-C^{14}, and direct enzymatic assays. The galactosemic cells show a pattern of galactose sensitivity similar to the transferaseless mutants of E. coli. Corresponding data have been obtained for one heterozygote, and here also the defect persists.

Part B included.

Wilson's disease and hypocatalasemia are also under study by this approach.

Our efforts to follow Gaucher's disease and certain other histiocytic states in culture (referred to in the last report) have not yet succeeded. Indefinite propagation of recognizably affected cells has not been achieved although the cells will attach to glass, spread, and appear to increase in number for a few weeks. Thereafter, though the cells can be made to survive for months, no evidence of growth is found.

A search is being made in our laboratory, as in others, for new chromosomal abnormalities in human disease. Cell lines have been developed from biopsies on 5 patients with congenital malformations, in each case of a kind not previously reported. In every instance thus far the chromosomes have been normal in morphology and number.

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Krooth, R. S., and Weinberg, A. (1960) Properties of Galactosemic Cells in Culture. Biochem. Biophys. Research Comm., in press.

Honors and Awards relating to this project: None.

LABORATORY OF INFECTIOUS DISEASES

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1960

Administrative Office - Edward F. Zadai

Introduction

The Administration of the Laboratory of Infectious Diseases is responsible for furnishing all services, except the actual conduct of scientific research, required for the numerous scientific investigation programs of the 4 sections. In fulfilling a mission of conducting integrated laboratory-scientific research, investigators must be provided with individual personal attention, an earnest interest in their work, and all the administrative support possible. This responsibility requires continuous evaluation of activities in all of the Laboratory of Infectious Diseases supplying the multiple services needed both directly and indirectly.

Personnel

During the past 3 months we have been fortunate in almost eliminating the problem of carrying vacant positions in our laboratory. We have added two professional members to our staff (Dr. Shug - from the Veterans Administration and Dr. Huff - from the Laboratory of Clinical Investigation, NIAID). We have further employed two additional Medical Biology Technicians and 2 more Laboratory Animal Caretakers, leaving only two nonprofessional vacancies in the entire laboratory which we hope to fill before the completion of this calendar year. Seventeen employees have been promoted during the year with three promotions still pending final action.

Physical Changes

This past year has seen the completion of planning and the beginning of construction of 3 major renovations in designing newly assigned laboratory space. Rooms 333 and 334 in Building 7 will be converted from animal rooms into laboratories with work scheduled for completion March 5, 1961. Also additional laboratory space will be acquired and renovated in Room 313 for use by a collaborative research study with the National Institute of Neurological Diseases and Blindness. Lastly Rooms 204 and 206 in Building 5 have been vacated by the Division of Biologics Standards and will be renovated for use by our Medical and Physiological Bacteriology Section.

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Scientific Exchange

An exchange of scientific personnel at the international level was carried out during the year by sending one of our scientists to Paris, France, to participate in research studies on auto-immunity in viral infections and related virus research. In turn we received Dr. Dietrick Falke, Senior Research Assistant in virus research from the Hygiene Institute, Marburg, Germany. Dr. Falke is presently spending a one year stay with us as a guest worker studying tumor-inducing viruses.

Training

Courses in outside training under sponsorship of NIH were completed by eight professional employees and it is certainly anticipated that this will bring promising results in our scientific projects.

Problems

The ever present scarcity of good personnel and adequate space has certainly affected our progress. Many paths toward completion of good scientific experiments have been narrowed or blocked due to the lack of facilities. This Administrative Office has just completed preliminary negotiations in purchasing two house trailers to be used as a field laboratory. In this manner we will be able to continue progress on a virus study that would have become useless without a means of isolating the mice necessary for experiments.

Research Associates

In the role of a leading research facility, the Laboratory of Infectious Diseases also conducts formal advanced training for young scientists in certain specialties. The Research Associate program provides exposure to techniques and methodology in microbiologic research. In 1960 there were several applicants from leading universities and hospitals in this country for these type positions which we were forced to reject. It would be of great value if we could expand our facilities to accept more of these applications because recognition of the quality of experience in our scientific programs is a significant factor in the recruitment and retention of staff members.

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Conclusion

The Laboratory of Infectious Diseases has earned the reputation of a progressive organization in the field of infectious disease research. There have been many problems in retaining extremely competent professional, technical, and administrative personnel. We are fortunate in having a devoted highly productive and intelligent senior staff made up of internationally known young workers with the large part of their careers still before them. Results of significant value in the conquest of disease have been achieved, and it is the desire of the Administrative Office to meet the challenge of the future with earnest and sincere efforts. The Administrative Office is fully aware of the job which lies ahead of us if we are to support rather than contain our scientists. We feel very strongly that the time is growing near when we will have to expand our facilities if we wish to continue maximum progress.

Below is a highlight summarization of some of the honors awarded LID scientists during the past year:

Dr. Chester W. Emmons

Elected President, Mycological Society of America
Elected Councilor-at-Large, Society of American Bacteriologists
Chairman, Second Conference on Medical Mycology, N.Y. Acad. Sci.
Appointed Board Member of Amer. Acad. of Micro. (3 year term)
Chairman, Standards and Examination Committee, Amer. Acad. Micro.
Convenor of Mycological Symposium, III National Congress of Microbiology, Mexico
Honorary mention for exhibit on Chemotherapy of Systemic Mycoses, AMA meeting in Florida
Appointed to WHO Expert Advisory Panel on Parasitic Diseases (5 year term)
Member of Committee on National Index of Fungus Cultures, Quartermaster Research and Development, National Academy-Research Council (3 year term)

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Dr. Robert J. Huebner

Elected to National Academy of Sciences
Appointed to Enterovirus and Adenovirus Committees of NIAID, NCI
Invited to give several honorary lectures, including the 1960 Harvey Lecture

Dr. Wallace P. Rowe

Awarded Eli Lilly Award at the Annual Meeting of the American Society of Bacteriologists
Appointed to Research Advisory Committee on Etiology of Cancer of the American Cancer Society

Dr. Robert M. Chanock

Elected to Society for Clinical Investigation
Full membership to the ARD Commission of the AFEB

Dr. Leon Rosen

Appointed to the Enterovirus Committee, NCI
Received a letter from the Governor General of French Polynesia to the Surgeon General of the United States Public Health Service citing him for his research on viruses and eosinophilic meningitis in French Polynesia

Dr. Arthur K. Saz

Chairman, President's Fellowship Committee, Society of American Bacteriologists

Dr. Roy Repaske

Elected to Membership, American Society of Biological Chemists

Dr. Arthur L. Schade

Invited to following European universities to deliver lectures: Vienna, Marburg and Geissen, Saar, Freiburg, Berne, Zurich, Tubingen, Frankfurt, Berlin, Lund

Dr. Robert C. Woodworth

Awarded NIH Fellowship)NHI) to spend a year at Lund University, Malmo, Sweden

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Virus and Epidemiology Sections

1.0 Introduction

In 1960 the Virus and Rickettsial and Epidemiology Sections continued integrated and comprehensive efforts to define the importance of virus infections in disease. Field investigations of human and animal virus infections were made possible through collaboration with a number of other organizations, including the Bureau of Medicine, USN; the District of Columbia Children's Hospital Research Foundation; the District of Columbia Welfare Department; the New York City Health Department; the National Cancer Institute; the National Institute of Allergy and Infectious Diseases; the Laboratory of Clinical Investigations, NIAID; and in Paris, France the Laboratoire des Virus, Hopital Saint-Vincent-de-Paul; and Le Centre Claude-Bernard de l'Hopital Saint Louis.

Natural events and opportunities afforded by our collaborators shaped the course of most of our field studies. Technical breakthroughs in the laboratory made it possible for us to take fuller advantage of these opportunities to study natural disease and thus acquire not only new information about specific virus infections, but also to move nearer our ultimate goal, namely, a clear view of the numerous viral causes of human diseases which is also sufficiently comprehensive to make concerted efforts to control them appear feasible and worthwhile.

In the long run, a research organization stands or falls on its published work, so that if we have anything deserving attention it is in the content of our scientific reports. Nearly 50 manuscripts were published or accepted for publication and 10 more papers are either in the Editorial Board or about to be submitted. It is not surprising therefore that even a summary makes for a rather long document. However, it may help if I "highlight" certain specific findings which while not necessarily more important have a more immediate bearing on disease, and merely mention others.

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2.0 Respiratory Virus Disease Studies

2.1 New Causes of Virus Pneumonia

Pneumonia and other lower respiratory tract infections continue to represent major causes of death and a large segment, presumed to be viral in origin, is still uncontrolled. Until recently it was wholly undefined. During 1958 and 1959 our studies at Children's Hospital and Junior Village helped define the relative importance of adenoviruses, para-influenza viruses, and influenza viruses in causing lower respiratory illnesses of childhood. The data suggested that as much as 40 per cent of croup bronchiolitis and pneumonia were explained by these viruses. In 1960, using more sensitive methods, we were able to explain a much larger percentage of such illnesses, chiefly because we were now able to assess the very significant contributions of respiratory syncytial virus (RS) to the respiratory disease problem. Early in the year large outbreaks of RS virus were intensively studied both at Children's Hospital and Junior Village. Over 80 strains of RS virus were isolated from children with pneumonia and 60 per cent with bronchiolitis yielded RS virus, whereas virus was recovered from less than one per cent of comparable control patients without respiratory illness.

Retrospective analysis of serologic surveys of respiratory illnesses in Children's Hospital since 1957 suggested that perhaps 20 per cent of all lower respiratory illnesses observed during the last three years was due to RS virus. Thus, considering the contributions of adenoviruses, para-influenza viruses, influenza viruses, and "PAP" virus it now appears that 50 to 60 per cent of the more severe respiratory illnesses of young children can now be explained and, hopefully, controlled. Except for influenza virus (which contributed probably less than 5 per cent of the total), the LID respiratory virus unit personnel played key roles in the discovery of the first representatives of each of the other virus groups - adenovirus, para-influenza, and RS. Delineation of still undefined viral causes of the respiratory disease syndrome represents the major challenge to respiratory disease investigators for 1961.

A good start toward meeting this challenge has already been made. Preliminary analysis of nearly 1800 enterovirus infections observed in our childhood study populations showed that certain of them such as Coxsackie B 3, poliovirus 2, and ECHO 3 virus were responsible for febrile respiratory illnesses. From recent results obtained by other workers, and previously by our own group, it is likely that newly recognized but extremely common enteroviruses closely related to the Coxsackie A virus, will be found to contribute a significant proportion of still unexplained respiratory illnesses.

2.2 Respiratory Virus Vaccines

The fact that a very significant proportion of the more severe respiratory illnesses occuring in childhood can now be explained makes the development of effective preventive vaccines against the known agents a worthwhile undertaking. The viruses involved (see above) are antigenic and, although reinfection can occur, evidence indicates that specific antibodies provide significant protection against the more severe manifestations of these viruses during subsequent infections. Unfortunately, progress towards the development of the complex vaccines containing many different viruses can be expected to be very slow unless more interest and attention can be focused on the public health importance of such efforts.

Unlike poliomyelitis and other dread and dramatic illnesses, common respiratory disease, although responsible for much more illness and possibly more deaths as well, do not have national foundations devoted to their control or eradication. The relative lack of large scale goal-oriented research activities contrasts vividly with the dimensions and technical needs of contemporary respiratory virus disease research; both exceeding by several magnitudes that of poliomyelitis.

During 1960 several experimental but commercially prepared killed vaccines containing various combinations of adenoviruses (6 types), para-influenza viruses (3 types), and Coxsackie B viruses (5 types) were tested in Junior Village. The evidence suggests that while modestly antigenic, the vaccines had insufficient potency to be regarded as satisfactory for larger scale studies. Additional studies of vaccines are planned for 1961, but only at a pilot study level, since the space and personnel available to our Virus and Epidemiological groups is scarcely sufficient to continue our laboratory and field studies of the viruses as causes of respiratory disease.

2.3 Pneumonia in Adults

The etiologic role of PAP (Eaton's virus) in primary atypical pneumonia suggested earlier by Eaton and Liu and explored by us in 1959, was finally fully established in 1960. In cooperation with the Bureau of Medicine, USN, the continuing "epidemic" of virus pneumonia in Marine recruits at Parris Island was studied in several ways. Serological studies showed that 51 per cent of 530 pneumonia cases had antibody rises to PAP virus; only 6 per cent revealed contemporary infection with adenoviruses. Serologic studies of infection showed PAP virus to be much more common than disease; approximately 30 recruits were infected for each case of pneumonia, information vitally important to fuller comprehension of the natural history of this important virus.

2.4 Treatment of Primary Atypical Pneumonia (PAP)

In 1959 treatment of Parris Island pneumonia cases with broad spectrum antibiotics (tetracyclines) appeared to reduce the severity and the duration of the Eaton pneumonias. In 1960 the efficacy of a new tetracycline drug, demethylchlortetracycline, was tested in a well-controlled double blind study including 290 pneumonia patients. The drug greatly reduced the severity and duration of pneumonitis and fever in those shown to have serologic responses to PAP virus. These findings, based on accurate laboratory diagnosis, fully confirm earlier but controversial reports of the efficacy of tetracyclines in atypical pneumonias. It also adds further support to the importance of the Eaton virus as a cause of virus pneumonia.

An additional link in the chain of evidence establishing the PAP virus as an important cause of pneumonia was achieved recently in collaborative studies with the Laboratory of Clinical Investigations, NIAID. Volunteers inoculated intranasally with PAP virus grown in tissue cultures reacted with a wide gamut of respiratory signs and symptoms, including pneumonitis characteristic of PAP.

2.5 Common Colds and Other Mild Respiratory Illnesses in Adults

Recent studies have served to clarify and enlarge existing concepts of the etiology of common mild respiratory illnesses in adults. It is now quite clear that instead of a few specific closely related viruses, numerous viruses belonging to different groups each contribute in part to the syndrome called the "common cold." Thus the newer viruses (adenoviruses, para-influenza viruses, respiratory syncytial virus and others), together with older agents (influenza viruses and certain bacteria), each contribute only a small proportion of the milder respiratory ailments of adults. They contribute a larger segment of more serious diseases, particularly in children. Very recent reports of common cold viruses from England, together with the prior reports of agents with somewhat similar properties in this country, served to focus our attention on these viruses in 1960. Together with investigators elsewhere, it was found that most, if not all, of these agents - the British HGP and FEB, the American 2060, JH, Coe and PETT viruses which grow selectively and rather "fussily" in human epithelial cell lines, really represent "fastidious" enterovirus strains which have (as do almost all Coxsackie A's and some ECHO viruses) special growth requirements. These viruses, as do a number of still unclassified agents found in Junior Village during the past several years, have properties very similar to the Coxsackie A viruses; indeed, several have been shown, on the basis of serologic markers and/or by suckling mouse pathogenicity, to be indistinguishable from Coxsackie A viruses.

Enteroviruses as Specific Causes of Mild Respiratory Disease

In previous years we have reported enteroviruses in relation to respiratory illnesses in Junior Village; JVI virus (now ECHO 20) was one such case in point.

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ECHO 28 (2060-JH Viruses)

Several years ago investigators at Great Lakes Naval Training Station and Johns Hopkins reported viruses called respectively "2060" and "JH" in association with mild respiratory illness. Subsequently these agents were classified as ECHO 28 in the enterovirus family. During 1960 our Virus Section developed a complement fixation test for this virus as well as for most of the 58 known enteroviruses. Serologic surveys of respiratory illnesses have revealed high prevalence of antibodies, but thus far rises in titer in relation to illness have been rare.

ECHO 3

During 1960 an outbreak of ECHO 3 virus infections in Junior Village was analyzed in relation to contemporary illness. The study showed a temporal relation to mild respiratory illness attended by brief febrile responses. A report is in preparation.

Coe Virus and Other New Viruses in Military Personnel

During 1960, in cooperation with the Bureau of Medicine and Surgery, USN, studies of mild respiratory illness in military recruits were conducted at Camp Lejeune, North Carolina. Initial findings suggested that new para-influenza viruses and respiratory syncytial virus, in addition to adenoviruses, were contributing in part to the syndrome. Although the clinical importance of these latter agents must still be determined, the fact that they are encountered in adults is of considerable interest.

However, more recently (since October 1960) we have observed large outbreaks of Coe virus - over 70 strains of virus were isolated from as many cases of mild respiratory illnesses. Although identical serologically with the prototype Coe virus, these new strains exhibit a hemagglutinin not previously reported. These strains furthermore produce effects in suckling mice which are indistinguishable from those produced by Coxsackie A viruses. Thus one more "new" virus, at first regarded as wholly unique, is now found with further study to belong to a well established virus family - namely the Coxsackie viruses.

At the same time and in the same recruit population numerous isolations of the so-called "fussy" enteroviruses also have been recovered from persons with respiratory illness, using the special techniques described last year by British investigators. The role of these newer enteroviruses in causing illness are now under study.

Future Studies of Colds due to Enteroviruses

We are making arrangements with the Bureau of Medicine and Surgery, USN, not only to continue but to extend our studies of the enteroviruses as causes of respiratory illnesses in the large military population at Camp Lejeune. We hope to include observations on the numerous dependents and on permanent cadre personnel as well. Thus our studies of enterovirus-caused respiratory illnesses will cover experiences in children as well as adults, and will include all seasons of the year.

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3.0 Virus Diagnostic Reagents and Their Public Health Importance

The public health importance of the more than 100 newly recognized viruses which commonly infect man is now beginning to be appreciated. However, only a handful of public health laboratories are currently making systematic attempts to diagnose viral infections and even these are limiting their surveillance to a pitifully small group of viruses, including usually no more than 3 polioviruses, 2 influenza viruses, and several viral zoonoses. This involuntary indifference to man's largest morbidity problem is largely due to the fact that they lack the necessary viral reagents and of course personnel trained to use them. Realizing that control of respiratory diseases must remain an unattainable objective unless and until acute respiratory diseases (see 1957-58 National Health Survey) can eventually be defined as a public health problem, we have made the development of simple and reliable virus diagnostic procedures a major part of our research program.

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3.1 New Serological Test Procedures

The laboratory section of the Epidemiology Section therefore concentrated on the development, application and evaluation of *in vitro* test procedures for the identification of new viruses as well as for detecting virus infection as expressed in antibody responses. Thus, using conventional complement fixation (CF) and newly developed hemagglutination inhibition (HI) procedures it has been possible for our group to type thousands of virus isolates belonging to the adenovirus, myxovirus, enterovirus, and reovirus groups. As was true during the past several years, LID in 1960 again described and characterized more new representatives of these viruses than all other virus laboratories in the world combined. This was made possible during 1960 because each of our various virus research units contributed new diagnostic techniques. Dr. Rosen's group developed additional specific HI procedures for identifying adenoviruses and adenovirus infections; and for reoviruses and enteroviruses as well. Similarly, Chanock, Johnson and Cook developed tissue culture procedures for isolating Eaton's PAP virus, while Rowe and Hartley not only discovered several "new" mouse viruses in tumor virus study systems, but developed serological procedures for recognizing their presence.

3.2 Serologic Reagents

But the availability of simplified procedures are of very little use unless the necessary reagents are also available. Although many virus research laboratories could do the tests, few laboratories are able to produce the necessary reagents. The magnitude and cost of producing and certifying them promises to continue to exceed any possible resources available. This fact has had a very depressing effect on research efforts aimed at the study of viruses as causes of disease, and serves as yet another deterrent to early delineation of the common virus diseases as public health problems. Consequently, with the help of NINDB and Microbiological Associates, LID in 1959 and 1960 accepted responsibility to develop and evaluate more than a hundred commercially produced virus antigens. LID, of course, has been active in the certification of virus prototypes and furnishes many to the Virus Registry of the American Type Culture Collection. We are also collaborating with the Enterovirus and Adenovirus national committees in setting up standards for large scale production of certified antiserums for serotyping and classification of viruses; perhaps the highest priority need of all virus laboratories concerned with human infection and disease.

3.3 LID as the Unofficial World Reference Laboratory for Viruses

Wholly through the operation of circumstances, the Virus Section of LID has become virtually the chief (in many instances only) reference laboratory for many of the newer viruses, including adenoviruses (about 30 human and several animal serotypes), myxoviruses (5 new para-influenzas occurring in 3 species), reoviruses (3 serotypes in 4 species), many of the newer and some older enteroviruses (5 - 10), salivary gland viruses (from 4 species), and new mouse viruses (6), the latter frequently found in tumor virus study systems.

Until virus reagents desperately needed for many extremely common viruses are made available either commercially, through government agencies, or both, LID as the sole custodian of many of these agents cannot avoid responsibility for assisting other excellent virus laboratories to identify their viruses, and on a pro-tem basis at least for keeping order in the general virus field. Unfortunately we have no specific commitment to provide such services and even worse, no specific budget to cover them, so that our involuntary, constantly growing and unavoidable service functions must be done at the expense of our research missions.

However, it must be admitted that the simpler virus diagnostic techniques and the availability of a complete supply of viral reagents in our laboratory (developed out of necessity) facilitate not only our own epidemiologic studies of naturally occurring virus infection but also enable us to evaluate the significance of the data furnished by other laboratories who come to us for technical assistance. As long as LID continues its policy of working on the frontiers of virus disease problems we have no way to avoid the responsibilities that devolve on us as a result, and for that matter no real desire to do so. However the dimensions of this frontier have grown geometrically in recent years, and unless LID and other virus laboratories can grow with it we cannot hope to continue to be effective and to play such a decisive role in the future. There may be some justification to the desire to see this happen, but unless other laboratories step into the breach (it will take many years to build other groups with the overall competence of the Virus units in LID), the effect of such a policy will be to slow up progress not only in LID but in many other virus laboratories concerned as we are with viruses as agents of human disease. This may seem a presumptuous statement, but the facts of the matter are clear and objective enough to justify it.

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4.0 Sero-epidemiologic Studies of Virus Disease

Comprehensive serological studies of over 75 common viruses* became feasible in 1960 as the result of successful commercial production of experimental lots of antigens. This was achieved collaboratively with the NINDB Collaborative Cerebral Palsy Study Program and Microbiological Associates, Inc. (MBA), with the Virus Section of LID serving as project director for the production items actually produced by MBA.

During 1960 all the antigens were shown to have homologous reactivity and many of them were evaluated in conventional complement fixation (CF) and hemagglutination inhibition (HI) tests, and some were employed in routine diagnostic tests in our epidemiological studies of respiratory disease.

However before definitive sero-epidemiologic surveys of virus infections in relation to birth defects can be monitored, all the antigens and test procedures must be put through "shake down" evaluations with standard human and animal serums representing experiences with each of the viruses, thus achieving quantitative information on sensitivity and specificity. Fortunately many of these standard sera are available; however, others must still be acquired in 1961.

Costs and Micro-techniques.

The development of many antigens required concentration and other special procedures, thus increasing the estimated cost of even routine production to as much as $10.00 per ml - a prohibitive cost when considered in relation to the large amount of testing which must be done in our sero-epidemiologic surveys. Consequently we have turned the attention and the efforts of our serology teams to the development and evaluation of micro-techniques.

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*Influenza, para-influenza, poliovirus, ECHO, Coxsackie A, Coxsackie B, adenovirus, reovirus, and other new virus groups.

The studies have been postponed because of the delay in obtaining the necessary precision instruments. However negotiations with an importer to obtain them from Hungary promises to solve the non-scientific obstacles to current progress. Preliminary studies suggest that the Takatsy micro-technique can be applied without modification in our HI tests and also suggest that slight modifications of the equipment will permit micro-CF tests as well.

Comment

The commercial production of numerous satisfactory complement-fixing and hemagglutinating antigens now makes possible broad and comprehensive sero-epidemiologic studies of virus infections which previously could not even be contemplated. However practical considerations, particularly their high production cost, and the lack of standard serums for evaluation (in which there is also a cost factor) will undoubtedly slow up transition from the stage of possibility to that of general feasibility, - perhaps until current concepts concerning acceptable costs for such research activities are revised upwards.

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5.0 Studies of Cancer Viruses

Our studies of cancer virus problems can be subdivided into several categories: (a) Laboratory studies of the properties of cancer viruses and development of laboratory tools for detecting and working with them; (b) field studies of the behavior in nature of those tumor viruses for which suitable detection tests are available; (c) studies of extraneous viruses ("background noise") now preventing high caliber virologic practice in the study of animal tumor viruses and obscuring interpretation of nearly all current observations on them; and (d) the study of general virus experiences in relation to human cancer - the "background noise" in the human cancer problem - which we feel must be done eventually if the role of viruses in human cancer is to be defined.

Our approach to these various interdependent studies is based on the following beliefs: 1) That the conventional methods of standard virology must be applied to cancer virus research if significant progress is to be made; 2) the study of cancer viruses obviously cannot be separated from general virology; and 3) that the "biologic point of view" rather than attitudes fostered by preoccupation with categorical disease, represents the best approach to a real understanding of the natural history of cancer viruses just as it does to other viruses (see Introduction).

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5.1 Laboratory Studies - Mouse Polyoma Cancer Virus*

New in vitro survey tools developed during 1959 (CF, HI, and MAP) were evaluated and applied in 1960 in studies of polyoma virus growth and excretion, its experimental epidemiology, and its natural history. This interesting and versatile cancer virus causes tumors not only in all strains of Mus musculus, but also in hamsters, rats, rabbits, and guinea pigs (Stewart and Eddy). Of equal interest is the fact that it can be studied and surveyed with the same facility as ordinary viruses, such as influenza and polioviruses. Virus isolation and serologic procedures, combined with epizootiologic studies have produced the following interesting observations:

Polyoma virus was found to be widely disseminated in mouse colonies nearly everywhere. Infection was found to be more commonly present than absent in laboratory strains raised in experimental or commercial laboratories and in wild strains found in city tenements. However the basic ecology or natural cycle appears to exist in rural areas - on farms and in feed mills in small towns.

In the laboratory the virus is maintained and disseminated by experimentally and spontaneously infected carrier mice which excrete virus in saliva, feces and urine, the latter appearing to be the most important vehicle of spread. Infected infant mice excrete so much virus (up to a million ID50's per ml of urine) into laboratory environments that much of the data on polyoma acquired in such environs is subject to question, particularly if adequate controls (uninoculated mice) are not included with every animal experiment. Polyoma virus was also shown to represent one of the more common extraneous agents, complicating interpretation of laboratory experimentation with other mouse tumor viruses.

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* Collaborators at the National Cancer Institute - Drs. L. W. Law, C. J. Dawe, W. G. Banfield and H. Kahler.

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5.2 Natural Behavior of Polyoma

Studies of the behavior of polyoma in Mus musculus were also carried out in three different field environments, in densely populated tenement areas of a large city, on farms producing grain and livestock, and in grain mills located in small rural towns. Each presents a different ecology but in each one focal environmental contamination appears to be quite important to the survival and persistence of polyoma infection in the mouse populations observed.

Harlem Studies

A full year's surveillance of Mus musculus infestation and polyoma infection of crowded tenements in Harlem revealed that virus infections persisted without exception in numerous separate foci. Three epidemiologic factors seem most important, namely, large mouse populations capable of furnishing adequate supplies of young susceptible mice, the extensive contamination of the tenement environment (virus was demonstrated in sweepings from areas showing signs of mouse activity), and finally the overcrowding which insures the continuous and extensive use of communal nesting areas (also demonstrated to be contaminated by virus). Apartment houses having smaller and less dense mouse infestation were generally free of infection and remained so during the study.

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Rural Studies

Systematic studies of polyoma in rural environments were undertaken during the last quarter of 1960. However, it appears from preliminary data that here we may have found the basic natural cycle of mouse polyoma. Mus musculus infestation and polyoma infection of Mus was found to be most intense in feed granaries on the farm and in cereal grain storage elevators in mills. As many as 30 per cent of several hundred mice trapped in these environs showed persistent evidence of polyoma infection, many of them apparently excreting virus in their urine. The virus has been found on cereal grains in the vicinity of mouse nesting areas, which appear to be very numerous in the granaries so far examined. The actual extent of cereal grain contamination by mouse excreta containing polyoma and no doubt other microbes must still be evaluated; however, present evidence suggests that it probably is very extensive, if not appalling.

The literature on the ecology of Mus musculus centers on the infestations of rural grain storage areas - particularly in grain "bins" and the traditional grain "ricks." Natural ecologic arrangements such as are reported here are no doubt much older and probably much more of a factor in the maintenance of natural mouse agents than the conditions that exist in infested urban areas or, for that matter, in production and experimental areas housing laboratory mice.

Since natural infection of wild mice is not limited to polyoma virus, but includes a number of other viruses known or suspected to infect man and domestic animals, the extension of these preliminary findings will likely prove very interesting.

Should our observations on Maryland farms prove not to be unique - and there is no reason to suspect that they would be - then extensive contamination of cereal grains with hardy viruses such as polyoma provides a logical vehicle of infection for laboratory mice with polyoma and no doubt with some of the other extraneous agents found in most production and experimental mouse colonies. It is interesting to consider the fact that man and his domestic animals have long been exposed to foodstuffs heavily contaminated by mice and other rodents and that the viruses and the cancers known to occur in these species are not remarkably different.

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Comment - Cancer a Possible Zoonosis?

The fact that polyoma virus, avian sarcoma and bovine papilloma, although well established tumor viruses, cross over species lines - polyoma infecting rats, hamsters, guinea pigs, and rabbits; Rous sarcoma, turkeys, ducks; and bovine papilloma, horses - seriously challenges we think prevailing concepts about the narrow species predilection supposedly exhibited by cancer viruses. It is clearly necessary now to examine the hypothesis that some cancers in man and domestic animals could be due to viruses which like the zoonoses have their basic natural cycle in lower commensal animals. Such studies can now be done on some of the known animal tumor viruses, such as polyoma.

Our field projects have developed much information of value in defining not only the natural behavior and the basic cycle of polyoma in nature, but also the probable sources of virus infections in both experimental and production colonies. Although serologic studies do not support the hypothesis that polyoma virus can infect man, widespread access of humans through mouse contaminated environments and contamination of food-stuffs with mouse excreta makes consideration of possible human infection rather more than academic. The lack of serologic correlations with cancer in man, such as occurs in mice, may not represent conclusive counter-evidence, since hamsters with polyoma induced tumors rapidly lose all evidence of infection with polyoma (Habel), despite the fact that the tumors persist.

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Proposed Course of the Natural History Studies

The natural history of polyoma and other animal tumor viruses are important subjects in their own right and our studies represent some of the first effective efforts to develop such information. As noted above, there can be no higher order of information about infectious agents than that derived from nature. Information derived from carefully planned longitudinal studies of tumor viruses are relevant to any consideration of possible human cancer viruses.

The possibility that cancer could be the result of a zoonotic infection no longer appears so very unlikely. We plan therefore to study possible polyoma infection in various animal species, particularly those having repeated exposure to environments and to materials known to contain tumor viruses, such as the polyoma and leukemia viruses of mice, and the leukemia and sarcoma viruses of chickens.

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6.0 Extraneous Viruses as "Background Noise" in Cancer Virus Studies

In 1960 the "background noise" problem in cancer virus research grew to almost "deafening" proportions and, in the opinion of LID virologists, constitutes the number one obstacle to intelligent and truly effective research on cancer viruses.

Nearly every animal tumor virus system currently under study was shown to be contaminated with extraneous agents and several viruses widely proclaimed as "tumor" viruses turned out to be fellow traveling ordinary viruses. To list a few examples: Friend leukemia was found contaminated with polyoma and mouse adenovirus; Gross leukemia by polyoma, K virus and mouse adenovirus; Schwartz leukemia with polyoma, K virus and mouse adenovirus; Moloney leukemia with mouse hepatitis and mouse reovirus; the polyoma virus itself became contaminated with mouse adenovirus, hepatitis and salivary gland viruses.

New "tumor viruses" grown in tissue culture systems or propagated in mice were reported and later found to be extraneous agents. Thus, to give one example, the tissue culture grown "VL" strain of lymphomatosis was shown to be an almost universally prevalent adenovirus of chickens now called "GAL" virus. LID virologists showed by serological and virus isolation methods the lack of association between most of these agents and the specific cancers with which they are associated, confirming in several instances similar findings in other laboratories.

6.1 Extraneous Viruses in Tumor Virus Study Systems

LID virologists showed that the "seeds" of the "background noise" viruses are commonly present in the animals used for the induction of tumors, and of course in the subsequent passage materials as mentioned above. The extraneous viruses most commonly encountered in cancer systems were the newer ones, such as polyoma, K virus, mouse reovirus and adenovirus; but this in part may be due to newly developed easily applied survey tools for these agents. Other viruses encountered less often (perhaps because of comparatively less sensitive tools) were mouse hepatitis, mouse salivary gland virus, the newly discovered "thymic agent" (TA). Except in newborns, most of these viruses occur subclinically and latently. Not found or seldom encountered in tumor systems were Theiler's virus, LCM, PVM and mouse pox (ectromelia), all familiar to most virologists. Since most of these older agents generally produce very obvious clinical effects in mice, they are perhaps more easily eliminated from production colonies.

All mouse colonies used for tumor virus induction were shown to contain at least one easily demonstrable extraneous virus; but more than one (usually three to five) was the rule. Discoveries of new mouse viruses and the development of techniques for selecting them enabled our scientists to survey and define many mouse production colonies and to some extent predict some of the problems encountered in current extensive efforts to establish cancer viruses in animal and tissue culture systems.

6.2 Proposed Course of "Background Noise" Studies

The first principle of microbiology calls for "pure culture" of microbes. The extraneous virus problem in virus study systems must be controlled before "good" virology can be done on cancer viruses in such systems. The extraneous agents are in most cases slow-growing, latent, subclinical agents. While they do not seriously handicap the study of the acutely virulent viruses such as the encephalitis and Coxsackie viruses, they do however pose serious problems in studies of the slow-growing and also generally latent cancer viruses.

LID scientists visualize several steps that must be taken: 1) Further development and application of sensitive techniques for detecting extraneous viruses; 2) Surveillance and monitoring of existing mouse colonies and experiments for known agents, thus helping to define current problems. This calls for the production and evaluation of diagnostic reagents; 3) Establishment of relatively "clean", "virus defined" colonies and animal research areas for pilot studies aimed at the elimination of extraneous viruses from study systems; 4) since all cancer virus passage materials passed through experimental animals either are known or suspected to contain extraneous viruses, they also must be "cleaned" up in order to utilize "clean" animals in "clean" areas and finally achieve the goal of "pure culture"; and 5) When sufficient information is available, "clean"animal production colonies and "clean" experimental areas must eventually be established wherever such animals are to be used for study of latent or tumor virus effects.

Comment. It is clear that the task described here will appear monumental to some, insuperable to others, and to still others too expensive. However, it is also clear that reluctance to meet this problem squarely and failure to eventually achieve its solution is to accept pre-Pasteurian concepts as guides for modern virology, to waste uncounted dollars and to accept in the beginning of new and very expensive enterprises on animal tumor viruses the probability of final failure.

LID scientists who have helped to develop and define this problem recognize the importance of its solution and accept responsibility to work towards that necessary goal. We must do so if we are to satisfy our desire to properly study the role of latent viruses in chronic and neoplastic diseases. It is our opinion further that the National Institutes of Health with its large commitment to support cancer virus research cannot avoid this responsibility and that the most effective action towards the solution of this critical problem depends on well directed and concerted action by NIAID and NCI.

7.0 Studies of Common Virus Infections in Animals Likely to be Important in Human Medicine

A. Bovine Virus Infections

During the past several years LID scientists in collaboration with other groups have helped to enlarge current notions of common virus infections of dairy and beef cattle. The occurrence of several "new" viruses, such as reoviruses types 1, 2 and 3, were reported in 1960, and additional viruses, chiefly bovine enteroviruses, are currently under study. Observations on the properties, clinical importance and prevalence of para-influenza 3 virus, infectious bovine rhinotracheitis and bovine adenovirus were also extended, and potencies of para-influenza 3 vaccines were explored in cattle. A clear view of the importance of these viruses in bovine disease and their significance for human health must await much larger and comprehensive efforts. Such an effort however far exceeds our currently available facilities and resources. Consequently, we plan in 1961 to continue only to a limited degree to develop our laboratory and natural history studies of these agents.

B. Virus Infections of House Mice (Mus musculus)

One of the more intimate of man's domestic creatures is the house mouse. It is perhaps no accident that the known viruses of mice closely resemble those of man; at least 13 mouse viruses have human counterparts and several are known to be identical with human agents. Thus, mouse reoviruses types 2 and 3 discovered during 1960 in both laboratory and wild mice, and in mouse tumor passage materials (see 6.0 Extraneous Viruses above) are indistinguishable from the prototype human strains. Of course LCM and Sendai viruses of mice and man were previously shown to be identical.

Although many of these viruses are now important because they produce "background noise" in cancer virus studies, they must be regarded as important in their own right, both as possible sources of infection to man and to other of his more desirable domestic animals. They also serve as interesting natural models of similar human infectious disease processes.

The contamination of cereal grains by polyoma virus have been mentioned above (in 5.0). Since other common viruses (such as mouse reoviruses and adenoviruses, etc.) are also excreted in saliva, urine or feces of mice, it seems inevitable that they will soon be found on grain, which of course is fed uncooked to livestock.

Human infection with mouse viruses due to exposure to cereal grains although less likely because they are almost always cooked, could also occur through occupational and household exposure to raw grains in the process of grinding, processing, cooking and baking with flour. Needless to say, we plan sero-epidemiological studies designed to answer the questions posed here.

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Medical Mycology Section

The Mycology Section reported 8 projects for calendar year 1960. All these projects have included broad fields of research and although definitive goals have been reached in most of them, all will be continued in order to further exploti productive lines of investigation. In most cases new or additional species of pathogenic fungi will be used in investigations, or techniques will be altered to permit further development of experimental studies. Results of investigations have been published in 9 papers.

Dr. George W. Lones, investigating antibiotic X-5079C, found that it is fungistatic but not fungicidal and that its apparent low degree of in vitro activity is due to its decay in culture medium. The yeast form of Histoplasma capsulatum is much more sensitive to X-5079C than the mycelial form and an assay method, sensitive to 1 ug/ml using H. capsulatum, was developed. X-5079C has low toxicity for HeLa cells and is active against H. capsulatum grown in HeLa cells.

Dr. Lones has converted a second strain of Coccidioides immitis to serial culture in the spherule form. He has made quantitative measurements of the ability of various carbon and nitrogen sources to support growth of spherule and mycelial forms of strain M-II of C. immitis. Only mannose is utilized as readily as glucose by spherules. Mannose and fructose support growth of the mycelial form as well as does glucose. A substrate which preferentially supports growth of the spherule form was not found in this study.

Dr. Herbert F. Hasenclever utilized spherules to immunize mice and found an increased survivor rate in immunized mice when challenged with a lethal infecting dose and earlier clearance of organs (negative cultures) in immunized mice challenged with a sublethal dose.

Dr. Hasenclever found two antigenic groups within the species Candida albicans. One is similar antigenically to C. tropicalis and one is antigenically indistinguishable from C. stellatoidea.

Dr. Hasenclever found by triturating and plating out organs of experimentally infected mice, that several minutes after Cryptococcus neoformans was injected either intravenously or intracerebrally into mice the largest numbers of yeast cells had been retained in the lungs. The fungus population in the lung then decreases and 2-3 days after infection multiplication in the brain is apparent. Although the interval from infection to death of infected mice varies with the strain of C. neoformans, the numbers of yeast cells per gram of brain tissue is approximately the same regardless of strain.

Dr. Hasenclever has found that many strains of Candida tropicalis are as pathogenic for mice as for rabbits and that multiplication in vivo is similar to that of C. albicans. The kidneys are the organs showing greatest tissue damage and the highest yeast cell population.

Dr. Chester W. Emmons and Mr. Willard Piggott, with the assistance of Mr. William Hill, have continued studies of the saprophytic occurrence in natural habitats of fungi which cause mycoses. Cryptococcus neoformans has been isolated from many additional collections of pigeon guano. When this material is collected from old pigeon nests and from roosting sites in hay mows of barns and upper floors of buildings, Histoplasma has never been found. There is increasing circumstantial evidence that a presently unstudied pneumonic form of cryptococcosis has occurred in men heavily exposed to such material and that such material and that such epidemics have been erroneously diagnosed histoplasmosis.

Dr. Emmons and Mr. Piggott designed a simple, convenient and clean device for exposing mice by inhalation to dry spores of pathogenic fungi. This has been used to stimulate natural conditions of infection.

Continuing in vivo testing of antibiotics and of drugs prepared by Dr. Benjamin Prescott have not yielded any chemotherapeutic agent equal to X-5079C in the treatment of several mycoses.

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Dr. Emmons has continued to collaborate with Dr. William J. Jellison, Dr. Lie-Kian-Joe, Dr. Charles Bridges and others, in the study of new or unusual mycoses and the fungi which cause them. Studies of phycomycoses have continued. With Dr. Bridges, a study of several cases of hyphomycosis destruens in horses and of its etiological agent has been submitted for publication. The fungus is described under the old name, Hyphomyces destruens. It appears to be a Phycomycete but its complete life history has not been determined.

In collaboration with Dr. Jellison a new species, Emmonsia crescens, was described. This fungus differs from the first species of Emmonsia (E. parva) in vivo and in vitro at 37° C by its multinucleate condition (instead of uninucleate), its ability to produce the in vivo form in vitro at 37° C, and its greater size. E. parva conidia when inhaled or incubated at 37° C increase in diameter from 2 - 4 u to 400 - 480 u. This 10^6-fold increase in volume of a single cell is very unusual in the fungi.

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Medical and Physiological Bacteriology Section
Dr. A. K. Saz

Introduction

Significant progress has been made during the past year on all scientific problems under investigation by the staff of the Medical and Physiological Bacteriology Section. All professional investigators in the Section have published in the past calendar year a minimum of one paper, and in most instances more, in reputable, first-line scientific journals. The cohesiveness of the Section has been further evidenced by the holding of regularly scheduled informal section seminars at which the various professionals present their current raw data for criticism. The program of the Section is varied but the one theme common to all projects is the pursuit of knowledge dealing with fundamental biological activity of various bacteria. The cross-fertilization which has occurred as a result of the many-faceted problems under investigation by the professional personnel of the section has been of prime importance in progress made and offers further confirmatory evidence for the validity of the philosophy that team projects are not the sole road to scientific knowledge.

It has been established that staphylococcal penicillinase is associated with particulate material in the cell and thus an explanation has been given for the refractoriness in preparation of this enzyme by conventional methods. New and more potent inhibition of Staph. penicillinase have been uncovered and the hope remains and is heightened for the ultimate finding of a chemically useful inhibitor. Further, sea water has been found to possess strong inhibitory activity against both penicillin-sensitive and penicillin-resistant staphylococci (phage type 80/81).

Real progress has been reported in the understanding of iron metabolism in the staphylococci. As a direct result of continuing work dealing with mechanisms of the development of non-specific immunity and in particular the function of the iron-transporting protein of plasma, siderophilin, fundamental observations on the effects of iron deficiency on the growth and metabolism of S. aureus have been reported. Work on the biology of the staphylococci so long neglected during the "antibiotic era" is cardinal to effective new therapy of staphylococcal infections.

Progress has also been made on the search for better methods of isolation and purification of M protein of streptococci. These results are of obvious importance in the understanding of Group A streptococcal virulence. Further, highly interesting observations have been reported dealing with the mechanism and significance of the long-chain test for determination of anti-streptococcal immunity.

Real understanding of the intimate mechanisms of energy metabolism in Hydrogenomonas in particular and other bacteria and higher form in general is closer as a result of work performed in this section this year. In an enormously complicated field, progress has occurred in the definitions of the essential reactions.

Detoxification studies on potentially useful chemotherapeutic agents have continued and new and promising leads have been uncovered for agents active against bacteria, fungi, parasites and it should be added, against cancer as well. Several of the aforementioned detoxified compounds have passed preliminary screening processes performed by the Cancer Chemotherapy Center.

Pinpointing of the enzymic locus of discrimination among hydrogen isotopes by Pseudomonas has been reported this year. The area lies in formic acid metabolism.

During the past year, we were unfortunate in losing the services of Dr. Robert C. Woodworth who has left this section, but this was compensated for by the selection of Dr. Austin Shug, Project Associate, Enzyme Institute, University of Wisconsin, to replace him. Dr. Shug will concern himself with studies on the iron metabolism of staphylococci.

We were saddened by the passing of Mr. Charles Offord, Animal Caretaker in this section. Mr. Jack Kelly has ably replaced Mr. Offord.

Dr. Eskin Huff, LCI, has been transferred to this section and will work on the mode of action of penicillin and the biochemical mechanisms of the development of resistance thereto. He is joined by Miss Harriet Milner, Biologist, and will occupy the space formerly assigned to Dr. Repaske, who in turn will have new quarters in the space left vacant by the departure of DBS personnel from the area.

Authorization still has not been obtained for the hiring of a microbial geneticist. It cannot be emphasized too strongly that this section's mission in bacteriological research cannot be completely fulfilled unless a program of genetic research is begun. This is a highly important, vital area of contemporary microbiological research and it is incomprehensible that an institute devoted to a study of the microbial world is not represented in this parameter.

Studies on the Biochemistry of Antibiotics.

Studies on the biochemistry of staphylococcal penicillinase are continuing on two parameters: 1) search for an effective inhibitor of the enzyme, 2) purification of the enzyme. Results reported last year indicated that various dipeptides, and particularly those with D-valine in the molecule were inhibitory. Other inhibitory compounds have now been found. These are an interesting group of substances prepared by condensing various amino acids, primarily of the D-configuration, into peptides with substances that show per se metal binding capacity but are not in themselves inhibitory to penicillin. On a molar basis, these compounds are by far the most inhibitory of any yet studied. The most active are D-dialanyl- and L-dialanyl-benzidine, D-valyl-4-aminobiphenyl and D-phenyl-L-alanyl-aminofluorene. Inhibitions up to 60 per cent have also been observed with other dipeptides, i.e., L-threonyl-D-alanine, L-allothreonyl-D-alanine and D-alloisoleucyl-D-alanine.

Various considerations had indicated that staphylococcal penicillinase conceivably was associated with particulate matter within the cell. Work performed this year has indicated that this is indeed the case. It has been possible to secure 12-fold purification of the cell-free enzyme by centrifuging in the Spinco Model L centrifuge at 144,000 x g for periods of 5-15 minutes. All activity is found in the pellet. It is hypothesized that the marked stimulation of penicillinase activity by various alcohols (reported last year) is due to the reversible solubilization of the particle mediated by the alcohols. This activity brings the enzyme and the substrate (penicillin into apposition).

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A corollary of the work reported has been the development of a new, rapid spectrophotometric method of penicillinase assay which depends upon the change in color of a pH indicator dye as a result of penicilloic acid formation arising from the action of penicillinase on penicillin. This method was adapted directly from the work of Dr. Nirenberg, NIAMD.

It has been found, as a result of collaborative studies with the Woods Hole Oceanographic Institution investigators that sea water has a marked anti-staphylococcal activity. The activity is manifest against both penicillin-sensitive and penicillinase producing, penicillin-resistant staphylococci. By contrast, the activity against Escherichia coli is minimal. The anti-staphylococcal factor(s) is heat labile and sea water can be diluted at least 1:8 with undiminished activity. It is hoped that this collaborative project with Woods Hole Oceanographic Institution will continue.

Microbiological Fractionation of the Hydrogen Isotopes by a Marine Pseudomonad.

A guest worker in this section from the Geological Survey has been concerned with this problem for the past two years. The principal organism under investigation is a marine Pseudomonad which was isolated from the Bahama Banks in 1956, although other known bacterial species including E. coli and various marine bacteria have been examined along parallel lines.

Both deuterium and tritium are fractionated from organic substrates and water by the Pseudomonad. Deuterium analyses were made with mass spectrometer and tritium analyses by a windowless counter coupled to a scalar and ratemeter using geiger counting gas.

During fermentation of various carbohydrates, hydrogen gas is evolved which is invariably deficient in the light hydrogen isotope, protium, according to expected concentrations from the isotopic equilibrium constant:

$$K = \frac{Q\ HDO\ Q\ H_2}{Q\ H_2O\ Q\ HD} = 3.88 \pm 0.05\ @\ 25^\circ\ C$$

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Fractionation of deuterium from protium apparently takes place in the step $HCOOH \longrightarrow CO_2 + H_2$. Of the three possible enzyme systems involved in equilibrium reactions of atomic and molecular hydrogen, viz. hydrogenlyase, dehydrogenase and hydrogenase, only the first one is inhibited by deuterium in concentrations equal or less than 66 per cent D_2O or by completely deuterated formate. These findings strongly suggest that at least part of the fractionation effect leading to light hydrogen gas can be attributed to inhibition of specific enzymes by deuterium.

Studies with tritium have involved the reactions between tritiated water and organic constituents in living cells. The Pseudomonad quickly absorbs tritiated water in various media and splits the water molecule containing tribium. Whereas the amino acids from bacterial protein show little or no tritium bonding, the carbohydrate fraction including polysaccharides appears to contain a large percentage of the bound tritium. Much of the tritium entering the cell as tritiated water diffuses out later as carbohydrate or polysaccharide. Further studies are contemplated to identify the tritium-bound organic matter. Since the equilibrium constant for tritium is $K = \frac{Q\ HTO\ Q\ H_2}{Q\ H_2O\ Q\ HD} = 5.93 \pm 0.08$ @ 25° C which is appreciably higher than deuterium, it may be expected that the fractionation factor involving atomic and molecular compounds of tritium will likewise be higher.

Aside from the demonstration of deuterium and tritium fractionation, these studies have revealed the rapidity which microorganisms absorb and split or bind water. Tracer methods using tritiated water reveal that the Pseudomonad pumps water molecules at the rate of 10^{13} molecules per hour at 25° C.

The implications from this study include the following:

1) The natural isotope, deuterium, appears to inhibit specific enzymes in living cells which may affect the overall metabolism.

2) Bacteria in marine sediments may play an important role in the concentration of deuterium in the marine environment.

3) Bacteria may prove to be effective agents for the concentration of natural and radioactive isotopes. The former has practical significance in the production of fusion fuel (HDO) and valuable trace elements; the latter may be important in problems dealing with water pollution from radioactive isotopes.

The Electron Transport System in an Autotrophic Hydrogenomonad.

Hydrogenomonas eutropha (Bovell) is an autotrophic bacterium which is capable of obtaining all of its energy for growth, maintenance, and repair from the oxidation of hydrogen gas by oxygen ($2H_2 + O_2$ ----> $2H_2O$). Although the oxidation of the particular substrate, hydrogen, is unusual, the reaction is fundamentally a general one in which hydrogen replaces the ordinary organic substrates such as glucose, glycerol or other complex compounds. Another unique feature of H. eutropha is its ability to synthesize all cell material from carbon dioxide with energy secured from the oxidation of hydrogen. Nitrogen is obtained from ammonium salts, sulfur from sulfate. This organism then is a complete biological synthetic machine which manufactures the most complicated structural proteins, enzymes, vitamins, carbohydrates and lipids from three gases (H_2, O_2 and CO_2) and simple inorganic compounds.

Tremendous amounts of energy are needed for these processes, yet the mechanism of energy production during oxidative metabolism is not known for this organism or any other aerobic organism.

It has been observed that various biological systems share many similar biochemical reactions; this has given rise to the concept of comparative biochemistry. Consequently it is assumed that the mechanism of oxidative phosphorylation in H. eutropha is analogous to that in other forms of aerobic biological life. Biochemists working with bacterial, animal and plant material have applied themselves to the problem of coupled oxidation and phosphorylation for the past 35 years, but the mechanism for trapping energy in the form of ATP during oxidations of the type mentioned has thur far defied resolution because (1) the enzymes of the electron transport system and the interacting energy yielding system are both associated with particulate cellular elements, and (2) resolution of one or the other system into a soluble state have generally resulted in loss of both activities.

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Hydrogenomonas eutropha is a bacterium with unusual possibilities for the study of oxidative phosphorylation. Present evidence indicates that the electron transport system is soluble; consequently, classical enzyme purification techniques should result in separation of the individual enzymes leading to an elucidation of their function. It seems probable that the enzymes responsible for trapping the resultant energy in the form of ATP are also soluble and they may be similarly studied.

Since an understanding of the electron transport system is prerequisite to a study of the energy trapping system, we have begun studies on the hydrogen oxidizing system. We have found that the initial steps involve the activation of hydrogen gas and the passage of electrons to the coenzyme diphosphopyridine nucleotide (DPN). A second coenzyme requirement, riboflavin phosphate (FMN) has also been found, and the data suggest that it functions as the cofactor between hydrogen and DPN. Extreme caution must be exercised in handling the enzyme because of its sensitivity to oxygen. This has necessitated the design of several new pieces of equipment and of techniques which minimize oxygen contamination during handling of the enzyme. To date the enzyme has been purified three to four fold from crude, cell-free extracts by protamine sulfate and calcium phosphate gel treatments. Additional purification will be necessary before investigating the next enzymic reaction, which is probably cytochrome linked.

Preliminary studies reveal that H. eutropha contains but one cytochrome of the "c" type; in contrast, most other aerobic organisms contain three of four cytochromes. This fact again indicates that this organism possesses a less complicated electron transport system than is usually encountered.

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Detoxification of potential Chemotherapeutic Agents:

Further studies are in progress to increase the tolerated dose of various chemotherapeutic agents, such as isoniazid (INH), streptomycin (SM) and Kanamycin for tuberculosis, Neomycin for other infections, Mystatin for fungal infections; Miracil D, piperazine and complexes of tetracycline for parasitic infections and certain agents in use in cancer therapy, by concomitant administration of various metabolites. Studies on the tolerance of mice for streptomycin and/or isoniazid have shown that a lethal dose of either drug dissolved in glycerol formal as solvent (25-30 per cent) permitted 90-100 per cent survival in white and DBA mice. Mixed with SM-INH, 85 per cent of the mice tolerated a lethal dose of 10 mg SM and 4 mg INH and 80 per cent survived with 10 mg SM and 8 mg INH. In addition, studies with steroids as adjuvants showed that a lethal dose of 30 mg SM and 4 mg INH (5 mg is the tolerated dose of SM) administered simultaneously with 25 mg of sodium taurocholate permitted 55 per cent survival in two strains of mice. With twice the amount of sodium taurocholate, 100 per cent of the mice tested tolerated a 30 mg dose of Miracil D (6 x lethal dose).

A number of potentially non-toxic new chemotherapeutic agents against fungal and parasitic infections have been synthesized. Among these, long chain thiosemicarbazones, piperazines and numerous tetracycline complexes of atabrine, chloroquine, sulfanilic acid and naphthoquinones have already demonstrated high in vivo activity and yet drew negligible toxicity for mice. Several of the long chain thiosemicarbazones have also passed initial tests in mice when tested by the Cancer Chemotherapy Screening Center.

Investigations of the antimicrobial activity of substances from shellfish are being considered. Two groups of fractions were isolated from abalone juice by passage through an anion-exchange (diethylamino-ethylcellulose) column followed by elution with a series of Tris-H_3PO_4 buffers. Antibacterial activity of a group of the early eluates has been reported (Proc. Soc. Exp. Biol. and Med., in press). These fractions showed no inhibition in tissue culture against Japanese 365 strain of influenza A virus and polyoma virus. A grouping of later eluates including a final M-NaCl wash from the column contained no antibacterial activity. However, these combined eluates exhibited definite inhibition against influenza virus and polyoma virus. When monkey kidney cells were treated for 24 hours before virus inoculation with a fraction of this group at a concentration of 50 mg per cent, virus multiplication could not be demonstrated.

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Studies on the Growth and Metabolism of Staphylococci and on the Iron Metabolism of the Host as Mediated by the Iron-binding Serum Protein Siderophilin.

Research on this project is oriented towards elucidation of the following problems:

1) The role of siderophilin as a non-specific immune factor in the control of bacterial infection through nutritional iron restriction.

In the circulatory system, both in blood and in the lymph, the iron availability to the bacterial cells as well as to the host tissue cells is regulated by the protein constituent siderophilin. Workers in this section have discovered that the growth of Staph. albus in serum is completely inhibited by siderophilin if, as occurs in normal and in many pathologic sera, the protein is not completely saturated with iron. Staph. aureus, on the other hand, grows in serum at a rate which is a function of the percentage iron saturation of the siderophilin. For example, if the siderophilin in serum is 100 per cent saturated, Staph. aureus grows ten times faster than when the siderophilin is only 30 per cent iron-saturated (the percentage saturation of normal serum). Further, the lag period of growth, under the experimental physiological conditions employed, is extended from 8 hours to 27 hours at the lower percentage saturation. Since, in various pathologic states, the percentage iron-saturation levels of serum siderophilin vary in a diagnostically significant manner far above and below the usual normal value to 30 per cent, the suitability of such sera as in vivo culture media for the growth of many pathogens and, in particular, Staph. aureus, is a factor in the defense mechanism of the host against the establishment of a given bacterial infection. A phenomenon associated with infection and worthy of further investigation is the response of the host to decrease the percentage iron-saturation of its siderophilin by lowering the concentration of circulating serum iron. One consequence of this response is to provide a more unsuitable nutritional environment for the infecting bacterium; another consequence is to increase the iron concentration in the reticulo-endothelial system. The mechanism by which the response is effected is unknown and the significance of the heightened iron concentration in the R. E. system likewise requires elucidation.

2) The effect of the siderophilin-controlled [illegible] iron concentration on the metabolic activities of Staph. aureus

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It has been found that the carbohydrate metabolism of cells of Staph. aureus grown in medium restrictive as to available iron (low percentage iron-saturation of siderophilin) is greatly different from those grown in media providing 5 to 20 times as much free iron (high percentage iron-saturation of siderophilin). One of the most striking effects, among others, is the failure of "low iron" cells to oxidize the important pivotal substrate, pyruvic acid. The concentration of certain iron enzymes, as cytochrome oxidase and catalase, is severely reduced in "low iron" cells.

The biochemical mechanisms by which iron controls the overall metabolism of Staph. aureus are being investigated and the changes in enzymatic function and localization of iron-containing components within various cellular structures are being studied.

3) A comparison of the mechanisms by which mammalian host tissues acquire iron from iron-siderophilin with that operating in the cells of Staph. aureus.

It has been determined that cells of Staph. aureus, grown in the presence of serum, obtain their iron from iron-siderophilin as free ionic iron. The source of this ionic iron is the natural dissociation of the metal protein complex under physiological conditions of pH and bicarbonate concentration. The relationship of pH and bicarbonate concentration changes to the dissociation rate of the metal complex have been investigated. It has been established that bone marrow tissue and reticulocytes take up iron from iron-siderophilin in serum, or from a bicarbonate buffer solution of the metal complex, not as ionic iron but by some type of direct transfer from the complex to the cells. Investigation of the basis for this direct transfer is contemplated. Whether other tissue cells than bone marrow and reticulocytes depend for their iron on similar direct transfer of iron from iron-siderophilin complex or whether they are similar to Staph. aureus cells in fulfilling their iron requirements through diffusion of ionic iron is a current problem of active interest.

Effective use of Staph. aureus and of rabbit reticulocytes have been made as indicators of the integrity of isolated, purified siderophilins on the basis of their efficacy in the donation of iron to the given cells. Further development of the applicability of these agents to such purpose is required.

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Streptococcal M Protein, Virulence, and Type-specific Immunity.

This unit has been interested in the bacterial flora of the upper respiratory tract, chiefly the gram-positive cocci, because of their prevalence and pathogenicity. Chief among these are the beta-hemolytic streptococci of Group A. Despite more than 15 years of antibiotic usage, these organisms appear to be as ubiquitous as ever. Widespread use of antibiotics and prophylactic programs have caused, however, decreases in severity and in non-suppurative sequelae such as rheumatic fever and acute glomerulonephritis. The continuing high incidence of Group A streptococcal infections is probably caused by many repeat infections with the same type, due in turn to the suppression of adequate type-specific antibody response by early administration of antibiotics. The role of the presumptive virulence factor, M protein, in such infections is moderately well-established by association, but the mechanism of its action is obscure.

For the past several years, work in the Respiratory Bacteriology Unit has emphasized cultural studies in field situations, balanced by increasing basic laboratory investigations into the nature of streptococcal virulence and of the protective type-specific antibodies. It is felt that such studies may ultimately aid also in determining the nature of virulence in, and of specific resistance to, other gram-positive cocci and particularly the staphylococci which, although they produce certain extracellular materials in common with the streptococci, are less well classified serologically or by antigenic dissection.

Cultural studies in calendar 1960 have been limited to serologic identification of beta-hemolytic streptococci isolated sequentially from personnel stationed in the Antarctic. The study, done in conjunction with a group at the School of Hygiene of Johns Hopkins University, has shown that delayed isolation of beta-hemolytic streptococci from frozen glycerine-broth is feasible; and that the majority of streptococci so isolated were of Group A. The correlation of serologic group with bacitracin sensitivity, however, was low. The latter thus appears to be a poorer screening procedure than indicated by previous workers. Continuing field studies of this sort are planned, both in the Antarctic and Arctic. In addition, the antibody responses of carriers will be studied by new methods.

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Basic investigations have been concerned with the development of new methods for extracting and purifying M protein; and with improving tests for type-specific antibody. M protein of several types have been prepared as the acid-alcohol soluble picrates. These preparations are simple to make, are antigenic, and cross-react only when the organism from which they are made is known to contain a cross-reacting "R" protein. Further purification and analysis by antigenicity, agar gel diffusion and immunoelectrophoresis are in progress. The use of picrates and of conventionally-prepared M proteins in enhancement of virulence or protection against phagocytosis, is being studied. Fragmentation of M protein by heat, pH, and enzymes to define the portion active in virulence, as well as in other reactions, is under way.

Improved methods for the detection of type-specific antibody are being pursued. These include the long chain test and inhibition of specific fluorescence. The former has been increased in simplicity, speed, sensitivity and statistical control by using chi-square analyses of chain-length frequency distributions. The importance of time-interval sampling, and the relation of time to antibody and antigen concentrations used in the test, have been shown. As a result, it is now possible to titrate readily antisera for type-specific antibody to a degree not previously possible.

The inhibition of specific fluorescence appears to be a sensitive screening test for type-specific antibody, but its use in titration is hampered by subjective determination of the degree of fluorescence as an end-point. Type-specific fluorescent antisera, needed for this test, were readily prepared by absorption of labelled antisera to whole cells, and did not cross-react. Such antisera have also been used, together with group antisera, to study the location of M protein in the cell wall. It has been shown that type-specific antibody will block group antibody, although the reverse is not true. These results appear to verify the superficial location of M and the deeper position in the cell wall of the group carbohydrate antigen.

Group A streptococci have been shown to grow well in, and to form long chains in, fluorescein-labelled homologous type-specific antiserum. Once antibody in such a system is depleted, however, subsequently-formed portions of the cell wall are not fluorescent, thus providing a sharp visible differentiation of old and new cell wall. Similar studies, using ferritin-labelled antibody and visualization under the electron microscope, are planned.

Antibody-labelling techniques are also being used in studies on the nature of type-specific antibody. Univalent antibody has been produced by pepsin and cysteine treatment, and is being examined by a variety of tests.

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Individual Project Report
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Part A.

Project Title: Studies on Cellular and Cell-Free Staphylococcal Penicillinase

Principal Investigator: Dr. Arthur K. Saz

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 22/12
Professional: 8/12
Other: 14/12

Project Description:

Objectives: To purify and study penicillinase from penicillin-resistant staphylococci. To investigate the possibility of strongly inhibiting cellular penicillinase.

Methods Employed: Isolation and purification of cell-free penicillinase from staphylococcal cells by standard biochemical techniques. Measurement of penicillinase activity manometrically and iodometrically.

Major Findings: In continuation of one aspect of this problem carried over from last year, the search for inhibition of penicillinase activity has continued. Various compounds prepared by condensing amino acids and metal binders have been tested for anti-penicillinase activity. Of these, the extremely insoluble compounds D-dialanyl benzidine and L-dialanyl benzidine show marked inhibitory activity. Due to the very low solubility of these compounds, an accurate inhibitory concentration has been difficult to determine. However, these compounds are, on a molar basis, by far the most active of all inhibitors thus far found. Lesser activities, but still potent, have been noted for D-valyl 4-aminobiphenyl and D-phenylalanyl L-aminofluorene. Inhibitions of penicillinase activity up to 60% have been observed with L-threonyl-D-alanine, L-allothreonyl-D-alanine and D-alloisoleucyl-D-alanine.

Efforts at purification of staphylococcal penicillinase have continued. To date, the most active preparations exhibit twelve-fold puri-

fication. Various considerations indicated that the penicillinase as it occurs intracellularly, was not a soluble enzyme, but rather was particulate. This has proved to be the case. Cell-free extracts of the penicillin-resistant staphylococci prepared by sonic or Nossal disintegration were subjected to ultracentrifugation in the Spinco Model L preparative centrifuge. After only five minutes centrifugation at 144,000 x g, all penicillinase activity appeared in a small pellet with a concomitant 4 to 6-fold purification. Electron microscopic observation of the pellet showed particulate matter, presumably though not definitively associated with penicillinase activity. This observation conceivably could explain the marked stimulation of penicillinase activity, as reported previously, by various alcohols, and particularly n-propanol and n-butanol. In this instance, the alcohol activity might parallel the stimulatory effects of various detergents and solvents on mitochondria derived from mammalian tissue. The solvents presumably render the particles more permeable to substrate. This possibility is under investigation.

Significance to bio-medical research and the program of the Institute: If a non-metabolizable non-toxic inhibitor of staphylococcal penicillinase could be found it would go a long way toward solving the penicillin-resistant staphylococcus problem. Any information concerning penicillinase is of interest to this Institute.

Proposed course of project: 1. To purify penicillinase further and to study its properties. 2. To continue the search for an effective inhibitor. Animal studies will be used as indicated. 3. To study the mechanism of the alcohol stimulation of penicillinase.

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Saz, Arthur K. and Martinez, L. Marina: Enzymatic Basis of Resistance to Aureomycin. III. Inhibition by Aureomycin of Protein-Stimulated Electron Transport in Escherichia coli. J. Bact., 79, 527-531, 1960.

Honors and awards relating to this project:

Chairman, President's Fellowship Committee, Society of American Bacteriologists.

Organizer and Convener of Panel on Mode of Action of Antibiotics, Conference on Anti-Microbial Agents - Mayflower Hotel, Washington, D.C. Oct. 26-28, 1960.

Lecturer (Professor) in Botany, Howard University, Washington, D. C.

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3. Bethesda, Maryland

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Part A.

Project Title: Antibiotic Activity of Sea Water

Principal Investigator: Arthur K. Saz

Other Investigators: Dr. Stanley Watson
Woods Hole Oceanographic Institution
Woods Hole, Massachusetts

Cooperating Units: Woods Hole Oceanographic Institution

Man Years (calendar year 1960)
Total: 6/12
Professional: 6/12
Other:

Project Description:

Objectives: To investigate the reported antibiotic activity of sea water. To isolate from sea water, compounds active against fecal organisms and penicillin-resistant staphylococci.

Methods Employed: Treatment of sea water by various methods (extraction, chromatography, electrophoresis) in order to isolate inhibitory factors present. Standard bacteriological techniques.

Major Findings: It has been possible to confirm a weak anti-coliform activity of sea water. Perhaps of more interest, however, is the unequivocal demonstration for the first time of an antistaphylococcal activity in sea water. The reputed strong anti-coliform activity of sea water is in large part due simply to the effect of increased salinity in a sea water environment. Most previous reports of this activity failed to use adequate controls as evidenced by close inspection of the data. Indeed, on occasion, Escherichia coli, inoculated into either filtered or raw sea water, increased in numbers during the observation period of 72 hours. On other occasions and with different specimens of water, a weak activity (survival of 50% after 72 hours) was evident.

Part B included: Yes

The problem of adequate controls for the demonstration of activity was investigated. A small amount of organic material, proper pH, temperature of 20-25° and the use of sea water in diluting inoculated samples for plate count proved to be essential for demonstration of activity. Further it was essential to run a control in each experiment of 2.5% buffered NaCl (the saline concentration of sea water) to eliminate the possibility of kill due to salinity alone.

Experiments reported hereafter were performed, because of crowded conditions at the Woods Hole Oceanographic Institution, with a strain of penicillin-sensitive S. aureus. However, preliminary experiments performed at N.I.H. indicate that a penicillin-resistant Staphylococcus (80/81) is killed by sea water. In a typical experiment, 2.6×10^6 staphylococci per ml were inoculated with raw sea water. After 24 hours, 5.7×10^3/ml organisms were viable and in 48 hours, 3.2×10^3 organisms/ml survived. There were only 10 viable organisms/ml after 72 hours. In contrast, the saline control for the same periods showed 1.1×10^6, 0.3×10^6, and 3.6×10^5 survivors/ml. Similar results were obtained using filtered sea water. By contrast, when organisms were inoculated into autoclaved sea water, the anti-staphylococcal activity was completely lost. In a preliminary experiment raw sea water, diluted 1:8, exhibited as much activity as whole sea water over a 24 hour period.

It was also observed that not all samples of sea water collected exhibited activity. To date, of three samples studied two have been active. These results are presently being written up for publication.

Significance to bio-medical research and the program of the Institute: The public health aspects of the mechanism of the killing of fecal organisms in sea water are obvious. An inhibitor of penicillin-resistant staphylococci would be of prime importance.

Proposed course of project: A cooperative arrangement has been worked out with Woods Hole Oceanographic Institution for continuation of the problem both here at the NIH and it is hoped at the Oceanographic Institution. Isolation of the substance(s) will be attempted. Since all the waters studied in this report were surface (10 M) it would be of interest to determine activity of deeper continental shelf water and deep, open ocean water. To this end, it is hoped a stay at W.H.O.I. this summer can be arranged so that use of the Oceanographic Institution research vessels can be utilized for this purpose.

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

none

Honors and awards relating to this project:

Appointed guest worker at the Oceanographic Institution, Woods Hole, Massachusetts.

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3. Bethesda, Maryland

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Individual Project Report
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Part A

Project Title: The electron transport system in an autotrophic hydrogenomad.

Principal Investigator: Dr. Roy Repaske

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 24/12
Professional: 12/12
Other: 12/12

Project Description:

Objectives: This research has as its object the identification of individual enzymes and intermediary cofactors which are operative in electron transport. This information may be transposable in part to mitochondrial systems and provide important clues about the electron transport sequence in these particulate structures. The long range plan will include an investigation of the energy yielding reactions coupled to electron transport.

Methods Employed: The methods employed will be those necessary for mass cultivation of organisms and for isolation and purification of the electron transport enzymes. Growth of large quantities of Hydrogenomonas eutropha is technically difficult because of the requirement for large volumes of an artificial gas mixture. Apparatus has been designed and is being built for this purpose. The assay of the initial enzymatic reactions in cell free preparations also requires a modified procedure because the enzymic activity must be measured in a hydrogen atmosphere in a spectrophotometer. Protein fractionation will be achieved by procedures involving ammonium sulfate precipitation, gel adsorption and elution, solvent precipitation, and chromatography.

Major Findings: It was readily apparent from the atypical kinetics and other indirect evidence that the enzymatic reaction being

studied, the oxidation of hydrogen by diphosphopyridine nucleotide (DPN), involved either a second enzyme or a cofactor. The cofactor which was first found in a boiled cell extract has been identified as riboflavin phosphate (FMN), but its requirement could be shown only when the enzyme was preincubated under hydrogen and reducing agent was added to the assay system.

Enzyme stability has been controlled by storing and handling the enzyme under an atmosphere of hydrogen and in the presence of cationic buffers of relatively high concentration (0.05 to 0.2 M). The pH optimum for stability is 8.0. A sharp pH optimum for enzyme activity was found at pH 7.5.

The enzyme catalyzing the initial reaction, i.e., DPN reduction, has been purified only three fold by calcium phosphate gel adsorption and ammonium sulfate fractionation. Unaccountable losses of enzyme activity during these procedures are at the moment unexplained, but may reflect inherently important characteristics of the complex. Consequently the reason for this loss of activity is under intensive investigation.

The most purified enzyme preparation has a very high affinity for riboflavin phosphate but it has not been possible to demonstrate FMN reduction of added exogenous FMN, indicating that the FMN is tightly bound by the enzyme and/it is not in equilibrium with the exogenous FMN pool.

Significance to bio-medical research and the program of the Institute: Hydrogenomonas species are unique organisms which obtain energy for all of their life processes through oxidation of hydrogen gas in the presence of oxygen. This is in contrast to animal cells and most bacteria which oxidize organic substances for energy. Although the initial substrates are different, there is every reason to believe that both types of systems have much in common. Both serve the identical function of providing energy for synthesis, growth and repair, and both utilize cytochromes and diphosphopyridine nucleotide to mediate the transfer of electrons to oxygen. Biochemical studies in the past have revealed that a common denominator exists between biochemical processes of various organisms, and this well supported concept of comparative biochemistry is the rational basis for investigating a given reaction in an organism most amenable to study.

Previous research on this organism was carried out at another institution by the principal investigator. The potential advantage of studying these reactions in H. eutropha lies in the fact that the system in this organism is soluble and therefore it can be fractionated by classical protein fractionation procedures.

The electron transport system in all other organisms studied to date has been associated with particulate elements of the cell. Efforts to dissociate some of these enzymes from the particles have met with limited success while other enzymes cannot be solubilized. Concomitantly with the occurrence of electron transport, ATP is generated; of the two processes, ATP generating system is the most labile during manipulation. As a result of these characteristics, our knowledge of electron transport and simultaneous ATP formation is sketchy and indirect. It is felt that the limitations associated with other systems may not exist in the H. eutropha system.

Proposed course of Project: More extensive purification of the DPN reducing system is planned to eliminate interfering enzymes and compounds and to demonstrate with reasonable assurance that only one enzyme participates in this reaction. The remaining reactions between reduced DPN and oxygen will be segregated so that the characteristics and requirements of each individual reaction in the sequence will be known. The system reconstructed with purified enzymes should have the same characteristics as those found in the crude extract. During these studies, the occurrence of ATP formation during electron transport will be explored as a preliminary survey to an investigation of this system.

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Part B. Honors,Awards, and Publications

Publications other than abstracts from this project:

Repaske, R. and Seward, C.: FMN as a cofactor in the enzymatic reduction of DPN by hydrogen. Biochem. Biophys. Res. Comm., 2, 397-401, 1960.

Litwack, G., Repaske, R. and Myrvik, Q.: Lysozyme as related to problems in Microbiology. Purdue University, 1960, 39 p.

Honors and awards relating to this project:

Gave the following invited seminars:

"Enzymatic Reduction of DPN by Hydrogen" - Gerontology Branch, NHI-NIH, at City Hospital, Baltimore, Maryland - Mar. 23, 1960.

"Enzymatic Oxidation of Hydrogen Gas by Extracts of an Autotrophic Bacterium" - Dept. of Bacteriology, Pennsylvania State University, State College, Penn. - Oct. 13, 1960.

"Enzymatic Reduction of DPN by Hydrogen with Extracts of Hydrogenomonas" - Dept. of Bacteriology, Indiana State University, Bloomington, Indiana, Nov. 16, 1960.

Panel Member in a Symposium on Lysozyme presented at Society of American Bacteriologists national meetings in Philadelphia, May 1960.

Elected to membership, American Society of Biological Chemists.

Nominated as candidate for Secretary of the General Division of the Society of American Bacteriologists.

Invited to participate in the 2nd International Symposium on Fleming's Lysozyme in Milan, Italy - April 7-9, 1961.

PHS-NIH
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Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Repaske, R. and Seward, C.: FMN as a cofactor in the enzymatic reduction of DPN by hydrogen. Biochem. Biophys. Res. Comm., 2, 397-401, 1960.

Litwack, G., Repaske, R. and Myrvik, Q.: Lysozyme as related to problems in Microbiology. Purdue University, 1960, 39 p.

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Elected to membership - American Society of Biological Chemists.

Nominated as candidate for Secretary of the General Division of the Society of American Bacteriologists.

Invited to participate in the 2nd International Symposium on Fleming's Lysozyme in Milan, Italy - Apr. 7-9, 1961.

Serial No. NIAID - 62-A
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Microbiological Fractionation of the Hydrogen Isotopes

Principal Investigator: Dr. F. D. Sisler

Other Investigators: Dr. Benjamin Prescott

Cooperating Units: U. S. Geological Survey

Man Years (calendar year 1960)
Total: 17/12
Professional: 9/12
Other: 8/12

Project Description:

Objectives: To determine the mechanism of hydrogen isotope fractionation by microorganisms; the divergent metabolic pathways of protium, deuterium and tritium; the effect of the heavy hydrogen isotopes on the physiology of the simple biological systems; the ecological and evolutionary significance of the isotope effect in the biosphere.

Methods Employed: Pseudomonas GLA and other bacterial cells and cell products, from culture media containing normal and enriched amounts of hydrogen isotopes (protium, deuterium, tritium)previously incubated under a variety of conditions simulating various environmental variables will be analyzed using methods involving the mass spectrometer, radioactive counting equipment designed for tritium compounds, paper chromatography, infra red and conventional biochemical analytical procedures.

Major Findings: The three isotopes of hydrogen, viz. protium, deuterium and tritium are selectively fractionated by microorganisms during metabolic processes involving hydrogen compounds. Samples of gas from marine sediments and from bacterial fermentation analyzed by mass spectrometer reveal a protium concentration in excess over that expected from non-biological processes. It would appear that in the marine environment various microorganisms

Part B not included.

particularly those containing hydrogenlyase and hydrogenase enzymes play an important role in hydrogen isotope equilibria. Mechanisms by which microorganisms may fractionate the hydrogen isotopes involve enzyme specificity, bond cleavage rates, cell wall diffusion and the formation of large insoluble molecules, e.g. protein-bound polysaccharides. The latter process resembles a sequestration effect in that the heavy isotope atoms are prevented from equilibrating with the medium so long as the cell wall is intact. After cell death and disintegration, predicted chemical equilibria is reestablished. Laboratory studies involving microbial activity on tritium compounds reveal a multi-staged process by which protium and tritium are fractionated. It is concluded that marine microorganisms are important geochemical agents in dynamic processes involving hydrogen and other isotopes in the sea. The evidence accumulated to date suggests that microbiological fractionation of the hydrogen isotope is a ubiquitous phenomenon throughout the biosphere.

Significance to bio-medical research and the program of the Institute: A knowledge of hydrogen metabolism of biological systems can be considered as one of the most fundamental of metabolic processes. Hydrogen metabolism (hydrogen transfer, electron transfer) is synonymous with the energetics of living processes.

With increasing use of both natural and radioactive isotopes in medical research, it is considered of utmost importance that attention be focused on fundamental studies which may contribute to the understanding of the isotope effects in biological systems. This study of the hydrogen isotopes in simple cell metabolism should, therefore, contribute basic information of value towards the solution of problems of public health and economic importance such as implications in radioactive waste disposal in marine environments, toxic effects of deuterium and other isotopes and further is of fundamental importance in studying new metabolic pathways in microbial and other systems.

Proposed course of project: To continue current research with the following objectives in view:

1. Isolation and identification of intracellular compounds primarily responsible for deuterium and tritium fractionation.

2. Examination of extracellular products showing heavy isotope enrichment.

3. Evaluation of the chemical thermodynamics involved in isotope fractionation, comparing experimentally established values with the theoretical.

4. Further studies on transport of isotopes across cell membranes; rate processes and steady state equilibria of all phases involved in isotope fractionation.

Serial No. NIAID 63
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Non-specific Immunity Associated with Serum Siderophilin and the Iron Metabolism of both Pathogen and Host.

Principal Investigator: Dr. Arthur L. Schade

Other Investigators: Dr. Lathrop E. Roberts

Cooperating Units: None

Man Years (calendar year 1960)

Total:	30/12
Professional:	18/12
Other:	12/12

Project Description

Objectives: To investigate the contribution of blood serum siderophilin to the natural resistance of the host; to investigate the iron requirements of selected pathogens and the effect of the iron-chelating siderophilin and conalbumin on in vivo iron accumulation, growth, and metabolism of these pathogens; to investigate the factors leading to changes in concentration of both serum iron and siderophilin in circulating blood as a result of infection and other abnormal conditions, and the consequence of such changes on resistance to infection, iron absorption by the host, and anemia.

Methods Employed: Bacterial and cultural procedures in specially deironized media; spectrophotometric, chromatographic, and radioisotopic methods; metabolic and enzymatic analytical procedures; and methods particularly suited to estimation of iron chelated either to siderophilin or conalbumin and to their relative unsaturations.

Part B included: Yes

Major Findings: Previous findings have established that some bacteria will not grow in serum unless the contained siderophilin is saturated with iron, while the growth rate of many others in serum is a function of the percentage iron-saturation of the siderophilin. We have, for our more intensive research investigations, restricted ourselves to Staphylococcus albus, whose growth in normal serum is negligible in the absence of excess iron, and to Staphylococcus aureus, whose growth rate in serum reflects the amount of bound serum iron compared with its total iron-binding capacity.

A- Proceeding from our preliminary studies on the metabolism of Staph. aureus cells grown in medium non-restrictive with respect to iron and those grown in a medium whose concentration of free ionic iron was severely limited by the presence of either siderophilin or conalbumin at low iron saturation (5%) we have observed the following facts:

1. The rates of oxygen consumption of both high and low iron cells in the presence of glucose are essentially the same but the aerobic and anaerobic glycolytic rates of the low iron cells are significantly increased over those of the high iron cells. Differences of rates up to three-fold have been observed. High iron cells oxidize pyruvate at a rate similar to that of glucose, while lactate is oxidized at approximately twice the rate as that obtained with pyruvate. Acetate and succinate are oxidized at rates 40% and 12% that found with pyruvate as substrate. Low iron cells, on the other hand, despite the similarity to high iron cells in their attack on glucose, fail to oxidize pyruvate but continue to oxidize lactate, albeit at a rate 1/3 to 1/4 that of the high iron cells. Low iron cells fail also to attack either acetate or succinate.

2. The extent of oxidation of several substrates by Staph. aureus cells cultured in media of "high" and "low" iron content was determined:

Molecules O_2 Taken up per Molecule of Substrate

Substrate	Theoretical Value for Complete Oxidation	Observed Values* "High" Fe Cells	Observed Values* "Low" Fe Cells
Glucose	6	3	1
Formate	0.5	0.5	0
Acetate	2	2	0
Pyruvate	2.5	1.5	0
Lactate	3	1.5	0.5

* Endogenous oxygen uptake subtracted.

3. The rate of anaerobic dismutation of 2 moles of pyruvate to one mole each of lactic acid, acetic acid, and CO_2 has been studied

manometrically and by chemical analysis. High iron cells carry out this reaction about $3\frac{1}{2}$ times as rapidly as low iron cells.

It is clear from these metabolic observations that the carbohydrate metabolism of Staph. aureus is profoundly affected by the availability to them of iron during their growth period. The results likewise indicate that iron, either directly or indirectly, participates in enzymatic activities not generally appreciated as being mediated by iron enzymes, e.g. the anaerobic dismutation of pyruvate. We recall that under physiological conditions, sera are often found in which the availability of iron to this and other pathogens is either greatly restricted or relatively unrestricted depending upon the percentage saturation of the siderophilin of the sera. Many of these results present new challenges to our understanding of the metabolism of Staph. aureus and require further investigation to elucidate the physiological response of this pathogen to the environmental changes that naturally occur in serum in vivo.

B. In an effort to prepare an iron-low, satisfactory growth medium for Staph. aureus, we devised a simple procedure for rapid and efficient iron removal from the complex culture medium, trypticase. The procedure, to be published, makes use of the difficultly water soluble bathophenanthroline as iron complexer followed by extraction of the remaining traces of bathophenanthroline in the medium with organic, immiscible solvents. The method reduces the labor of preparing an iron-low medium from a tedious, uncertain, time-consuming chore to a 3 to 4 hour treatment to yield large quantities of final medium whose iron content is below the sensitivity of the most sensitive colorimetric test available. Use of isotopic iron Fe^{59} indicates that the iron concentration is below 0.001 µg per ml of final medium.

C. In association with Dr. Knight of the Laboratory of Clinical Investigations we participated in an extended study of a case of Lupus Nephrosis which, upon examination, disclosed an unusually low concentration of siderophilin in the serum. The serum iron was extremely low because of the severely reduced siderophilin concentration and the low percentage iron-saturation of that present. We suggested the possibility of siderophilin loss in the urine and proceeded to analyze uring samples for this protein. At the height of the proteinuria, we found approximately 10% of the total protein lost in a 24 hour period (ca. 20 grams) to be siderophilin. Iron was also being lost through the kidney along with the siderophilin. Calculation showed that the patient was losing an amount of her iron-binding protein in 24 hours which was equivalent to that in the circulation at any given moment. The details of this investigation and the accompanying clinical data were presented by Dr. David Rifkind, et al, at the recent (Nov. 5, 1960) Regional Meeting of the American College of Physicians.

D. Our method employing Staph. aureus for the evaluation of the integrity of isolated siderophilins has been improved by the use of the ultrafiltrate of normal human serum plus 0.4% iron-free trypticase as bacterial growth medium. This medium better approximates whole serum employed as a control than previously used media. Three new isolated siderophilin preparations have now been found by this method to be equivalent in their iron-donation capacities to the native siderophilin present in serum. There is now considerable hematological interest in the possibility that human tissues other than bone marrow and reticulocytes may obtain their iron from the siderophilin-iron complex by the mechanism for which Staph. aureus is the model.

Significance to the bio-medical research and the program of the Institute: The study of the naturally occurring iron-chelating serum protein, siderophilin, contributes to our understanding of the non-specific immunity mechanism available to humans for protection against microbial infection. Investigation of the qualitative and quantitative effects of siderophilin on the growth and metabolism of the pathogen Staphylococcus aureus will provide useful information on the pathogenicity of this bacterium especially under conditions closely approximating in vivo conditions.

Proposed Course of Project: We shall investigate in detail the growth, metabolism, and enzymatic capabilities of S. aureus when grown under conditions approximating the in vivo state with especial regard to the effect of different serum iron-saturation levels on such characteristics. Special emphasis will also be placed upon the biochemical mechanisms by which iron controls the overall metabolism of the organism. In these studies, changes in the enzymatic function and the localization of iron containing components within various cellular structures will be investigated. Additional biological features such as hemolysis, effectiveness of phagocytosis, and possible differences in response to antibiotics of high and low iron-containing cells will be studied.

Part B. Honors Awards, and Publications

Publications other than abstracts from this project:

Schade, Arthur L.: The microbiological activity of siderophilin. Clinica Chimica Acta, in press.

Schade, Arthur L.: Methods applicable to the study of siderophilin. Behringwerk Mitteilungen, in press.

Honors and awards relating to this project:

Lectures:

By invitation, visited the laboratories and gave lectures appropritate to the interests of the audience on different aspects of, "Iron metabolism of bacterial pathogens and host tissues as mediated by siderophilin", at the following institutions:

1. Univ. of Vienna; Dept. of Physiology, Prof. Auerswald - Oct. 21 & 24.
2. Universities of Marburg and Giessen; Medical School, Prof. Schultze. Nov. 3 and 4.
3. Univ. of the Saar; Medical School, Prof. Rummel - Nov. 4 and 5.
4. Univ. of Freiburg; Medical School, Prof. Heilmeyer. Nov. 7 and 8.
5. Univ. of Berne; Dept. of Organic Chemistry, Prof. Nitschmann. Nov.9 & 10.
6. Univ. of Zurich; Medical School, Dr. Hitzig. Nov. 11 and 12.
7. Univ. of Tübingen; Medical School, Prof. Bennhold. Nov. 14 and 15.
8. Univ. of Frankfurt; Dept. of Pharmacology, Prof. Heinz. Nov. 17.
9. Berliner Mikrobiologische Gesellschaft Meeting; Prof. Höring of the Free University of Berlin. Nov. 22.
10. Univ. of Lund; Dept. of Clinical Chemistry (Malmö), Prof. Laurell. Nov. 28 and 29.

Serial No. NIAID 63-A
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Chemical Aspects of the Specific Binding of Iron to Serum Siderophilin and Egg White Conalbumin.

Principal Investigator: Dr. Robert C. Woodworth

Other Investigators: Dr. Arthur L. Schade

Cooperating Units: None

Man Years (calendar year 1960)
Total: 12/12
Professional: 7/12
Other: 5/12

Project Description:

Objectives: The goals of this project are to investigate the chemistry of the reactive groups in the siderophilin molecule responsible for the specific binding of iron and to determine the physico-chemical means by which the iron-siderophilin complex can be dissociated under physiologic conditions obtaining in pathologic and normal hosts.

Methods Employed: Methods include the employment of immunological assays for purity and for content of the iron-binding proteins, radioactive tracers, spectral analyses, ion-exchange chromatography, electrophoresis, polarography, and polarimetry.

Major Findings: The previously reported technique of determining the iron-release rate of the iron-siderophilin complex by means of iron^{59} isotope exchange has been applied to a detailed study of various parameters affecting the dissociation of iron from siderophilin.

Reaction: siderophilin-Fe_2^{56} + $2Fe^{59}$ ---> siderophilin-Fe_2^{59} + $2Fe^{56}$.

Assay: antibody + siderophilin-$Fe_2^{(59 + 56)}$---> antibody-siderophilin-$Fe_2^{(59 + 56)}\downarrow$. Count precipitate for Fe^{59} activity.

Part B included. Yes

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After requisite mathematical manipulation, the data were plotted and consistently showed two intersecting straight lines, rather than a single straight line. The occurrence of the two intersecting lines may be interpreted to mean that the two irons bound to a siderophilin molecule are attached to two distinct sites whose properties differ in such a way that a specific site must release its iron first in order that the other site may release its iron. A mathematical analysis of the system under study has been generously provided by Dr. Clifford S. Patlak, Biometrics Branch, NIMH. By setting up and solving the applicable differential equations, Dr. Patlak has provided us with a method for obtaining from our data the different dissociation rates of the two bound iron atoms.

The effect of pH on these dissociation rates has been of considerable interest to us. Studies of the two iron-release rates were made in whole serum at various pH's which were established by varying the percentage composition of the carbon dioxide-nitrogen atmosphere above the serum. It appeared that the first dissociation rate was dependent on the square of the hydrogen ion concentration, ie., if the hydrogen concentration were doubled, the rate quadrupled; and that the second dissociation rate was independent of pH. A study of the effect of buffer concentration at constant pH revealed, however, that the first dissociation rate was dependent on the first power of the concentration of the carbon dioxide-bicarbonate buffer system. Since this buffer system had been used for the pH studies, it became apparent that the rate of dissociation of the first iron was actually dependent on the first power of the hydrogen ion concentration and on the first power of the carbon dioxide-bicarbonate buffer.

In an attempt to determine whether the iron release rate is subject to "general acid catalysis" we examined the concentration effects of two other buffer systems, namely tris-(hydroxymethyl) amino methane and glycylglycinate, both in the region of pH 7. Not only were the isotope exchange rates considerably slower in these buffers than in the carbon dioxide-bicarbonate system, but they were not appreciably affected by large shifts in buffer concentration. It would thus appear that hydrogen ion and carbon dioxide-bicarbonate buffer are specific acid catalysts for the dissocation of iron from iron-siderophilin.

Associated with each iron in the iron-siderophilin complex is a bicarbonate ion. We found we could tag this bicarbonate with C^{14} by supplying $C^{14}O_2$ as the sole source of bicarbonate when forming the complex from iron (ferrous) and iron-unsaturated siderophilin. The C^{14} tag remained with the siderophilin during precipitin and washing procedures. A "double-tagging" experiment in which we followed both the rate of

dissociation of $HC^{14}O_3^-$ and the rate of incorporation of Fe^{59} showed that the bicarbonate may be released from the complex 10 to 100 times faster than the iron released. However, this experiment should be repeated under conditions more favorable to accurate determination of the desired rates.

The effect of temperature on both iron dissociation rates was studied. Both rates appeared to decrease with decreasing temperature, but no quantitative treatment of the data has been carried out.

Significance to bio-medical research and the program of the Institute: The shifts in bound iron levels occurring as a result of infectious diseases, acute infections, and allergic reactions are recognized. The availability of plasma iron to bacterial pathogens is a function of the siderophilin molecule's affinity for iron and of its degree of iron-saturation. Knowledge of the chemical bases of this affinity should elucidate the manner in which siderophilin governs the transfer of iron to the host as well as to the pathogen.

Proposed course of project: Since Dr. Woodworth is presently on an N.I.H. Research Fellowship with Prof. C.-B. Laurell in Malmö, Sweden and since he may choose to seek a University professorship rather than apply for reassociation with N.I.H. it appears that the course of this project will be: first, to prepare in comprehensive form for publication the accumulated data thus far obtained, and, second to pursue, as opportunity permits in his absence, the problem of the rates of association of iron with siderophilin in serum, particularly under physiological conditions, so that from the rates of dissociation and association, the concentration of free ionic iron in serum can be approximated.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Woodworth, Robert C. and Schade, Arthur L.: Immunological Precipitin Titrations based on Radioactive Tagginf of the Iron Naturally Chelated by the Proteins Siderophilin and Conalbumin. Accepted for publication, Biochimica et Biophysica Acta.

Honors and Awards relating to this Project:

Dr. Robert C. Woodworth was awarded an N.I.H. Fellowship (National Heart Institute) to study during 1960-61 with Prof. Dr. C.-B. Laurell, Head of the Kemiska centrallaboratoriet, Lund University, Malmö, Sweden.

Serial No. NIAID 63-B
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biochemistry of the acquisition of iron by mammalian tissues as mediated by the iron-binding protein, siderophilin

Principal Investigator: Dr. Arthur L. Schade

Other Investigators: Dr. Robert C. Woodworth

Cooperating Units: None

Man Years (calendar year 1960)
Total: 17/12
Professional: 6/12
Other: 11/12

Project Description:

Objectives: To investigate the mechanism by which mammalian tissues acquire from serum siderophilin the iron required for normal growth and function and to study the effect of physiological conditions upon this exchange.

Methods Employed: Hematological procedures, radioactive tracer techniques, manometric techniques and immunologic analyses.

Major Findings: Reticulocytes remove iron from siderophilin up to 67 times as fast as the natural dissociation rate of iron-siderophilin complex under physiologic conditions of pH and ionic strength. The rate of removal of iron from siderophilin by reticulocytes is independent of percentage saturation of siderophilin. Both iron atoms are removed by reticulocytes from this chelating protein even in the presence of excess ionic iron while iron chelated with conalbumin or complexed with ethylenediamine tetraacetic acid (versene) or in an ionic form is not removed or taken up at significant rates. The absolute rate of iron uptake by reticulocytes appears to be a function, inter alia, of the concentration of iron-siderophilin in such manner as to suggest that the cells possess a finite number of specific sites for accommodating the iron-siderophilin complex. The amount of iron taken up by a given blood sample is dependent on the number of reticulocytes present and no demonstrable iron is absorbed by the mature red cells. Reticulocyte uptake of iron is markedly restricted by anaerobic conditions. This fact suggests either that the iron exchange

Part B included: Yes

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from siderophilin to the cell is an oxidative, energy requiring process or that the synthesis of an iron acceptor system leading to hemoglobin production is oxygen dependent. Attempts thus far to approximate the iron uptake of the intact reticulocytes by a cell-free hemolysate prepared from reticulocytes have not yet yielded definitive results.

A significant practical consequence of this work is our development of a simple physiological test of the "integrity" of isolated, purified serum siderophilins in terms of their iron-donating or iron exchange capabilities. Progress in the study of the clinical uses of such isolated siderophilins has been stifled by the absence of such a test. Now, the employment of our reticulocyte iron-uptake method promises to stimulate the production of isolated siderophilin and its application to a great variety of experimental and clinical investigations. Our reticulocyte and Staphylococcus aureus iron-uptake tests together have served as guides to the American National Red Cross in its efforts to produce, on a large scale, purified siderophilin for such investigations. New methods of siderophilin isolation are being sought so as to preserve these physiological activities intact.

Significance to bio-medical research and the program of the Institute: As a first approximation, the normal functioning of host tissues requires iron for synthesis of their heme enzymes. Hence, the conditions of availability of serum iron to host tissues and the mechanism of its transfer from siderophilin are of significance to host-parasite relationships obtaining in infections.

Proposed course of project: We propose to investigate with the satisfactorily active isolated siderophilin, which heretofore has not been available but is now at hand in small quantity, the relationships of the absolute concentrations of iron-siderophilin to iron exchange and metabolism by reticulocytes and Staphylococcus aureus cells. We wish further to investigate the basis for the indicated catalysis of iron exchange by bone marrow and reticulocytes and to determine whether such a mechanism applies to non-erythropoietic tissues as well.

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Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Honors and Awards relating to this Project:

Named consultant to the American Red Cross to gather information for them on the serum protein fraction, siderophilin, at Wiesbaden and Bruges internists' and scientists' meetings in the Spring of 1960.

Gave invited lecture on "Siderophilin, Its Characteristics and Functions" to the research laboratory personnel of Parke, Davis & Co. on August 5, 1960.

Serial No. NIAID 63-C
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Development of an Economical 280mμ Light Source Suitable for Detecting Proteins in Effluents from Chromatographic Columns.

Principal Investigator: Dr. Robert C. Woodworth

Other Investigator:

Cooperating Units: None

Man Years (calendar year 1960)
Total: 2/12
Professional: 1/12
Other: 1/12

Project Description:

Objectives: We desired to develop a relatively low-cost light source for continuous photometric monitoring of chromatographic column effluents at λ 280mμ.

Methods Employed: Spectral analyses of filters developed in the course of the research and of the light source itself with various filters.

Major Findings: Filter combinations have been described by others for the isolation of various regions of the ultraviolet line spectrum of mercury. These filters provided a starting point for our investigations, but the combination suggested for the 280mμ region provided too wide a band-pass for our needs. This particular filter combination consists of (1) a 2mm Corning red-purple Corex A filter No. 9863, (2) a 1M $NiSO_4$ aqueous solution in a 1 cm light path vycor or quartz cuvette, and (3) a 0.02% aqueous solution of 2,7-dimethyl-3,6-diazocycloheptadiene-1,6-iodide (hereinafter referred to as "cyanine" iodide) in a 1 cm light path vycor or quartz cuvette. These three are placed in series between the lamp and photocell. The "cyanine" iodide possesses an absorption minimum at 263mμ. Dr. E. Kravitz of the National Heart Institute provided a pure sample of phenazine-α-carboxylic acid (produced by Pseudomonas aureofaciens). A 0.02% aqueous solution of this substance (neutralized with KOH to make it soluble) possesses an absorption minimum at 290mμ. An equal-weight mixture of "cyanine" iodide and phenazine-α-carboxylic acid made to a total of 0.02% in water (with KOH just sufficient to dissolve the acid) possesses an absorption minimum at 280mμ. This

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Part B not included.

solution in a 1 cm vycor or quartz cuvette, in series with a Corning red-purple Corex A filter and 1 cm of 1M $NiSO_4$, as described above, provides a spectrum with only a single, narrow transmission band with a maximum of 280mμ. Appreciable transmission appears again only in the far red (750mμ), a wavelength region to which commonly-used phototubes, i.e., No. 935, are completely insensitive. The filter has proved stable to ultraviolet radiation over an in-service period of six months, i.e., the transmission spectrum of the filter has remained unchanged.

When the output of a General Electric H3FE low-pressure mercury arc lamp is passed through this filter system and analyzed with a Beckman DU spectrophotometer, one finds many mercury emission lines other than 280mμ. This output spectrum is closely similar to that obtained from the same lamp filtered by a Corning red-purple Corex A filter together with a Baird Atomic 280mμ interference filter. This latter filter system and light source are used in a commercially available protein-monitoring unit. A vast improvement in the monochromicity of filtered light from this lamp is obtained from our filter system by increasing the "mixed-organic" filter (50% each of "cyanine iodide--phenazine-α-carboxylic acid) concentration three-fold to 0.06%. If the 280.4mμ transmission is now adjusted to 100%, the only other significant transmission lines found are 34% at 275mμ and 21% at 289.2mμ, both of which are strongly absorbed by proteins.

The "mixed-organic" filter has the additional virtue of possessing a variable transmission maximum between 263 and 290mμ, which is dependent on the per cent composition of the filter. Thus, it may be useful for isolating other spectral lines than 280mμ in this region.

The shops at NIH have done preliminary work on a stable photometric-recording system for use with this light source, but it is as yet not ready for use.

Significance to bio-medical research and the program of the Institute: The availability of an economical photometric analyzing system for protein-containing chromatrographic effluents makes possible the release for more important work of an individual, professional or technical, from the time-consuming, routine task of reading individual fractions in a manual spectrophotometer. Further, the use of a light source which will be specifically absorbed by proteins is of paramount importance, in order to provide adequate sensitivity for minor components and to make possible quantitative estimations of protein content, if this should be desired.

The 254mμ line of mercury utilized in some commercially-available ultraviolet monitoring devices is not suitable for use with proteins because of its demonstrated denaturing effects.

Proposed Course of Project: A more satisfactory light source might be found, i.e., one with fewer emission bands close to the 280mμ line. Such a lamp would allow for a lower concentration of the "mixed-organic" filter and thus permit a higher intensity of usable light. Search for such a light source will continue.

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Serial No. NIAID 64
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Detoxification of Potential Tuberculostatic, Fungistatic, Parasiticdal and Viricidal Agents.

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 13/12
Professional: 3/12
Other: 10/12

Project Description:

Objectives: Further studies on toxicity and detoxification of the tuberculostatic drugs streptomycin (SM), isoniazid (INH) and mixtures of streptomycin-isoniazid (SM-INH) in two strains of mice. In addition, to find means of reducing the toxicity of the widely used therapeutic agent Neomycin and of several parasiticidal drugs.

Methods Employed: The effect of various chemicals on the toxicity of INH and/or SM and Neomycin was measured by the effect on survival time of normal mice following simultaneous subcutaneous administration of a given adjuvant with a lethal dose of drug (both single and multiple administrations). The in vitro bacteriostatic action of the drug-adjuvant combination was also determined.

Major Findings:

1. With glycerol formal as solvent. Studies initiated with glycerol formal as solvent for INH and/or SM have shown that with a lethal 6 mg oral dose of INH in a 25% glycerol formal solution (in water) permitted 95% survival of a group of 60 white mice and 90% survival in DBA mice (50). However, on repeated daily administration,

Part B included: Yes

optimal results with no toxic manifestations were found in mice given a 5 mg dose (LD_{50}) in 35% solvent.

The use of this solvent with SM has thus far shown that a single subcutaneous administration of a lethal 15 mg dose of SM in 35% solution permitted 100% survival of mice. Decreasing the concentration of adjuvant to 25% permitted 100% survival and a 15% solution 90%. The detoxifying action of this solvent was tested on SM-INH mixtures in two strains of mice. 85% of the mice tolerated a lethal mixture of 10 mg SM and 4 mg INH and 80% survival with 10 mg SM and 8 mg INH.

2. With steroids: Further studies with steroids showed that 55% of both white and DBA mice tolerated a lethal dose of 30 mg SM and 4 mg INH when administered subcutaneously with 25 mg of sodium taurocholate. With a lethal dose of 20 mg SM and 6 mg INH or 15 mg SM and 8 mg INH, the same amount of adjuvant permitted 80 and 90% survival.

3. With L-argininyl-L-glutamate: Mice survived a lethal 15 mg dose of SM when it was administered in combination with 58 mg of the peptide L-argininyl-L-glutamate in both single and repeated doses.

4. With Miracil D: Further studies with this drug included the use of various steroids as possible detoxifying agents. Four steroids (cholic acid, sodium taurocholate, sodium glycocholate and sodium glycotaurocholate) were tested as adjuvants with various doses of Miracil D. Of the four steroids tested, sodium taurocholate showed good detoxifying activity. 100% of the mice tolerated a 30 mg dose of Miracil D (6 x lethal dose) when it was administered simultaneously with 50 mg of sodium taurocholate.

5. Detoxification of Neomycin: Since sodium glucuronate and glycine proved effective in permitting mouse survival when administered simultaneously with toxic doses of INH, SM and mixtures of SM-INH, the detoxifying activities of these adjuvants were tested with Neomycin in two strains of mice. When 10 mg of Neomycin per 20 g mouse (singly lethal) was injected simultaneously with a mixture of 50 mg glycine and 50 mg sodium glucuronate, 99% of the mice survived. Even if the Neomycin dose was increased to 20 mg, 90% of the mice survived. In chronic toxicity tests, 60% of the animals tolerated 15 repeated injections of the 10 mg dose of Neomycin if 50 mg glycine and 50 mg sodium glucuronate were injected simultaneously. No interference by the detoxifying agents tested was observed on the in vitro bacteriostatic action of Neomycin on one strain of Staphylococcus aureus.

6. With certain vitamins: Of 16 vitamins similarly tested with Neomycin, nicotinic acid and calcium pantothenate exhibited detoxifying activity. The last named in a molar ratio adjuvant and drug gave optimal results in combination with 10 mg of Neomycin. Survival was 100% in two strains of mice. Up to 15 daily doses of the Neomycin with calcium pantothenate were tolerated by 100% of the mice.

7. With amino acids: On single administration of a 10 mg dose of Neomycin per 20 gram mouse, D-glutamic, L-aspartic, and acetyl DL-methionine permitted 70 to 86% survival of white mice; 18 other amino acids tested showed little or no activity.

Significance to bio-medical research and the program of the Institute: The widespread use of the chemotherapeutic agents streptomycin and isoniazid in the treatment of human tuberculosis is limited by their toxicity. Similarly use of Neomycin is also restricted. Means of increasing the tolerance of these toxic chemotherapeutic drugs should permit more effective therapy.

Proposed course of the Project: This study will be extended to include in vivo tests and to a few more adjuvants that show promise.

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Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Prescott, B., Kauffmann, G. and Stone, H. J.: Means of increasing the tolerated dose of isoniazid-streptomycin mixtures in mice. II. Certain Vitamins. Antibiotics and Chemotherapy 10, 163-168, 1960

Honors and Awards relating to this Project:

Invited to participate in "Symposium uber experimentelle und klinische Pharmakologie der Antibiotika" to be held in Aachen, Germany, May 18-19, 1961.

Serial No. NIAID 64-A
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Potential Fungistatic and Parasiticidal Agents.

Principal Investigator: Benjamin Prescott

Other Investigators: None

Cooperating Units: Section on Mycology, LID-NIAID
Dr. Chester W. Emmons - Serial No. 76-B

Section on Chemotherapy, LPC-NIAID
Dr. George Luttermoser - Serial No. _____

Man Years (calendar year 1960)

Total:	13/12
Professional:	3/12
Other:	10/12

Project Description:

Objectives: Synthesis of new potential non-toxic chemotherapeutic agents against fungal and parasitic infections.

Methods Employed:

Antifungal agents: A series of 30 dithiooxamide derivatives were synthesized by treating a solution of dithiooxamide with hydrazine hydrate forming a dihydrazide. The resulting compound was then condensed with various aliphatic and aromatic aldehydes in 95% ethyl alcohol. The final recrystallized products were analyzed for carbon, hydrogen, nitrogen and melting points obtained. Toxicity studies were performed in white mice. Since thiosemicarbazones have been shown to have chemotherapeutic activity (tibione against tuberculosis and isatin thiosemicarbazone against viruses), a series of 50 long chain thiosemicarbazones were synthesized by first preparing octadecylthiosemicarbazide and condensing this compound with various aliphatic, aromatic and heterocyclic aldehydes.

Part B not included.

Additional derivatives of thymol, naphthoquinones and 4,4'diaminodiphenylsulfone were synthesized. In mice, toxicity of these compounds was of a very low order. Dr. Chester W. Emmons, LID-NIAID, will test the fungistatic activity of these compounds.

Parasiticides: A new series of piperazine derivatives were synthesized by condensing N-aminoethyl piperazine with aldehydes, carboxylic acids, sulfonic acids, isocyanates and isothiocyanates. In addition, 45 derivatives of tetracycline were prepared by coupling tetracycline with compounds like atabrine, chloroquine, piperazine monocarboxylic acid, sulfanilic acid, hetrazan and several naphthoquinones.

Major Findings: Several of the tetracycline derivatives demonstrated considerable in vitro activity against parasites and showed negligible toxicity in mice.

Significance to bio-medical research and the program of the Institute: Because of high tolerance and effectiveness, the tetracycline derivatives may be useful in the chemotherapy of human parasitic infections.

Proposed course of Project: Tests for usefulness of the antifungal and antiparasitic compounds are to be performed in vivo in laboratory animals (mice and dogs) to determine their effectiveness in experimental infections. All the compounds synthesized for antifungal and antiparasitic activity are also being tested for anti-tumor activity. About 400 compounds have been sent to the Cancer Chemotherapy screening program. Several long chain thiosemicarbazones have passed the initial tests in mice.

Serial No. NIAID 64-B
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Isolation of Antibacterial and Antiviral Substances from Shellfish

Principal Investigator: Benjamin Prescott

Other Investigators: None

Cooperating Units: Laboratory of Virology and Rickettsiology, Division of Biologics Standards
Dr. Chen Pien Li - Serial No. DBS_____

Man Years (calendar year 1960)
Total: 16/12
Professional: 6/12
Other: 10/12

Project Description:

Objectives: To isolate and purify potential therapeutic agents from shellfish.

Methods Employed: Juice from fresh frozen abalone and extracts of oysters and clams were each dialyzed against distilled water, the residue adjusted to pH 7.8 and applied to anion-exchange (diethylaminoethylcellulose)columns set up on automatic fraction collectors in the cold (4° C). Elution of material from the column was carried out with a series of tris-H_3PO_4 buffers of varying ionic strength and pH (3.7 - 7.6) and a final wash with M-NaCl. Eluates were consecutively collected. Several pools were made of various fractions, dialyzed against distilled water and lyophilized.

Major Findings: It was found that the early eluate pool fractions contained antibacterial activity against both penicillin-sensitive and -resistant strains of Staphylococcus aureus, a beta-hemolytic strain of Streptococcus pyogenes, Salmonella typhi, and S. paratyphi A and B. The growth of 10,000 or more organisms per ml was inhibited by these fractions in a concentration of 10 mg per cent. These fractions showed no antiviral activity. The fraction isolated from the final wash of the column with M-NaCl showed no antibacterial activity. However, there was definite inhibitory activity against influenza A virus and polyoma virus in tissue culture.

Part B included: Yes

Significance to bio-medical research and the program of the Institute: The abundance and variety of shellfish is a potential source of antibacterial and antiviral agents. Since fractions isolated from this source were found to possess marked antibacterial activity, they may be valuable as therapeutic and prophylactic agents.

Proposed course of Project: Preparation of large quantities of active fractions and chemical identification of the agent are in progress. In addition, the therapeutic efficiency of the agents is being studied in protection experiments in mice experimentally infected with streptococci and pneumococci.

Serial No. NIAID 64-B

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Prescott, B. and Li, C. P.: Abalone juice: Fractionation and antibacterial spectrum. Proc. Soc. Exp. Biol. & Med. (in press)

Honors and Awards relating to this Project:

None

Serial No. NIAID 65
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Longitudinal Studies of Beta-hemolytic Streptococcal Isolations.

Principal Investigator: Roger M. Cole

Other Investigators: Edythe J. Rose

Cooperating Units: Dept. of Pathobiology
School of Hygiene and Public Health
Johns Hopkins University
(Dr. Wm. J. L. Sladen)
Bacteriology Laboratory
Johns Hopkins Hospital
(Dr. Jack Causton

Man Years (calendar year 1960):

Total:	30/12
Professional:	12/12
Other:	18/12

Project Description:

Objectives: An aspect of studies continued in this laboratory for several years, to extend knowledge of human experience with streptococci through culture, serologic and epidemiologic methods.

Methods Employed: In general, similar to laboratory and epidemiologic methods described in projects for other years. In particular, throat swabs taken routinely from personnel in isolated situations in Antarctica were mixed in glycerine broth which was then deep-frozen and transported to the United States. Some months later, samples were plated from the thawed fluids and colonies of beta-hemolytic streptococci picked for serologic identification and tested for bacitracin sensitivity at a level said to distinguish Group A from other beta-hemolytic streptococci. Sera from the same personnel were taken at intervals and kept frozen for future reference. Simultaneous throat swabs were taken for virus isolation by another group (Virus & Rickettisal Section, LID). In the past,

Part B included: Yes

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somewhat similar studies have been made in institutionalized children (Jr. Village, D.C.); and studies of other methods of transport of streptococci prior to culturing were made in collaboration with investigators in California (U. Cal. Berkeley: see publication, Part B).

Major Findings: Approximately 240 cultures of beta-hemolytic streptococci have been received from the collaborating laboratory for identification. Most of these represent the same persons, repeatedly positive for streptococci of the same group or type of Group A. About 38% of the cultures were Group A, 25% Group B, 6% Group C, 30% Group G, and the small remainder failed to grow or to react serologically. About 64% were bacitracin-sensitive by the test used: 98% of Group A were sensitive as expected, but so were 57% of Group C, 49% of Group G and 31% of Group B. The test as used is obviously not an adequate screening method for distinguishing Group A from other beta-hemolytic streptococci.

Significance to bio-medical research and the program of the Institute: Only in isolated or semi-isolated situations with limited possibilities for introduction of new organisms can the persistence of throat flora be adequately examined. Whether, under such conditions of carriage, beta-hemolytic streptococci of Group A induce antibody formation (and if so, how or why they persist) is a matter of considerable interest in evaluating the various aspects of streptococcal virulence and resistance to streptococcal infections.

Proposed Course of Project: Continue. Antibody responses will be tested by improved methods described under another project. Additional culture and serum specimens are expected from Arctic areas within the next year.

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Hollinger, N. F., Lindberg, L. H., Russell, E. L., Sizer, H. B., Cole, R. M., Brawne, A. S., and Updyke, E. L.: Transport of streptococci on filter paper strips. Pub. Hlth. Rep., 75: 251-259, 1960.

Honors and Awards relating to the project:

None

Serial No. NIAID 65-A
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Streptococcal M protein, virulence, and type-specific immunity.

Principal Investigators: Dr. Jerome J. Hahn & Dr. Roger M. Cole

Other Investigators: None

Cooperating Units: Laboratory Histology and Pathology, NIDR (Dr. David Scott) (Dr. Marie Nylen) NIDR - 4

Laboratory Biology of Viruses, NIAID (Dr. C.F.T. Mattern)

Man Years (calendar year 1960):
Total: 50/12
Professional: 20/12
Other: 30/12

Project Description:

Objectives: In general, to study Group A streptococcal M protein and of antibody thereto, in order to better understand virulence and resistance to infection among gram-positive cocci. Specifically; (a) to improve methods of preparing antigenic M protein and to determine the differences between M proteins which account for type specificity; (b) to investigate and improve methods for detecting antibody to M protein; (c) to study the relation between M protein, or other possible cellular components, to streptococcal virulence; and to determine more precisely the cell wall localization of M protein and its significance.

Methods Employed:

(a) Neutralized acid extracts of cells of 5 streptococcal types were precipitated with saturated picric acid, and the precipitates extracted with 80% acid ethanol: supernates were pooled

Part B included: Yes

and further precipitated by acetone. These washed and dried precipitates were dissolved in saline and gradually neutralized, with removal of precipitates as formed. Active material was in the supernates, which were analyzed for M protein immunologically, for carbohydrate by the Molisch reaction, and for nucleic acid by the orcinol method. The immunologic tests were made by antigenicity in rabbits and by type-specific precipitation in liquid and in agar gel diffusion systems.

M proteins, prepared by both the conventional (acid hydrolysis-ammonium sulfate precipitation) method and the picrate method, have been subjected to immunoelectrophoresis. In addition, attempts to fragment M protein into immunologically reactive portions are being made by differential heating with pH control and by fractional enzymatic degradation.

(b) Detection of type-specific antibody to M protein has been examined by two methods: (1) the long chain reaction, in which streptococcal chains are significantly longer in homologous antiserum than in heterologous or normal serum, and (2) inhibition by unlabelled test antisera of fluorescence on addition of fluorescein-labelled specific antiserum. Both methods have been compared with the usual precipitin and bactericidal tests. The nature of type-specific antibody is being examined by conversion to univalent fragments by pepsin-cysteine or papain treatment. Such univalent antibody will be tested by blocking of precipitation, blocking of fluorescence, precipitation, specific fluorescence after labelling, bactericidal activity, and the long chain reaction.

(c) Virulence of streptococcal strains is tested by intraperitoneal injection of culture dilutions in mice, with subsequent determination of 50% lethal doses. Bactericidal and phagocytic tests in rabbits or human whole blood with or without added specific sera, or with mouse leukocyte suspensions, are also used to detect presence in living cells of the presumptive virulence factor, M protein. Addition of homologous and heterologous, partially purified, M proteins to phagocytic systems, has been used to determine if the extracted proteins enhance virulence by protection against phagocytosis.

Differential blocking of fluorescence by group and type-specific antisera applied in different sequences, is being used to aid in localization of M and of group carbohydrate in the streptococcal cell wall. Sections of fixed and embedded streptococci have been made and examined under the electron microscope, as a preliminary to the use of ferritin-labelled antibody for the electron microscopic localization of cell wall components.

Major Findings:

(a) M protein picrates, made as described, contain less than 0.1% each of carbohydrate and nucleic acid. During alkalinization in their final preparation, picrates of Types 1 and 18 form precipitates at a lower pH (3.7) than do those of Types 2, 4, and 28 (pH 4.5). Picrates of Types 1, 4, and 18 give precipitin reactions only with their homologous type antisera, whereas that of Type 28 reacts also with antisera to Types 2, 13 and 44. The possibility of the presence of "R" protein, instead of, or in addition to, M is thus raised; and preliminary studies in agar gels indicate that at least two reactive antigens may be present in the picrate preparations. Early results from intramuscular injection of a Type 1 picrate indicate that the material is weakly antigenic, requiring 4 weekly injections of 5 mg. each to produce antibody detectable in undiluted serum by the long chain test.

Immunoelectrophoresis of conventionally prepared M proteins demonstrates differences between Types 1 and 23. A single precipitin band is obtained when the former is tested against homologous antisera, whereas Type 23 always shows two bands or spurs which move together and cannot be separated by variations in buffers, pH, voltage, nor time of run.

Boiling M protein at an acid pH for between 30 and 60 minutes appears to produce a fragement which binds with but does not precipitate, specific antibody. Similar studies, using enzymes, are in progress.

(b) The long chain test for type-specific antibody has been statistically improved by a more rapid and simpler method, utilizing the chi-square-analyzed differences in frequency distributions of chains above and below a predetermined length, in test and normal sera. Interval sampling has shown that time of incubation prior to reading the test is of prime importance: chains increase in length, and long chains increase in frequency, to a maximum with time and then decrease. The decrease depends on antibody depletion; and maximum length or frequency is reached sooner and is lower in low concentrations of antibody than in high. As a result, time interval sampling allows titration of antibody by determining the highest dilution which gives a positive result according to predetermined statistical criteria. The effect of dilution of streptococcal inoculum in test is opposite to that of antibody dilution. Total coccal growth is unaffected by the presence of antibody. The Size Class Frequency method of determining a positive long chain reaction has been shown to be reproducible and more sensitive than the previous method using mean chain lengths.

Fluorescein-labelled group-specific and type-specific antisera have been successfully prepared. No cross reactions among antisera to several

different types have been shown by this method. Inhibition by unlabelled antisera of specific fluorescent antisera appears to be a simple and sensitive method for determining the presence of type-specific antibody. Its use in titration is being compared with the long chain and bactericidal tests, but appears somewhat limited by the subjective determination of the fluorescent end point.

A method of labelling portions of the streptococcal cell wall by growth in fluorescent antiserum with subsequent removal of excess antibody, has been devised. Its use in following cell wall formation and in analysis of the mechanism of the long chain test is being investigated.

Univalent antibody, produced by pepsin-cysteine treatment of whole antiserum, has been shown capable of blocking precipitin and fluorescent reactions, but does not produce long chains. Additional studies of the nature of streptococcal type-specific antibody are under way.

(c) M protein of Type 23 has been reported to enhance phagocytosis when added to a mouse leukocyte system containing antibody and virulent Type 23 streptococci. This experiment was repeated and confirmed, but similar enhancement in a Type 1 system was not found. No increase in phagocytosis by heterologous M protein occurred. Because M, as the presumptive virulence factor, was expected to prevent phagocytosis rather than the reverse, the possibility of a different virulence factor -- at least in Type 23 -- arose. To see if removal of anti-M from a serum made against virulent organisms would leave an "anti-virulence factor", M+ avirulent variants were derived from Types 1 and 23, and then used to absorb sera prepared against virulent organisms. These antisera contained "anti-virulence factor", as shown by their mouse-protective capacities. After absorption to the point of removal of type-specific precipitins, but not of long chain producing antibodies, mouse protective antibody was still present. Further absorption removed both long-chain producing and mouse protective antibodies from both Type 1 and Type 23 antisera. The effect appears to be a quantitative one, and this method failed to distinguish a different virulence factor than M protein.

The suspected surface location of M protein on the streptococcal cell wall was verified by fluorescence-inhibition experiments. Unlabelled group-specific antiserum failed to block the fluorescence of labelled type-specific antiserum, whereas unlabelled type-specific antiserum inhibited the fluorescence of added group-specific antiserum. The type-specific antigen (M) therefore appears to be superficial to the group antigen, or a peculiar steric relationship may exist. Similar experiments, using ferritin-labelled antibody and the electron microscope, are being initiated.

Significance to bio-medical research and the program of the Institute: Group A streptococcal infections in man are common, even today with the widespread use of antibiotics. Immunity to such infections, shown in a limited number of instances and by use of difficult and tedious methods, has been said to be type-specific. The use of rapid and improved methods such as we have described will greatly facilitate verification and expansion of these findings and allow epidemiological and clinical serologic studies on a scale not previously possible.

The role in virulence of the M protein which stimulates such type-specific antibodies is widely accepted but not understood. Mouse virulence is the usual measure, but many M+ strains freshly isolated from man are not mouse-virulent without passage: other discrepancies occur, and the possibility of other virulence factors requires further study. Investigations of streptococci, which are well characterized antigenically, may serve as useful models for other gram-positive cocci such as staphylococci which are at present poorly defined antigenically.

Proposed course of Project: Continue along lines indicated.

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Kantor, F. S., & Cole, R. M.: Preparation and antigenicity of M protein released from Group A, Type 1 streptococcal cell walls by phage-associated lysin. J. Exp. Med., 112: 77-96, 1960

Honors and Awards relating to the project:

None

Serial No. NIAID 66
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Epidemiologic Studies of Illnesses and Microbial Experience of Junior Village Nursery Children

Principal Investigator: Dr. Joseph A. Bell, Dr. Albert Z. Kapikian

Other Investigators: Dr. Francis M. Mastrota and Dr. Robert J. Huebner

Cooperating Units: Virus and Rickettsial Diseases Section, LID, (Dr. Chanock, Dr. Johnson), Epidemiology Laboratory Unit, (Dr. Rosen), Oncolytic and Oncogenic Virus Unit, (Dr. Rowe), Virology Section, Perinatal Research Branch, NINDB (Dr. Sever), Parasitic Disease Service, LCI, NIAID (Dr. Beye), Infectious Disease Service, LCI, (Dr. Utz), Pediatrics Service, D. C. General Hospital, (Dr. Reichelderfer)

Man Years (Calendar year 1960):
Total: 62/8
Professional: 19/8
Other: 43/8

Project Description:

This is a long term, very intensive study of the illness and microbial experiences of nursery children at Junior Village, a District of Columbia Welfare Institution. It began in July 1955 and provides research material for many projects, e.g. 66-A, 66-B and others.

(A) Basic Objectives: The general objective is to maintain under observation a population group suitable for epidemiologic study of occurrence of infection and disease and host-parasite-disease-relationships as they occur naturally in this group and as they can be altered by chemo prophylaxis and new vaccines. One of the chief interim objectives is the development of epidemiologic, clinical and laboratory tools and methods for studying infectious diseases. A concerted effort is being made to find new microbiologic agents which cause disease, methods of identification of these agents, modes of spread and methods for prevention and treatment of acute illnesses, particularly respiratory illnesses. The study is designed to shed light on the nature and scope of studies which should be pursued in further search for methods of control of acute infectious diseases particularly, the large mass of acute respiratory diseases, including the common cold.

Part B included [X] Yes [ ] No

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Methods Employed and Patient Material:

The study children are located in Southwest Washington, D. C. The daily population is now close to 130 white and negro babies six to 40 months of age who are in residence in Eisenhower Cottage and the Infirmary for domiciliary care. The mean duration of residence is approximately 17 weeks per child. Children with illnesses are studied either in their domicile or the infirmary at Junior Village, or the Clinical Center at N.I.H., or at the D. C. General Hospital, depending upon the severity of illness and study interest.

A full-time pediatrician, three nurses, and four nurses' assistants maintain constant medical surveillance of the children, record rectal temperatures twice every day, collect specimens for laboratory study and prepare clinical records on each child each day, regardless of whether or not ill. Both throat and anal specimens for virus study are collected on admission, and three times weekly from every child, and otherwise when indicated. Blood specimens are collected on admission, on discharge, six weeks after admission, every three months during residence, and under special circumstances.

Major Findings:

Epidemiologic methods have been devised for recognition of febrile departure from normal health and illnesses have been classified as (A) questionable fever, (B) definite fever with clinical findings (FUO) and (C) definite illnesses, i.e., fevers associated with clinical findings.

Two major epidemics of interest have occurred in the past year in Junior Village.

In a period from April 24 to May 13, 36 or 40% of the 90 residents developed pneumonia. In the Eisenhower Cottage where the older children resided, 16 (25%) of the 65 children contracted pneumonia while in the infirmary where the younger children resided 20 (80%) of 25 developed pneumonia. Respiratory syncytial virus isolation from throat swabs was associated with the pneumonia illness indicating an etiologic relationship. Also 91% of 80 infants and children tested showed 4-fold or greater rises to RS antigen by complement fixation and/or neutralization tests. A four-day incubation period of RS pneumonia was also clearly shown. The pneumonia illness was severe with the average of the highest fever in each patient being 103°F.(Rectal) and with the duration of a fever of 100.6°F. or greater being about 4 days.

An interesting outbreak of infectious lymphocytosis was recognized in August 1960 when 4 of the children were discovered to have white blood counts over 60,000 per cu. mm with 95% lymphocytes on differential smear. This initiated an intensive effort to study the epidemiology, the clinical course, and the laboratory aspects of the disease. White blood counts were done once weekly on all the 130 residents. Appropriate material has been studied in laboratory animals and in tissue culture in attempts to find the etiologic agent. Stools have been tested for ova and parasites. Patients have been admitted to the Clinical Center and D. C. General Hospital to study the

clinical findings. Thus far, no clinical finding except for the lymphocytosis has been significantly associated with the disease. Over 25 children have contracted the lymphocytosis with no relationship seen to age, or length of time in residence at Junior Village. An incubation period of 11-13 days is postulated. With the paucity of literature on the subject of infectious lymphocytosis, this study should add measurably to the definition of the disease.

Significance to Bio-medical Research and the Program of the Institute:

This project provides a source of specimens for finding new causes of illness, for studying their etiologic significance and for testing methods of control. It is significant that long term Junior Village studies of host-parasite-disease-relationships are being carried out on the bulk of diseases which commonly cause misery and absenteeism from schools and industry. The studies are of such a nature that they are not likely to be conducted by other than a government group. The studies are progressing meticulously and fairly satisfactorily to the investigators.

Proposed Course of Project:

Clinical epidemiological observations are being continued together with collection of throat and anal specimens one to three times weekly for virus study and collection of routine blood samples. It is planned to continue the study for as long as it continues profitable.

Serial No. NIAID 66

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Part B Honors, Awards, and Publications

** A Study of the Hemadsorption (Para-Influenza)* And Other Viruses in Children with and without Respiratory Disease

Albert Z. Kapikian, M.D., Robert M. Chanock, M.D., Joseph A. Bell, M.D., Dr. P.H., Thomas E. Reichelderfer, M.D., M.P.H., Robert J. Huebner, M.D.

** Published in PEDIATRICS August 1960

Serial No. NIAID 66-A
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Epidemiologic Studies of Host Parasite Disease Relationships

Principal Investigator: Dr. Joseph A. Bell

Other Investigators: Dr. Francis M. Mastrota, Dr. Albert Z. Kapikian, Dr. Robert J. Huebner

Cooperating Units: Virus and Rickettsial Diseases Section, LID, (Dr. Chanock, Dr. Johnson), Epidemiology Laboratory Unit, (Dr. Rosen), Oncolytic and Oncogenic Virus Unit, (Dr. Rowe)

Man Years (Calendar year 1960):
Total: 15/8
Professional: 5/8
Other: 10/8

Project Description:

(1) Objectives: To delineate which of the many microbial agents are causing acute illness in Junior Village nursery and describe clinical and epidemiological nature of etiologic entities found.

Patient Material:

The Junior Village nursery studies are described in project number 66. Epidemiologic methods are being devised to determine which of the 53 typed viruses and the many typed bacteria, which have been isolated, were causing illness.

The high frequency of illness occurrence (mean weekly definite illness attack rates of 25 %) and the even higher frequency of new and overlapping infections with potentially pathogenic microbial agents presents an extremely complex epidemiological problem to establish specific etiologic relationships. One of the chief problems is serological determination of susceptibility and immunity to the many specific infections, or illness attributable thereto; with the limited amounts of serums available. To guide the most profitable use of these serums and for other purposes preliminary analyses of temporal relationships between onset of specific infection and onset of acute undifferentiated illnesses have been

Part B included [ ] Yes [x] No

completed and top priority for use of available serums can be established on the basis of resolving the etiologic relationship of the more serious illnesses, study of the many newly discovered viruses and study of new vaccines. Within these limits the meticulous and time consuming analysis of specific etiologic relationships is in progress.

Major Findings:

The preliminary analyses of temporal relations between onset of specific infection and onset of acute undifferentiated febrile illness using both horizontal and cross sectional controls have shown no evidence that the following agents were causing illness in the nursery babies: Adenovirus 2; Polio 3; Coxsackie B 5; ECHO 7, 8, 11, 12, 13, 14, 18, 19, 20, 25 and JV 5; nontypable Group A Streptococci; Hemophilus Influenza B; Pneumonia Type 19, 23; Staphylococci having Phage Type 81 in their antigenic pattern; Enteropathogenic coli of various types, and alkalescens dispar. Infection with the following agents were significantly associated with the occurrence of acute, febrile, undifferentiated illness; Adenovirus 1, 3, and 5; Influenza virus Asian; parainfluenza virus 1 and 3; Poliovirus 2, Coxsackie virus B 3; Group A Streptococci types 4, 12, 23, and 1, 2, and 5; and Shigella Sonnei. In general, it is crudely estimated that some 45% of the more severe illnesses of the nursery group may be accounted for by the above agents and that measles and adenovirus infections account for the bulk of the more severe illness.

Significance to Bio-medical Research and the Program of the Institute:

Although the study is deliberately limited to a group of young children who have a high proportion of susceptibles to various microbial infections, it shows some of the potentialities of such infections in older susceptible persons. This project represents a systematic approach to determining the various etiologies of acute febrile illnesses so as to guide research on development of methods for control.

Proposed Course of Project:

It is planned to continue the study for as long as continued collection of data and data analyses appear profitable.

Serial No. NIAID 66-B
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Epidemiologic Studies of New Vaccines and Chemoprophylactic Agents

Principal Investigator: Dr. Joseph A. Bell

Other Investigators: Dr. Francis M. Mastrota, Dr. Albert Z. Kapikian, Dr. Robert J. Huebner

Cooperating Units: Virus and Rickettsial Diseases Section, LID, (Dr. Chanock, Dr. Johnson) Epidemiology Laboratory Unit, (Dr. Rosen), Oncolytic and Oncogenic Virus Unit, (Dr. Rowe)

Man Years (Calendar Year 1960)
Total: 29/8
Professional: 4/8
Other: 25/8

Project Description:

Objectives: (A) To design and have prepared monovalent and multipolyvalent vaccines with inactivated viruses which have shown evidence of producing important illnesses. (B) To carry out preliminary trials of such vaccines for determination of antigenicity and untoward reactions. (C) To observe whether such vaccines substantially reduce the occurrence of infection or illnesses in the Junior Village Institution.

Patient Material:

The Junior Village nursery studies are described in project numbers 66 and 66-A. The rather intense daily clinical observation permits an evaluation of vaccine induced reactions. The vaccines which have been used are: (1) Adenovirus types 1, 2, 3, and 5; (2) Adenovirus types 3, 4, and 7; (3) Coxsackie B virus types 1, 2, 3, 4, and 5; (4) Rubeola virus; (5) Respiratory vaccine containing 12 virus strains, namely, monkey kidney cell grown influenza A1, A2, and B viruses; Adenovirus types 1, 3, 4, 5, 7; Parainfluenza virus types 1, 2, and 3; (6) Poliovirus types 1, 2, 3 - three different products; (7) Parainfluenza 1 and 3 - egg grown. In addition, a daily oral dose of 300,000 units of benzathine Penicillin has been given to a 20% random sample of all children. The vaccines are given at time of admission, with the second dose three weeks later and a third dose at three months. The vaccines are

given in one ml doses to preselected random samples of all children admitted to the Junior Village nursery group. Blood sera for antibody studies is collected on admission, at six weeks, and every three months thereafter and at discharge.

Major Findings:

Analysis of the previously mentioned products is in progress currently. Preliminary analysis showed the inactivated rubeola monkey kidney vaccine to offer no protection in preventing rubeola. Serologic studies on this vaccine are in progress.

The respiratory vaccine containing 12 virus strains did not alter the illness pattern in those receiving it with the exception of a period in January 1959 when Asian influenza was prevalent at Junior Village. At that time, those children who had received the vaccine had significantly less illness than those without the vaccine. The ability of the vaccine to produce CF antibody to Asian influenza antigen was shown. Differences between vaccinees and controls at other times of the year were not evident.

Significance to Bio-medical Research and the Program of the Institute:

It is obvious that the many acute febrile respiratory diseases and fevers of undetermined origin which are a major public health problem are caused by a great multiplicity of etiologic agents. To prevent or ameliorate these illnesses by vaccine prophylaxis requires a multipolyvalent vaccine in as much as no one of these known agents are producing a very large proportion of these diseases. It seems timely to initiate controlled epidemiologic studies in the use of multivalent vaccines and their possible enhancement or interference with each other.

Proposed Course of Project:

It is planned to continue the study for as long as collection of data and data analyses appear profitable.

Serial No. NIAID 66-C
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Etiology of Respiratory Disease on a College Campus

Principal Investigator: Dr. Albert Z. Kapikian

Other Investigators: Dr. Karl Johnson, Dr. Robert Chanock, Dr. Joseph Bell
Dr. Robert Huebner

Cooperating Units: University of Maryland Infirmary

Man Years (Calendar Year 1960)
Total: 0/8
Professional: 0/8
Other: 0/8

Project Description:

Objectives: (1) To determine the role of known viruses as etiologic agents in a young adult population; (2) To find new agents which might be associated etiologically with respiratory disease.

Patient Material:

With the cooperation of the University of Maryland Infirmary, a study was undertaken in October 1958 to determine the role of viruses in the etiology of respiratory diseases in a young adult population. Patients who appeared with complaints referable to their respiratory tract were examined. Throat swabs and acute and convalescent bloods were also obtained. An attempt to have a control group of nonrespiratory patients was unsuccessful. The throat specimens were tested for viruses in appropriate tissue culture and the blood was tested for CF antibody against a battery of respiratory virus antigens.

Major Findings:

The finding of a new parainfluenza virus type 4 was presented previously.

Two hundred twenty-four patients with respiratory disease were seen--among these, approximately 20% had fever of 99°F. or greater. Virus isolation attempts were markedly unsuccessful, possibly a result of problems of storing of specimens enroute from Maryland University to N.I.H. However, paried bloods were obtained from 129 of the total. Thirty or 23%

Part B included [ ] Yes [x] No

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of this latter group showed a four-fold increase to one of the following respiratory viral antigens by CF test: Parainfluenza 1, 3, 4,; Adenovirus; Influenza A and B; Respiratory Syncytial Virus. Of the 30 showing CF antibody rises over one-half (52%) were to Influenza B, 17% to Respiratory Syncytial, 17% to Parainfluenza 1, and 3% to each of the other antigens. The illnesses of the patients with the rises to each of the antigens was not associated with any specific clinical syndrome.

Significance to Bio-medical Research and the Program of the Institute:

This project attempted to show the etiology of respiratory diseases with respect to viruses. It is seen clearly that serologically, only 23% of the respiratory diseases could be associated with viruses and if this had been a year when influenza B was not prevalent, the figure might have been even lower. The task of further attempting to find those agents which are causing viral respiratory diseases is apparent.

Proposed Course of Project:

There are no plans to renew this study at the present time.

Serial No. NIAID 66-D
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Role of Viruses in the Etiology of Ear Disease

Principal Investigator: Dr. Albert Z. Kapikian

Other Investigators: Dr. Joseph A. Bell, Dr. Robert J. Huebner

Cooperating Units: Washington Hospital Center, Ear, Nose and Throat Department

Man Years (Calendar Year 1960)
Total: 0/8
Professional: 0/8
Other: 0/8

Project Description:

For many years, it has been stated that viruses were responsible for many nonbacterial ear infections such as serous otitis media. It was attempted to study patients with serous otitis media to determine the role of viruses in this disease.

Patient Material:

Patients at the Washington Hospital Center ENT clinic with serous otitis media were examined. Fluids from the middle ear were tested in appropriate tissue culture lines. Acute and convalescent bloods were obtained.

Major Findings:

Thus far only a limited number of specimens from the ear have been tested with no virus yet isolated. Serologic tests on the sera are in progress.

Significance to Bio-medical Research and the Program of the Institute:

The role of viruses in ear disease is uncertain. This project attempts to clarify their role in the etiology of middle ear disease, especially serous otitis media.

Proposed Course of Project:

It is planned to renew attempts to obtain appropriate specimens during the year.

Part B included [ ] Yes [x] No

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Serial No. NIAID 66-E
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A Study of the Influence of Influenza A and B Mineral Oil Adjuvant Vaccines on Influenza Antibody Patterns, 8-9 Years after Vaccination

Principal Investigator: Dr. Joseph A. Bell

Other Investigator: Dr. Robert J. Huebner

Man Years (Calendar Year 1960)
Total: 0/8
Professional: 0/8
Other: 0/8

Project Description:

Objectives: (1) To see if Mineral Oil Adjuvant Influenza A and B vaccines influenced influenza antibody patterns, 8-9 years after vaccination.

Patient Material:

Approximately 100 N.I.H. personnel were given one dose of a mineral oil adjuvant influenza vaccine between 1951 and 1952. Twenty-five hundredths ml of a 100 CCA unit vaccine was administered. Influenza type A and B products were assigned by a strictly random sampling process so that any follow-up of the group would give two strictly comparable groups of near equal size, each of which had received one dose of either type A or type B Influenza adjuvant product 8-9 years previously. The A and B groups should have remained comparably equal (within the range of sampling variation) with respect to all attributes including influenza infection, disease, vaccination, antibody response, etc., except for the influence of the different study vaccines given. The 100 N.I.H. personnel who were given 1 of the 4 adjuvant products and who were all pre-bled before vaccination are in the process of being bled currently. Hemagglutination inhibition tests are to be done to see if the adjuvant vaccines given 8-9 years ago are still influencing the antibody pattern.

Major Findings: None

Significance to Bio-Medical Research and the Program of the Institute:

The study attempts to show the effectiveness of mineral oil adjuvant vaccines in producing long lasting immunity. This may be of importance in future vaccine programs.

Part B included [X] Yes 1 [ ] No

Proposed Course of Project:

No further field work is contemplated currently, but the laboratory analysis will be carried on.

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Part B

Honors, Awards and Publications

Bell, J.A., Craighead, J.E., and James, R.G.

Epidemiologic Observations on Two Outbreaks of Asian Influenza in a Children's Institution

American Journal of Hygiene - Jan. 1961

Philip, R.N., Bell, J.A., Davis, D.J., Beem, M.O., Beigelman, P.M., Engler, J.I., Mellin, G.W., Johnson, J.H., Lerner, A.M.

Epidemiological Studies on Influenza in Familial and General Population Groups 2. Characteristics of Occurrence

American Journal of Hygiene - Mar. 1961

Davis, D.J., Philip, R.N., Bell, J.A., Vogel, J.E., Jensen, D.V.

Epidemiological Studies on Influenza in Familial and General Population Groups 3. Laboratory Observations

American Journal of Hygiene - Mar. 1961

Bell, J.A., Philip, R.N., Davis, D.J., Beem, M.O., Beigelman, P.M., Engler, J.I., Mellin, G.W., Johnson, J.H., Lerner, A.M.

Epidemiological Studies on Influenza in Familial and General Population Groups 4. Vaccine Reactions

American Journal of Hygiene - Mar. 1961

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INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: STUDIES ON HUMAN ENTEROVIRUSES, ADENOVIRUSES, AND REOVIRUSES

PRINCIPAL INVESTIGATOR: DR. LEON ROSEN

OTHER INVESTIGATORS: DR. JOSEPH A. BELL, DR. ROBERT J. HUEBNER, DR. ALBERT B. SABIN, DR. D. MENDEZ-CASHION, MR. JEROME KERN, AND MRS. JANET HOVIS

COOPERATING UNITS: D. C. WELFARE DEPARTMENT; CHILDREN'S HOSPITAL RESEARCH FOUNDATION, CINCINNATI, OHIO; SCHOOL OF MEDICINE, UNIVERSITY OF PUERTO RICO, SAN JUAN.

MAN YEARS: (CALENDAR YEAR 1960)

TOTAL:	72/12
PROFESSIONAL:	32/12
OTHER:	40/12

PROJECT DESCRIPTION:

OBJECTIVES: TO DETERMINE THE ROLE IN HUMAN BIOLOGY OF ENTEROVIRUSES, ADENOVIRUSES, AND REOVIRUSES. TO ELUCIDATE THE NATURAL HISTORY OF THESE VIRUSES.

METHODS EMPLOYED: THE PRINCIPAL POPULATION GROUP UNDER STUDY IS THE JUNIOR VILLAGE NURSERY GROUP WHICH HAS BEEN DESCRIBED IN DETAIL IN PREVIOUS REPORTS OF THE EPIDEMIOLOGY SECTION.

RECENTLY, A COLLABORATIVE STUDY WAS INSTITUTED WITH THE DEPARTMENT OF PEDIATRICS OF THE UNIVERSITY OF PUERTO RICO. IN THIS STUDY, SPECIMENS FROM CHILDREN WITH A VARIETY OF ACUTE NEUROLOGICAL DISORDERS WILL BE STUDIED IN BETHESDA.

A COLLABORATIVE STUDY WAS ALSO UNDERTAKEN WITH DR. A. B. SABIN OF THE CHILDREN'S HOSPITAL RESEARCH FOUNDATION OF CINCINNATI. DR. SABIN HAS SUPPLIED SEVERAL THOUSAND NON-POLIO ENTEROVIRUS ISOLATES FROM MEXICO.

PART B INCLUDED YES

PART A METHODS EMPLOYED (CONTINUED)

THESE ARE BEING STUDIED FOR THE PRODUCTION OF HEMAGGLUTININS IN AN ATTEMPT TO OBTAIN HEMAGGLUTINATING STRAINS OF VIRUS TYPES WHICH ARE NOT NOW KNOWN TO HAVE THIS USEFUL PROPERTY.

COLLABORATIVE STUDIES WITH OTHER INSTITUTIONS OUTSIDE OF THE NATIONAL INSTITUTES OF HEALTH AND WITH VARIOUS INTRAMURAL LABORATORIES ARE UNDERTAKEN FROM TIME TO TIME AS OPPORTUNITIES ARE PRESENTED.

MAJOR FINDINGS: AN EXTENSIVE OUTBREAK OF INFECTION WITH ECHO TYPE 3 VIRUS OCCURRED IN THE JUNIOR VILLAGE NURSERY POPULATION. THIS IS THE FIRST KNOWN OUTBREAK OF INFECTION WITH THIS VIRUS - THE PROTOTYPE HAVING BEEN DESCRIBED FROM A SINGLE ISOLATE. PRELIMINARY ANALYSIS OF DATA INDICATES THAT INFECTION WITH THIS AGENT WAS ASSOCIATED WITH A MILD FEBRILE DISEASE.

HEMAGGLUTININS WERE FOUND FOR THE FIRST TIME FOR ECHO VIRUS TYPE 21, COXSACKIE B VIRUS TYPES 1 AND 5, AND A PREVIOUSLY UNRECOGNIZED ENTEROVIRUS (JV-9) WHICH IS CYTOPATHOGENIC ONLY IN HUMAN TISSUE CULTURES. SIMPLE H-I PROCEDURES WERE DEVELOPED FOR IDENTIFYING ENTEROVIRUSES AND ALSO FOR PERFORMING SEROLOGIC TESTS WITH SERA FROM PERSONS SUSPECTED OF HAVING ENTEROVIRUS INFECTIONS.

TWO NEW ENTEROVIRUS SEROTYPES (IN ADDITION TO THE PREVIOUSLY REPORTED JV-1) HAVE BEEN CHARACTERIZED AND ACCEPTED FOR NUMBERING BY THE ENTEROVIRUS COMMITTEE. SEVERAL PROBABLY NEW SEROTYPES ARE CURRENTLY UNDER STUDY.

FOUR NEW ADENOVIRUS SEROTYPES HAVE BEEN CHARACTERIZED. SIMPLE SEROLOGIC PROCEDURES WERE DEVELOPED FOR IDENTIFYING ADENOVIRUSES AND FOR PERFORMING SEROLOGIC TESTS WITH SERA FROM PERSONS SUSPECTED OF HAVING ADENOVIRUS INFECTIONB.

HEMAGGLUTINATION OF RHESUS ERYTHROCYTES BY MEASLES VIRUS WAS DEMONSTRATED AND A SIMPLE H-I TECHNIQUE FOR WORK WITH THIS VIRUS WAS DEVELOPED.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: THE ROLE OF THE NUMEROUS ENTEROVIRUSES, ADENOVIRUSES, AND REOVIRUSES IN HUMAN BIOLOGY IS ONLY BEGINNING TO BE STUDIED. DATA OBTAINED THUS FAR INDICATE THAT A NUMBER OF THESE VIRUSES ARE QUITE IMPORTANT IN HUMAN PATHOLOGY. FURTHER PROGRESS IN THIS FIELD DEPENDS TO A GREAT EXTENT ON THE DEVELOPMENT OF SIMPLE LABORATORY PROCEDURES TO COPE WITH THE MORE THAN 90 VIRUSES NOW KNOWN TO BELONG TO THESE GROUPS.

PROPOSED COURSE OF THE PROJECT: THE STUDY OF THESE THREE FAMILIES OF VIRUSES WILL BE CONTINUED BOTH IN THE FIELD, AS OPPORTUNITIES ARE PRESENTED, AND IN THE LABORATORY. LABORATORY STUDIES WILL BE DEVOTED PRI-

PART A PROPOSED COURSE OF THE PROJECT (CONTINUED)

MARILY TO THE ORDERLY CLASSIFICATION OF EXISTING AND NEWLY RECOGNIZED SEROTYPES AND TO THE DEVELOPMENT OF SIMPLE IN-VITRO TECHNIQUES.

PART B INCLUDED YES

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Rosen, L.: Serologic Grouping of Reoviruses by Hemagglutination-Inhibition. Amer. J. Hyg., 71:242-249, 1960

2. Rosen, L., Hovis, J. F., Mastrota, F. M., Bell, J. A., and Huebner, R. J.: Observations on a Newly Recognized Virus (Abney) of the Reovirus Family. Amer. J. Hyg., 71:258-265, 1960

3. Rosen, L., Hovis, J. F., Mastrota, F. M., Bell, J. A., and Huebner, R. J.: An Outbreak of Infection with a Type 1 Reovirus among Children in an Institution. Amer. J. Hyg., 71:266-274, 1960

4. Rosen, L.: A Hemagglutination-Inhibition Technique for Typing Adenoviruses. Amer. J. Hyg., 71:120-128, 1960

5. Rosen, L., Bell, J. A., and Huebner, R. J.: Enterovirus Infections of Children in a Washington, D. C., Welfare Institution. In "Viral Infections of Infancy and Childhood," Rose, H. M., ed., Hoeber-Harper, New York, 1960, pp. 119-127

6. Rosen, L.: Hemagglutination and Complement Fixation. In "Perspectives in Pediatric Virology," Thirty-third Ross Conference on Pediatric Research, Cramblett, H. G., ed., Columbus, Ohio, Ross Laboratories, 1959, pp. 53-56

7. Philipson, L. and Rosen, L.: Identification of a Cytopathogenic Agent called U-Virus Recovered from Patients with Non-Diphtheritic Croup and from Day-Nursery Children. Archiv. fur die Gesamte Virusforschung, 9:25-30, 1959
(Not included in 1959 report)

8. Rosen, L.: Hemagglutination and Hemagglutination-Inhibition with Measles Virus. Accepted for publication in Virology.

Honors and Awards relating to this project:

1. Visiting lecturer in Epidemiology, School of Public Health, University of California

2. Appointed to the Enterovirus Committee of the National Cancer Institute

SERIAL NO. NIAID - 67A
1. INFECTIOUS DISEASES
2. EPIDEMIOLOGY
3. BETHESDA, MARYLAND

PHS-NIH
INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: STUDIES ON VIRUSES OF THE ENTERIC TRACT OF CATTLE

PRINCIPAL INVESTIGATORS: DR. F. R. ABINANTI AND DR. LEON ROSEN

OTHER INVESTIGATORS: NONE

COOPERATING UNITS: DAIRY HUSBANDRY DEPARTMENT, UNIVERSITY OF MARYLAND; HOME OF CORRECTION, JESSUP, MARYLAND

MAN YEARS: (CALENDAR YEAR 1960)

TOTAL:	6/12
PROFESSIONAL:	3/12
OTHER:	3/12

PROJECT DESCRIPTION:

OBJECTIVES: TO INVESTIGATE THE BIOLOGIC PROPERTIES AND NATURAL HISTORY OF THE VIRUSES INHABITING THE ENTERIC TRACT OF CATTLE

METHODS EMPLOYED: A LONGITUDINAL STUDY OF DAIRY CATTLE ON THREE DIFFERENT FARMS HAS BEEN CARRIED OUT FOR APPROXIMATELY TWO YEARS. MONTHLY FECAL SWABS AND SERUM SPECIMENS ARE OBTAINED FROM EACH ANIMAL AND THESE ARE STUDIED IN THE LABORATORY BY A VARIETY OF VIRUS ISOLATION AND SEROLOGIC PROCEDURES.

MAJOR FINDINGS: REOVIRUSES OF THREE DIFFERENT TYPES SEROLOGICALLY INDISTINGUISHABLE FROM THE THREE TYPES FOUND IN HUMANS HAVE BEEN RECOVERED ON NUMEROUS OCCASIONS DURING THIS STUDY. CALVES HAVE BEEN INFECTED WITH EACH OF THE THREE TYPES OF HUMAN ORIGIN. NO EVIDENCE OF DISEASE WAS NOTED IN EITHER THE NATURALLY OR EXPERIMENTALLY INFECTED ANIMALS.

AN APPARENTLY NEW HEMADSORBING VIRUS WAS RECOVERED FROM CATTLE ON ALL THREE FARMS. THE CHARACTER OF THE HEMADSORPTION PRODUCED BY THIS VIRUS APPEARS TO BE DIFFERENT FROM THAT OF ANY OF THE OTHER HEMADSORBING VIRUSES.

PART B INCLUDED YES

PART A MAJOR FINDINGS (CONTINUED)

SEVERAL HUNDRED ISOLATES OF ENTEROVIRUSES HAVE BEEN RECOVERED FROM CATTLE IN THIS STUDY. THE RELATIONSHIP OF THESE VIRUSES TO EACH OTHER AND TO THE ENTEROVIRUSES OF MAN IS CURRENTLY UNDER STUDY.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: THE RECOVERY OF VIRUSES (SUCH AS REOVIRUSES) FROM CATTLE WHICH ARE ANTIGENICALLY IDENTICAL WITH THOSE WHICH OCCUR IN MAN IS OF OBVIOUS INTEREST. ASIDE FROM THE POSSIBLE IMPORTANCE OF CATTLE AS POTENTIAL SOURCES OF INFECTION, A NUMBER OF ASPECTS OF THE NATURAL HISTORY OF THESE AGENTS CAN BE CLARIFIED BY THEIR STUDY IN LOWER ANIMALS. IN ADDITION, VIRUSES RECOVERED FROM CATTLE WHICH ARE ANALOGOUS, BUT NOT IDENTICAL, TO THOSE OF HUMANS ALSO PROVED A MECHANISM FOR GAINING USEFUL INFORMATION ABOUT THE LATTER AGENTS.

PROPOSED COURSE OF THE PROJECT: THE ANIMALS IN THE LONGITUDINAL STUDY WILL BE FOLLOWED FOR ABOUT ANOTHER SIX MONTHS. HOWEVER, A CONSIDERABLE AMOUNT OF LABORATORY WORK WILL REMAIN TO BE FINISHED AFTER THIS TIME.

PART B INCLUDED YES

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PART B HONORS, AWARDS, AND PUBLICATIONS

PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT:

ROSEN, L. AND ABINANTI, F. R.: NATURAL AND EXPERIMENTAL INFECTION OF CATTLE WITH HUMAN TYPES OF REOVIRUSES. AMER. J. HYG., 71:250-257,1960

SERIAL NO. NIAID - 67B
1. INFECTIOUS DISEASES
2. EPIDEMIOLOGY
3. BETHESDA, MARYLAND

PHS-NIH
INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: STUDIES ON THE ETIOLOGY OF EOSINOPHILIC MENINGITIS IN FRENCH POLYNESIA

PRINCIPAL INVESTIGATOR: DR. LEON ROSEN

OTHER INVESTIGATORS: NONE

COOPERATING UNITS: SCHOOL OF MEDICINE, UNIVERSITY OF CALIFORNIA, LOS ANGELES AND INSTITUT DE RECHERCHES MEDICALES DE LA POLYNESIE FRANÇAISE, PAPEETE, TAHITI

MAN YEARS: (CALENDAR YEAR 1960)

TOTAL: 4/12
PROFESSIONAL: 3/12
OTHER: 1/12

PROJECT DESCRIPTION:

OBJECTIVES: TO DETERMINE THE ETIOLOGY OF EOSINOPHILIC MENINGITIS

METHODS EMPLOYED: A COMPREHENSIVE EPIDEMIOLOGIC STUDY WAS UNDERTAKEN OF THIS UNUSUAL DISEASE WHICH OCCURRED ON TAHITI. BLOOD, SPINAL FLUID, AND STOOL SPECIMENS WERE COLLECTED FOR A VARIETY OF LABORATORY STUDIES.

MAJOR FINDINGS: BEGINNING IN MARCH 1958 MANY HUNDREDS OF CASES OF AN UNUSUAL TYPE OF MENINGITIS OF UNKNOWN ETIOLOGY OCCURRED ON THE ISLAND OF TAHITI IN FRENCH POLYNESIA. SINCE THE MOST CHARACTERISTIC FEATURE OF THIS MENINGITIS WAS A PLEOCYTOSIS CONSISTING IN LARGE PART OF EOSINOPHILS, THE DISEASE WAS CALLED EOSINOPHILIC MENINGITIS.

THE MOST CHARACTERISTIC CLINICAL FEATURES OF THE DISEASE WERE HEADACHE, STIFFNESS OF THE NECK AND BACK, AND PARESTHESIAS OF DIFFERENT TYPES. APPROXIMATELY FIVE PERCENT OF CASES HAD A FACIAL PARALYSIS OF THE PERIPHERAL TYPE. THE DURATION OF ILLNESS VARIED FROM SEVERAL DAYS TO SEVERAL MONTHS AND REOCCURRENCES WERE COMMON. NO DEATHS OCCURRED.

PART B INCLUDED YES

PART A MAJOR FINDINGS (CONTINUED)

MORE THAN ONE-HALF OF THE CASES HAD A PLEOCYTOSIS OF 500 OR MORE CELLS PER CU. MM. OF CEREBROSPINAL FLUID, AND IN 85 PERCENT OF ALL CASES EOSINOPHILS ACCOUNTED FOR MORE THAN ONE-FOURTH OF THE PLEOCYTOSIS.

EXAMINATIONS OF SPINAL FLUID, BLOOD, AND FECES WERE NEGATIVE FOR THE PRESENCE OF VIRUSES AND PATHOGENIC BACTERIA AND FUNGI. NO HELMINTHIC PARASITES WERE FOUND OTHER THAN THOSE COMMONLY INFESTING THE INHABITANTS OF THE AREA. SEROLOGIC EXAMINATION OF CONVALESCENT SERA AGAINST A NUMBER OF DIFFERENT ARTHROPOD-BORNE VIRUSES, ENTEROVIRUSES, LEPTOSPIRA, AND OTHER MICROBIAL AGENTS FAILED TO PROVIDE AN INDICATION OF THE ETIOLOGIC AGENT.

THE DISEASE OCCURRED PRIMARILY IN ADULTS AND AFFECTED BOTH SEXES EQUALLY. PERSONS OF POLYNESIAN OR PART-POLYNESIAN ORIGIN APPEARED TO HAVE A HIGHER ATTACK RATE THAN EUROPEANS OR CHINESE.

THERE WAS NO SHARP SEASONAL DISTRIBUTION AND CASES OCCURRED IN EVERY MONTH OF THE YEAR.

THE GEOGRAPHIC DISTRIBUTION OF CASES CORRESPONDED ROUGHLY TO THE DISTRIBUTION OF THE POPULATION AND THERE WAS NO DEFINITIVE EVIDENCE OF A HIGHER ATTACK RATE IN EITHER RURAL OR URBAN AREAS.

NO EVIDENCE WAS FOUND TO SUGGEST TRANSMISSION OF THE DISEASE FROM PERSON TO PERSON OR FROM ONE GEOGRAPHIC AREA TO ANOTHER. THE AGGREGATION OF CASES BY HOUSEHOLD SUGGESTED THE EFFECT OF A COMMON EXPOSURE THAN PERSON-TO-PERSON TRANSMISSION.

THE INCUBATION PERIOD OF THE DISEASE WAS ESTIMATED TO BE BETWEEN TWO AND FOUR WEEKS.

ALTHOUGH THE ETIOLOGY OF THE DISEASE WAS NOT DETERMINED, THE SUM OF THE CLINICAL AND EPIDEMIOLOGIC EVIDENCE SUGGESTED THE HYPOTHESIS THAT THE DISEASE WAS CAUSED BY A HELMINTHIC PARASITE OF THE OCEANIC BONITO OR SKIPJACK TUNA (KATSUWONUS PELAMIS) WHICH IS COMMONLY EATEN RAW IN THE AREA.

SIGNIFICANCE TO PROGRAM OF THE INSTITUTE: OUTBREAKS OF EOSINOPHILIC MENINGITIS HAVE OCCURRED IN NEW CALEDONIA AND THE CAROLINE ISLANDS, AS WELL AS IN FRENCH POLYNESIA. IT IS OBVIOUSLY OF GREAT PRACTICAL INTEREST TO DETERMINE THE ETIOLOGY OF THIS DISEASE IN ORDER THAT APPROPRIATE PREVENTIVE MEASURES CAN BE INSTITUTED. FURTHERMORE, IF IT IS SHOWN THAT THE DISEASE IS INDEED ACQUIRED FROM A MARINE FISH, IT WILL BE THE FIRST INDICATION THAT A PARASITIC DISEASE OF MAN CAN BE ACQUIRED FROM SUCH A SOURCE.

PART B INCLUDED YES

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PART A (CONTINUED)

PROPOSED COURSE OF PROJECT: ADDITIONAL FIELD STUDIES WILL BE UNDERTAKEN EARLY IN 1961 IN AN ATTEMPT TO OBTAIN FURTHER DATA ON THE ETIOLOGY OF THE DISEASE - ESPECIALLY WITH REGARD TO THE HYPOTHESIS THAT THE DISEASE IS ACQUIRED FROM EATING RAW FISH.

PART B INCLUDED YES

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

None

Honors and Awards relating to this project:

1. Letter from Governor-General of French Polynesia to Surgeon General of the U.S. Public Health Service expressing appreciation for the studies carried out on Tahiti by Dr. Leon Rosen.

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1. Infectious Diseases
2. Virus and Rickettsial
3. Bethesda, Maryland

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Part A

Project Title: Studies of Tumor Viruses in Nature.

Principal Investigators: Dr. Robert J. Huebner and
Dr. Wallace P. Rowe

Other Investigators: Dr. Janet W. Hartley, Mr. William T. Lane and Mr. John D. Estes

Cooperating Units: New York City Health Department
Dr. David Johnson, Smithsonian Institute
Dr. E. Baker, Smithsonian Institute
Dr. B. Burmester, Regional Poultry Research Laboratory, Agricultural Research Service, East Lansing, Michigan

Man Years: (Calendar Year 1960)

Total:	48/12
Professional:	16/12
Other:	32/12

Project Description:

Objectives: This project is concerned with elucidating natural behavior of tumor viruses in their natural animal hosts in natural environments. We have devoted most of our attention so far to those viruses for which survey tools have been developed, namely, polyoma and papilloma.

Methods Employed: Survey tools - direct virus demonstration and isolation techniques plus serologic procedures (CF, HI, MAP, N tests) are evaluated and used in examining specimens collected in field studies for evidence of contamporary and prior virus infection.

Part B included: Yes

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Field Studies: The natural history of polyoma virus infection of Mus musculus, the common house mouse, is under study in a densely populated urban area (Harlem, N.Y.C.), and in a rural area on Maryland farms and grain mills. The distribution of rabbit papilloma virus (independent of tumors) in cottontails was studied in an area known to be heavily infected (Kansas) and in a presumably tumor-free area (Maryland).

The natural occurrence of "VL" virus initially reported as a tissue culture grown avian lymphomatosis virus was determined by serologic surveys of commercial and experimental populations.

Specimens: Mice were live trapped, weighed, sexed, bled, marked, and generally released (in same area as caught). Tissues and urine were collected for virus isolations. Environmental materials contaminated by mouse excreta such as trash and grain were collected in sterile containers for virus study. Rabbits and chickens, both with and without tumors, were bled and the sera studied for papilloma and "VL" antibodies.

Certain Problems: Both virus demonstration and serological techniques required extensive evaluation to determine the reliability, accuracy and sensitivity of the available techniques. For polyoma serology the HI test was shown to be more sensitive than the CF test, and when sera was treated with RDE and heat, almost entirely equivalent to the more cumbersome neutralization tests; hence it was selected, not as the sole serologic survey instrument, but as the test of choice.

The most severe laboratory problem that had to be solved was the problem of spontaneous infection of laboratory mice with laboratory strains of polyoma virus in our Bethesda laboratory; this, plus the intrusion of other extraneous agents in the mouse study systems (see Project NIAID-7I B) invalidated most efforts to demonstrate virus in the MAP test, our most sensitive system. To obtain high order information and insure bona-fide field isolations, we eventually were required to set up field laboratories complete with constant monitoring and adequate controls. Fortunately, we were finally able to obtain isolations of virus which surely originated in the field specimens.

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Major Findings:

Polyoma: Polyoma was found to be widely distributed in natural colonies of Mus musculus, not only in Harlem but on livestock farms and grain mills furnishing feed for livestock. As with laboratory mice, virus was shown to be excreted in saliva and excreta by carrier wild mice. Not only the tenement environments and community mouse nests in Harlem, but even the cereal grains in the feed mills were found contaminated; presumably the farm granaries where infected mice also abound are heavily contaminated as well. Polyoma virus has been easily recovered from mice in all three studies; however, the relative extent of grain and environmental area contamination must be determined.

Polyoma, a ubiquitous and persistent natural infection in Mus musculus has now been studied in three separate ecologies - laboratory breeding colonies, urban tenement houses, and in rural agriculture establishments. The basic cycle in nature would appear to be the latter, where extensive contamination of cereal grains on the farm and the feed mill not only explains persistent foci of this highly resistant virus in these areas, but also suggests that the wide distribution (and occasional sudden outbreaks) of polyoma virus infection in laboratory colonies are induced and maintained by uncooked cereal grains commonly fed to such mice.

Longitudinal studies continued in Harlem tenements suggest that large and very dense populations of mice, plus contaminated foci, particularly "community nests", are the chief factors in maintaining continuous infections through several succeeding generations of mice. Polyoma virus was isolated from debris and dust in one Harlem kitchen cabinet near a nesting area over intervals exceeding eight months. During the year of surveillance in New York, negative premises tended to remain negative, and positive premises without exception remained positive.

Despite wide distribution and high infection rates, the role of polyoma virus in the genesis of naturally occurring cancers in mice is still undetermined, despite the fact that field isolates are quite as oncogenic as established strains when given to infant laboratory mice and hamsters. Several thousand mice have now been live trapped - some of them several times over periods of several months. To date only one naturally occurring tumor has been observed - a mammary tumor in a mouse negative for polyoma. However, it appears that few wild mice live beyond six months of age in the urban environment where polyoma is prevalent.

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Additional Findings: These studies promise to provide interesting information on the natural histories of other latent viruses of Mus musculus including mouse salivary gland virus, K virus, and reoviruses. It is probable as we intensify our laboratory study of field specimens that other latent and possibly tumor viruses may come to light.

Observations on the ecology of Mus musculus have led to interesting findings. In the grain mill and the feed barn, just as in the tenement house, mice tend to inhabit the upper floors presumably because rats are more common in basements and lower floors; female mice are more numerous than males, apparently live longer, and as adults are more frequently positive for polyoma. The latter presumably can be explained by greater exposure of females to infected communal nesting areas, which in turn is due to the much greater amounts of virus excreted by mice infected as infants (as much as 10^6 virus/ml of urine may be excreted for several weeks).

Rabbit Papilloma: Differential centrifugation combined with several washings to free antibody bound papilloma virus and complement-fixing antigens in cottontail papillomas obtained from Kansas provided more sensitive tests for live virus and CF antibody. Complement-fixing antibody was found to be present in 50 Kansas rabbits carrying visible papillomas; Kansas rabbits free of papillomas were most often negative but occasionally they also were positive in the CF test. The CF antibodies were shown to correlate with the presence of neutralizing antibodies in the domestic rabbit test. Maryland rabbits are reportedly free of papilloma and indeed none were seen on some 30 rabbits live trapped in this area. To date all Maryland rabbits have been negative in the papilloma complement fixation test.

This test appears to be very sensitive and quite specific, thus permitting surveys for virus prevalence independently of tumor production. Thus far, unlike polyoma (which it resembles in many physical and biologic traits) papilloma virus would seem to induce tumors in the majority (If not all) Kansas cottontail rabbits which are successfully infected.

Studies of modes of spread - a search for attenuated or non-tumorigenic strains appears feasible; similarly, studies of possible antigenic relationships with papilloma agents of other species, Homo sapiens included, are indicated.

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"VL" (GAL) Virus: This tissue culture grown virus was proven during the year to be an extremely prevalent extraneous adenovirus-like chicken virus and not the agent of avian visceral lymphomatosis as originally reported by Burmester and Sharpless. This dismal discovery was made simultaneously by Burmester, Sharpless in laboratory experiments, and by our own sero-epizootiologic studies of the prevalence of antibodies to this agent in chicken flocks. Virtually all commercial flocks and 90 per cent of the chickens in such flocks revealed neutralizing antibody to the "VL" or "GAL" virus (Gallus adenovirus-like). Studies of isolated outbreaks of visceral and neural lymphomatosis revealed that while most were associated with GAL infection, some typical leukemia outbreaks occurred in the complete absence of evidence for GAL infection.

Surveys of Burmester's 15 I strains of RPL-12 susceptible birds revealed that nearly all of them had antibodies to GAL virus. Fortunately, additional surveys have revealed a substrain of 15 I chickens free of GAL virus, which will be used for a re-examination of visceral lymphomatosis in a system free of an extraneous virus possessing many of the properties of lymphomatosis itself.

Significance to the Program of the Institute: In LID our approach to cancer research is based on a "biological" instead of the more prevalent clinico-pathologic point of view. We do not disdain the use of laboratory models (we employ them as indicated); but our major interest is conditioned by a concept which we think is fundamental to an intelligent and logical approach to the study of cancer viruses. We take it as axiomatic that if a cancer is caused by a specific virus, then as is true of all other microbial illnesses, the microbe is the central issue and the natural behavior of such a virus the most needed and highest order information achievable on the subject.

This project has already developed much information of value in defining not only the natural behavior and the basic cycle of polyoma in nature, but also the probable sources of infection in both experimental and production colonies.

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The demonstration by Eddy and Stewart of the tumorigenic activity of polyoma in hamsters, rats, guinea pigs, and rabbits reveals an alarming capacity for polyoma to cross species barriers. Although serologic studies do not support definite infection of man with this virus, widespread access of humans through mouse contaminated environments and apparently extensive contamination of food-stuffs, renders the question of possible human infections rather more than academic. The lack of serologic correlation with human cancer such as would be expected in mice may not represent conclusive counter-evidence, since hamsters with polyoma induced tumors lose their antibodies to polyoma rapidly despite the persistence of eventually fatal tumors.

Proposed Course of the Project: The natural history and behavior of polyoma and other animal tumor viruses are important subjects in their own right and studies of them represent some of the first extensive efforts to develop such information about latent viruses. As noted above, there can be no higher order of information about infectious agents and such information derived from careful exhaustive longitudinal studies are directly relevant to the question of human cancer viruses.

The possibility that cancer could be the result of a zoonotic infection no longer appears so very unlikely. We plan, therefore, to study possible polyoma infection of man and other animal species, such as domestic animals (cattle, hogs, dogs, and cats), or other wild rodents likely to be exposed repeatedly to infection with known cancer viruses of mice (such as polyoma) and chickens.

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Huebner, R.J.: Some questions about possible approaches to research on viruses as a cuase of cancer. Cancer Res., 20:669-830, 1960.

Huebner, R.J.: Viruses in search of cancer. Monograph - PERSPECTIVES IN VIROLOGY II, 1960.

Huebner, R.J.: New possibilities in virus disease control. Medical Section Proceedings of American Life Convention, 1960.

Halonen, P. and Huebner, R.J.: ECHO and poliomyelitis virus antisera in guinea pigs with fluorocarbon-treated cell culture antigens. Proc. of Soc. for Exper. Biol. and Med., 105:46-49, 1960.

Honors and Awards relating to this project:

Participated in Variety Children's Research Foundation Symposium, and dedication ceremonies of new research building; talk: "Viruses in Children, 1960". Miami, Florida, January 1960.

Participated in the Gustav Stern Symposium Perspectives in Virology II. Talk: "Viruses in Search of Cancer." New York, New York, January 1960.

Participated in NCI Viruses and Cancer Panel. New York, New York, January 1960.

Invited speaker, Epidemiology for Veterinarians course, - "New Horizons in Domestic Animal Virology." Communicable Disease Center, Atlanta, Georgia, February 1960.

Participated in International Conference on Asian Influenza. Discussant, "Methods of Diagnosis." National Institutes of Health, Bethesda, Maryland, February 1960.

Participated in Phenomena of Tumor Viruses Symposium, New York, New York, March 1960.

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Invited speaker, Frederick County Tuberculosis and Public Health Association, Frederick, Maryland. April 1960.

Invited speaker, American College of Physicians - "Viruses and Cancer." San Francisco, California, April 1960.

Society of American Bacteriologists. Talk: "Viral Agents in Relation to Tumors". Philadelphia, Pennsylvania, May 1960.

Annual Health Conference, Inc. Talk: "The Growing Importance of Virology in Public Health." New York, New York, May 1960.

Invited speaker, American College of Obstetricians and Gynecologists - "Virus Infections in 1960." Cincinnati, Ohio, April 1960.

Invited speaker, The Medical Section, American Life Convention - "New Possibilities in Virus Disease Control." White Sulphur Springs, West Virginia, May 1960.

Invited speaker, Kansas Trudeau Society - "Viral Respiratory Diseases." Kansas City, Kansas, September 1960.

Invited speaker, The American Academy of General Practice - "What is Non-Paralytic Polio." Kansas City, Kansas, September 1960.

Participant in discussions, University of Illinois Center for Zoonoses Research. Urbana, Illinois, September 1960.

Invited speaker, NCI Staff Conference - "Background Noise in Virus Study Systems." National Institutes of Health, October 1960.

Section chairman, Southwest Section of American Association for Cancer Research. Talk: "Viruses as a Cause of Cancer." Galveston, Texas, October 1960.

Invited speaker, Academy of Medicine of Cincinnati - "Viruses and the Common Cold." Cincinnati, Ohio, November 1960.

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Invited speaker, The Harvey Society - "Cancer as an Infectious Disease." New York, New York, November 1960.

Invited speaker, Chicago Medical School, Abbott Laboratories and Marquette University - "Respiratory Disease due to Viruses - A Comprehensive View." Chicago, Illinois, November 1960.

Chairman and discussant, AMA Clinical Session Symposium on Respiratory Virus Disease. Washington, D.C., November 1960.

Elected to membership, The National Academy of Sciences, April 1960.

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1. Infectious Diseases
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3. Bethesda, Maryland

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Part A.

Project Title: Sero-epidemiology of virus infections.

Principal Investigator: Dr. John L. Sever, Dr. Robert J. Huebner

Other Investigators: Anita Ley, Flora Wolman, Renee Traub, Joan Austin

Cooperating Units: NINDB, Collaborative study on cerebral palsy, mental retardation, and other neurological and sensory disorders of infancy and childhood (Dr. Richard Masland).

MBA - Dr. Gabriel Castellano

Man Years (calendar year 1960):

Total:	74/12
Professional:	38/12
Other:	36/12

Project Description:

Objectives: To utilize available serologic technique in an intensive study of newly recognized viruses as to their relation to the high incidence of acute undifferentiated respiratory diseases, chronic diseases, birth defects, and cancer. To develop, wherever technically possible, the refinements of serologic methods necessary for a large scale investigation of the natural course of the disease as caused by viral infections.

Methods Employed: Serologic techniques, including complement fixation tests, hemagglutination-inhibition, hemadsorption, viral neutralization, and tissue culture neutralization tests are now developed for the identification of over 100 viral infections.

Part B Included: No

Two major phases of this project have been pursued. First, the commercial production and standardization of antigens and antisera suitable for the performance of the serological tests. Second, the development of an integrated laboratory facility, employing trained technicians, capable of handling large scale testing.

Human sera for viral serologic analysis and identification of previous viral experiences are available from studies of common acute undifferentiated respiratory diseases, and various detailed studies of special virus disease problems of current interest to the Laboratory of Infectious Diseases. A large number of serial bleedings are being obtained from mothers during the course of pregnancy and from infants 4 months after birth in the Collaborative Study on cerebral palsy, mental retardation, and other neurological and sensory disorders of infancy and childhood of NINDB. These sera are now becoming available for testing.

Major Findings: Complement fixing antigens for more than 70 viruses have now been prepared by Microbiological Associates, Inc., in consultation with the Virology Section, LID and NINDB. The antigens which have been prepared are titered in both laboratories and when found acceptable are produced in quantities of 100 to 1000 ml. These include complement-fixing antigens for: Adenoviruses (common antigen), Coxsackie A and B viruses (25 types), Influenza A, B, C, mumps, Parainfluenza (4 types), Polioviruses (3 types), ECHO viruses (28 types), measles, and Herpes Simplex and Respiratory syncytial. Viral antigens for hemagglutination tests have also been produced for 24 adenoviruses, 4 parainfluenza viruses, respiratory syncytial virus, 3 Reoviruses, mumps, salivary gland virus, Q Fever, Psittacosis, and other viruses which are utilized in routine tests in our laboratories.

A large scale program for producing specific immune antisera in human volunteers has been initiated and will be pursued during the next calendar year. After the completion of safety tests, groups of volunteers will be given purified viral antigens. The pedigreed stock antisera obtained in this way will be standardized for evaluating the potency of antigen preparations.

The application of a micro technique to both hemagglutination and complement fixation tests will permit the use of considerably less sera and antigen for the performance of the various tests. The technique has now been refined and developed to the point where it may be applied in lieu of standard techniques whenever screening information is desired. Through the use of highly developed spiral loops, accurate dilutions may be made rapidly in the system. The technique should be of particular value in studying the serological background of animals from which only a small amount of sera is available.

Significance to the Program of the Institute: The availability of reliable standard and micro serological techniques for a large group of new viruses provides an opportunity to investigate the course of human disease caused by viruses which are either difficult to isolate or are resistant to evaluation because the clinical effects are delayed until a long time after infection has subsided. This is particularly true in the case of birth defects and animal cancers. The application of this tool for analysis should provide considerable information on the epidemiological aspects of virus infections.

The program conducted in association with the NINDB should provide the tools necessary to help establish the clinical importance of over 100 new viruses of man.

Proposed Course of the Project: During the year 1961, the serological program will be expanded both in terms of antigenic materials and space for the performance of an increased testing program. The space available has already been increased and will be enlarged to include a well equipped laboratory and office facilities. The continuing process of evaluating new antigens and the volume production of antigens will be continued. The evaluation of serial specimens from the NINDB study will proceed in conjunction with information on birth defects being supplied by the Collaborating Institutions.

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1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

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Part A.

Project Title: Study of viruses as causes of respiratory illness in infancy and early childhood.

Principal Investigator: Dr. Robert M. Chanock

Other Investigators: Dr. K.M. Johnson, Dr. R.J. Huebner

Cooperating Units: Dr. R.H. Parrott, Children's Hospital Research Foundation, Washington, D.C.

Man Years (calendar year 1960):
Total: 30/12
Professional: 5/12
Other: 25/12

Project Description:

Objectives: 1) To search for new agents responsible for respiratory illness in infancy and childhood. 2) To delineate the epidemiology of virus which have recently been associated with respiratory illness. 3) To continue the study of certain well established respiratory viruses, i.e., their epidemiology and contribution to the overall respiratory disease experience during infancy and childhood. 4) To determine how much pediatric respiratory illness can be associated with known viruses as a guide to future immunoprophylaxis.

Methods Employed: Infants and children with respiratory illness and suitable controls without such illness will be studied at Children's Hospital, District of Columbia. The severe lower respiratory syndromes will be investigated in hospitalized patients, while the milder febrile respiratory disease syndromes will be studied among clinical patients.

Part B Included: Yes

The main emphasis will be on the use of various tissue culture systems and the fluorescent antibody technique for virus isolation. An attempt will be made to inoculate specimens from patients directly into tissue culture without prior freezing and thawing since such treatment appears to rapidly inactivate respiratory syncytial virus and the current strains of influenza B. Fluorescent antibody techniques will be applied to the search for agents which may possibly grow in tissue culture without causing cell destructive effects.

After the various isolates are identified, their contribution to the different respiratory disease syndromes will be estimated by comparing the recovery rate in such groups with that observed for healthy children free of respiratory symptoms. Children with severe illness admitted to the hospital, as well as control subjects, will be studied serologically for evidence of infection with 15 - 20 known respiratory viruses as well as any new agent which may emerge as a potentially important pathogen.

Major Findings:

Respiratory syncytial (RS) virus: During the past 3 years RS infection was detected by serologic means in 11% of children with severe lower respiratory tract illness. The agent was found to be extremely labile and virus recovery was rarely accomplished until specimens were immediately inoculated into tissue culture without prior freezing. Employing this technic, 57 strains of RS virus were recovered from children with respiratory illness from March through July, 1960. Virus was recovered most frequently from infants less than 7 months of age who were hospitalized for pneumonia (54%) or bronchiolitis (59%). The RS agent was isolated from 32% of children of all ages with bronchiolitis or pneumonia during this period. Virus was recovered significantly less often (1%) from control subjects.

Serologic studies condirmed the virus isolation data and provided additional evidence that the RS virus is a major respiratory pathogen of infancy and childhood. When the serologic technics were re-examined in the light of the virus recovery data, it was found that the CF test was only 50% efficient in detection of infection. This suggested that 22% of the total severe pediatric respiratory illness seen in Washington, D.C., during the past 3 years was associated with RS infection.

Para influenza viruses: Similar to the pattern established during the past 2 years the para influenza types 1 and 3 viruses were active in infants and children of the Washington, D.C., area during most months of the current year. These agents continued to play an important role in all types of pediatric respiratory disease, especially infantile croup which is the most serious respiratory emergency of childhood. For the first time evidence was obtained which

associated type 2 (CA) virus with illness. This agent was associated with 10 cases of croup hospitalized during November through January.

Adenoviruses: During the past 3 years 344 adenoviruses have been recovered from 3624 patients with respiratory disease - isolation rate = 9.5%. These viruses are currently being typed. An analysis of their contribution to pediatric illness must await completion of typing. It is important to assess the role of each adenovirus type separately since the recovery rate for all adenoviruses from control subjects is so high (5%), and since adenoviruses not only cause acute illness but persist for long periods in a latent form in lymphoid tissues.

Enteroviruses: Currently under investigation is the importance of the recently described enterovirus-like agents which grow only in human tissue culture cells and which have very fastidious conditions for such growth. The agents have been recovered from 6% of children with various types of respiratory disease. The recovery rate, however, from control subjects was the same (6%). Numerous conventional enterovirus infections have also been observed in this study population during the past few years. Analysis of their clinical importance awaits specific identification of the many strains of virus recovered.

Significance to the Program of the Institute: Respiratory disease is the most common infectious ailment of man. Such infections occur commonly and in their severest form during infancy and early childhood. Detailed knowledge of the agents responsible for respiratory disease and their natural history are a necessary prelude to attempts at either immunoprophylaxis or chemotherapy. As has been shown with the respiratory syncytial and para influenza viruses, the significance to be derived from newer respiratory agents discovered in childhood illness is also increased because they are associated with infections which may produce a generally milder illness in adults.

Proposed Course of the Project:

Proposed Course of the Project: It is planned that this study will continue for a period of several years, since a large segment of childhood respiratory illness remains to be elucidated. In addition, viruses responsible for respiratory illness vary at different times and in different localities. Therefore, surveillance should continue in order to comprehend the larger picture.

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Chanock, R.M., Bell, J.A., and Parrot, R.H.: Natural history of para influenza infection. Monograph in: PERSPECTIVES IN VIROLOGY II. In press, 1961.

Chanock, R.M., and Johnson, K.M.: Infectious Diseases (respiratory viruses). In: Annual Review of Med. Pub.: Annual Reviews, Inc. In press, 1961.

Honors and awards related to this project:

Invited to attend The Gustav Stern Symposium on Perspectives in Virology II, and present paper entitled, "Observations on natural history of infection with certain recently recognized respiratory viruses." January 25, 26, 1960, New York City, N.Y.

Invited to participate at Asian Influenza Conference (International), and present paper entitled, "Hemadsorption". February 17, 18, 19, 1960, National Institutes of Health, Bethesda, Md.

Invited to participate in Armed Forces Epidemiology Board meeting, April 4,5,6, 1960, Kenwood Country Club, Bethesda, Md.

Elected Full Member, Armed Forces Epidemiology Board-Commission on Acute Respiratory Disease, June, 1960.

Invited to participate in the American Medical Association Clinical Sessions, and present paper on respiratory virus infections, November 28, 29, and December 1, 1960, Washington, D.C.

Invited to contribute chapter on Respiratory Viruses for publication in ANNUAL REVIEW OF MEDICINE - 1961.

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1. Infectious Diseases
2. Virus & Rickettsial
3. Bethesda, Maryland

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Part A.

Project Title: Viral pneumonia: etiology, therapy, and prevention.

Principal Investigator: Dr. Robert M. Chanock

Other Investigators: Dr. Karl M. Johnson

Cooperating Units: Dr. J. Kingston, Dr. H. Bloom, Dr. M. Mufson, and Dr. F. Gordon, Bureau of Medicine and Naval Medical Research Institute, U.S. Navy

Man Years (calendar year 1960):
Total: 50/12
Professional: 10/12
Other: 40/12

Project Description:

Objectives: 1) To define the etiology of viral pneumonia as it occurs in persons of all ages. 2) To determine the relative importance of various newly recognized viruses in the pneumonia syndrome in different populations and at different times. 3) To define the natural history of certain agents which appear to be responsible for a significant proportion of pneumonia in the young (para influenza 3, respiratory syncytial, adenovirus, and Eaton agent) and in the adult (Eaton agent). 4) To continue the search for other as yet unrecognized viral agents which cause pneumonia. 5) To evaluate the effect of tetracycline chemotherapy in Eaton pneumonia.

Methods Employed: Current emphasis has been placed on the role of Eaton agent in human pneumonia. The presence and quantity of antibody for the Eaton agent was determined by the indirect fluorescent antibody technique, employing frozen sections of infected chick embryo lung.

Epidemiologic field studies were carried out at a Marine Recruit Training Center (Parris Island, S.C.) which has a high rate of Eaton infection. Patients with pneumonia, febrile respiratory illness without pneumonia, afebrile respiratory illness and comparable control subjects free of respiratory illness were studied serologically for evidence of

infection with Eaton agent, as well as other respiratory viruses.

The effect of demethylchlortetracycline on the course of Eaton pneumonia was determined in a double blind therapy study in which the clinical observers were unaware of the patients diagnostic or therapy status. Laboratory tests were performed without knowledge of which patients received drug and which patients received placebo. The patients were given a daily physical examination, temperature was recorded four times a day, and chest X-rays were taken every third day.

Major Findings:

A. Epidemiologic findings. Over a 1-year period Eaton infection was associated with 51% of the 530 pneumonias admitted to the Naval hospital. Adenovirus infection contributed significantly less to the pneumonia syndrome (6%).

Eighty-three per cent of the recruits lacked detectable antibody for Eaton agent when they entered recruit training. Fifty-three per cent of these sero-negative men developed antibody during the 3-month training period. For the group of incoming recruits as a whole, the risk of infection during training was 44%. Over a 6-month period the risk of pneumonia during training was 2% and the risk of an Eaton positive pneumonia was 1.5%. The infection to clinical pneumonia ratio was estimated to be 30 to 1.

Infection was widely disseminated through the recruit center, and occurred in almost every platoon of recruits. Movement of infection was slow; onsets of Eaton pneumonia occurred over a 9-week period in certain platoons. Long incubation period and slow movement of infection are attributes ideally suited to maintain this agent in an ever changing recruit population. The fluorescent antibody test proved to be twice as sensitive as the cold agglutinin technic in the diagnosis of Eaton pneumonia.

B. Chemotherapy. In a controlled double blind study involving 290 patients, demethylchlortetracycline significantly reduced the duration of fever, rales cough, malaise, and fatigue. Therapy stopped the progression of pulmonary infiltration and accelerated its clearing. Fever did not return when therapy was stopped. These findings strongly suggest that the drug exerted a direct action upon the pulmonary disease process.

Demethylchlortetracycline had no apparent effect on the course of a small mixed group of illnesses associated with other known respiratory viruses.

Significance to the Program of the Institute: As seen during the past 2 years, the consequences of infection with Eaton agent appear to be of considerable public health importance in both children and adults. The demonstration of a marked beneficial effect of demethylchlortetracycline on Eaton pneumonia thus constitutes a finding of major importance in the therapy of viral pneumonia.

Proposed Course of the Project: The search for other as yet unrecognized viruses which cause pneumonia will continue.

An unexpected finding in the chemotherapy study suggests that another tetracycline sensitive agent was responsible for a significant proportion of the pneumonias which were investigated. Efforts to recover this agent are currently in progress.

In addition to the unexplained pneumonias of early life, future studies will concentrate upon the heretofore completely neglected problem of pneumonia in the aged. Emphasis will be placed in these two areas since it is here that the problem of pneumonia mortality is greatest.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Johnson, R.T., Cook, M.K., Chanock, R.M., and Buescher, E.L.: Family outbreak of primary atypical pneumonia associated with the Eaton agent. New England J. Med., v: 262, 817-819, 1960.

Chanock, R.M., Cook, M.K., Fox, H.H., Parrott, R.H., and Huebner, R.J.: Serologic evidence of infection with Eaton agent in lower respiratory illness in childhood. New England J. Med., v:262, 648-654, 1960.

Cook, M.K., Chanock, R.M., Fox, H.H., Buescher, E.L., Johnson, R.T., and Huebner, R.J.: Studies on the role of Eaton agent in lower respiratory tract illness. Evidence for infection in adults. Brit. Med. J., v:I, 905-911, 1960.

Chanock, R.M., Mufson, M.A., Bloom, H.H., James, W.D., Fox, H.H., Kingston, J.R.: Eaton agent pneumonia. I. Ecology of infection in a military recruit population. J.A.M.A. In press, 1960.

Serial No. NIAID - 69B
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study of the laboratory aspects of respiratory virus illness in a welfare orphanage.

Principal Investigator: Dr. Robert M. Chanock

Other Investigators: Dr. K.M. Johnson, Dr. A.Z. Kapikian, Dr. R.J. Huebner, Dr. J.A. Bell

Cooperating Units: None

Man Years (calendar year 1960):
Total: 62/12
Professional: 12/12
Other: 50/12

Project Description:

Objectives: To elucidate the natural history of certain newly discovered respiratory viruses by means of a longitudinal study of an orphanage population. This study supplements our cross-sectional studies in pediatric hospitals, thus adding additional dimensions to our observations and increasing the scope of our comprehensive study of respiratory infections.

Methods Employed: The population under study is an orphanage nursery in the District of Columbia (see Project Report NIAID-66) whose average population is 90-100 children between the ages of 6 months and 3 years. The turnover of infants and children is rather rapid, and, as a result, a great deal of respiratory illness occurs in this population throughout the year.

Rectal temperatures are taken twice a day, and the population is carefully observed by a full-time pediatrician who also provides medical care. Throat and rectal swabs are collected three times a week. Serum specimens are obtained on admission and discharge, and at various intervals between. Throat swabs are tested in monkey kidney tissue culture for the presence of various myxoviruses and in Hep-2 cultures for respiratory syncytial (RS) and adenoviruses. Serum

Part B Included: Yes

specimens are tested for antibody to the various myxoviruses and other respiratory agents by the neutralization, hemagglutination-inhibition, or complement fixation technique. Laboratory data are then correlated with clinical, bacteriological, and epidemiological information in an effort to determine qualitative and quantitative virus disease relationships. As was true in previous years with the adenoviruses and the newer myxoviruses (para influenzas) and certain enteroviruses, Junior Village provided in 1960 additional opportunities for defining the clinical importance of viruses in childhood disease.

Major Findings:

Respiratory Syncytial (RS) Virus: An abrupt outbreak of pneumonia occurred during the last week of April and the first 2 weeks of May, 1960. Thirty-six (or 40%) of the 90 infants and children in residence at Junior Village developed pneumonia. The usual rate of pneumonia at the nursery is 1 - 2 cases per month.

The widespread occurrence of RS infection in the general Washington, D.C. community at the same time, and its association with lower respiratory tract illness,(see Project Report NIAID 69-B 1959) suggested that the nursery outbreak might also be caused by RS virus. Laboratory techniques were modified accordingly, and throat swab specimens were tested within a few hours after collection without prior freezing. The inoculation of fresh specimens is necessary because of the extreme lability of RS virus. Unfortunately this type of testing was not initiated until the last half of the outbreak. Nevertheless, 24 strains of RS virus were recovered, 18 of them from patients in the acute phase of a pneumonia illness. An analysis of the virus recovery and illness data strongly suggested that the RS agent was etiologically associated with pneumonia. Serologic tests indicated that approximately 90% of children in residence during the outbreak developed a rise in antibody for RS virus.

Significance to the Program of the Institute: The information gained during the RS virus outbreak has yielded additional evidence that this agent is a respiratory pathogen of major importance in infancy and early childhood. Study of the RS pneumonia outbreak provided an unusual opportunity to observe the clinical consequences of infection, and to determine the risk of lower respiratory tract involvement in a young population. The finding that 40% of the infants and children in residence developed pneumonia constitutes high order information which has implications both in the natural history of infection with this agent, and in attempts to control its more severe effects by immunoprophylaxis.

Proposed Course of the Project: The project will continue in an effort to gain a better understanding of the newer respiratory viruses. A retrospective serological analysis of the pneumonia experience at Junior Village will be undertaken with special emphasis on the role of para influenza 3 and respiratory syncytial viruses since these agents have been associated with the majority of much illness in the past 2 years.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Chanock, R.M., Wong, D.C., Huebner, R.J., and Bell, J.A.: Serologic response of individuals infected with para influenza viruses. Am. J. Pub. Hlth., 1960.

Johnson, K.M., Chanock, R.M., Cook, M.K., and Huebner, R.J.: Studies of a new human hemadsorption virus. I. Isolation, properties, and characterization. Am. J. of Hyg., v:71, 81-92, 1960.

Serial No. NIAID - 70
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Laboratory studies of newly recognized viruses associated with respiratory illness.

Principal Investigator: Dr. Robert M. Chanock

Other Investigators: Dr. K.M. Johnson, Dr. A. Kisch, Dr. F.R. Abinanti

Cooperating Units: None

Man Years (calendar year 1960):

Total:	20/12
Professional:	10/12
Other:	10/12

Project Description:

Objectives: 1) To investigate the biologic and antigenic properties of the newly recognized para influenza viruses. 2) To study the properties of Eaton agent and its growth in various types of tissue culture. 3) To develop simple procedures for the recovery, identification, and serologic study of Eaton agent.

Methods Employed: The standard tools of virology--neutralization, complement-fixation, and hemagglutination-inhibition are employed to determine the antigenic specificity and existence of shared antigens among the para influenza viruses.

The fluorescent antibody technique employing frozen sections of chick embryo lung is used as the standard of reference in all studies with Eaton agent. Attempts are made to cultivate the agent in various types of tissue culture and laboratory animals. Growth of Eaton agent is determined by titration of tissue culture fluid or organ suspension in embryonated eggs combined with immunofluorescent examination of embryo lung tissue. Cytopathic effects, cytochemical changes, and inclusion bodies are searched for in the inoculated tissue cultures. Fluids harvested from inoculated tissue cultures are tested

Part B Included: Yes

for antigens which might fix complement with potent convalescent serum from patients with Eaton pneumonia.

Major Findings:

1. Para influenza 3. The hemadsorption type 1 (HA-1) virus recovered from humans with respiratory disease and the SF-4 virus recovered from cows with shipping fever were shown to be distinct antigenically. Previously, these agents had been considered very closely related or identical. Guinea pigs infected with the HA-1 or SF-4 viruses developed a hemagglutination-inhibition and neutralization antibody response which permitted differentiation of these agents. Complement fixation tests employing viral or soluble antigens indicated that the two viruses were sufficiently related to be classified together as sub-types of para influenza 3. Each of 23 human isolates from various parts of the world resembled the HA-1 prototype. Each of the 7 bovine isolates from different parts of the United States were indistinguishable from the bovine prototype SF-4 virus. These findings suggest that these viruses do not cross species boundaries.

2. Eaton agent. The Eaton agent was shown to multiply without cytopathic effect in chick embryo entodermal, chick kidney, Hep-2, human kidney, and monkey kidney tissue cultures. The highest level of replication occurred in monkey kidney cultures where infected fluids contained up to 10^4 egg infectious doses of the agent. Antigen concentration within the infected tissue culture cells was insufficient to permit visualization by immunofluorescence. Complement fixing antigens were not detected in fluids from infected tissue cultures.

An eclipse phase was demonstrated during replication in monkey kidney cells. This suggests that the agent should be classified as a virus according to a recent definition proposed by Burnet.

In a simultaneous test, monkey kidney tissue culture was found to be as sensitive as embryonated eggs for the recovery of naturally occurring strains of Eaton agent. Fourteen strains were recovered in tissue culture from 17 patients with serologically positive pneumonia.

Preliminary studies indicate that the agent is inhibited in tissue culture by 1.5 micrograms per ml. of demethylchlortetracycline (Declomycin). This concentration is approximately 1/2 that found in the blood 24 hours after oral administration of a small dose (0.5 gm) of the drug. These findings are consistent with the therapeutic effect which we have recently demonstrated for demethylchlortetracycline in Eaton pneumonia. Preliminary studies suggest that the drug inhibits intracellular synthesis of Eaton agent, and has no effect upon the agent when it is in the free extracellular state.

Significance to the Program of the Institute: Knowledge of the antigenic properties of the para influenza viruses is required in order to perform meaningful epidemiologic studies with these agents. Information bearing upon the species specificity of the para influenza viruses greatly adds to our understanding of the natural history of these agents.

The growth of Eaton agent in various types of tissue culture and the recovery of naturally occurring strains in monkey kidney culture represents a significant forward step in the study of this respiratory pathogen. This technique has made it possible to recover strains which were suitable for administration to human volunteers, and to study the behavior of the agent in such infected individuals. Tissue culture studies with demethylchlortetracycline provide an experimental basis for understanding the chemotherapeutic effect of this drug in Eaton pneumonia, and offer a tool for further analysis of this phenomenon.

Proposed Course of the Study: Efforts will continue to maintain surveillance of the antigenic properties of the para influenza viruses.

The search for simpler laboratory procedures for the study of Eaton agent will continue. Various types of tissue culture will be tested for the capacity to support multiplication of this agent, preferably with cytopathic effect. Complement fixing antigen and hemagglutinin will be sought.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Chanock, R.M., Johnson, K.M., Cook, M.K., Wong, D.C., and Vargosko, A.: The hemadsorption technique with special reference to the problem of a naturally occurring simian para influenza virus. Proc. of the International Conf. on Asian Influenza. In: AM. REV. OF RESPIR. DISEASES (book). In press, 1961.

Craighead, J., Cook, M.K., and Chanock, R.M.: Infection of hamsters with para influenza 3 viruses. Proc. Soc. Exptl. Biol. & Med., v:104, 301-304, 1960.

Chanock, R.M., Fox, H.H., James, W.D., Bloom, H.H., Mufson, M.A.: Growth of laboratory and naturally occurring strains of Eaton agent in monkey kidney tissue culture. Proc. Soc. Exptl. Biol. & Med., v:105, 371-375, 1960.

Gordon, F.B., Quan, A.L., Cook, M.K., Chanock, R.M., and Fox, H.H.: Growth of the Eaton agent of primary atypical pneumonia in chick entodermal tissue culture. Proc. Soc. Exptl. Biol. & Med., v:105, 375-377, 1960.

Serial No. NIAID - 71
1. Infectious Diseases
2. Virus & Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies of tumor-producing viruses.

Principal Investigator: Dr. Wallace P. Rowe

Other Investigators: Dr. J.W. Hartley, Dr. R.J. Huebner, Mr. L.W. Smith

Cooperating Units: Dr. L.W. Law and C.J. Dawe, NCI, NIAID

Man Years (calendar year 1960):

Total:	45/12
Professional:	16/12
Other:	29/12

Project Description:

Objectives: To characterize the viruses which produce tumors in animals from the standpoints of their laboratory properties, natural behavior, and modes of spread.

Methods Employed: Application of standard and newly developed virologic procedures for detection and quantitation of animal tumor viruses.

Major Findings:

A. Mouse polyoma virus. Work with this agent has continued along the lines of the past year. The newer emphasis has been primarily on the natural history in wild mouse populations as described in Project No. 68.

1. The patterns of infection in individual animals have been studied in greater detail. It has been found that the virus produces a prolonged chronic infection when inoculated into weanling mice. The weanling mice sporadically excrete the virus in the urine.

Part B included: Yes

2. Serial passage lines of the virus have been carried in newborn mice with passages made at the peak of viral infectivity. In this way strains have been obtained which produce extremely high hemagglutinin titers in suckling mouse tissue, and which have altered patterns of disease production in newborn mice, producing greater tendencies for inducing the runting form of infection, and an increased tendency to produce bone tumors.

3. The laboratory aspects of the epidemiological work in wild mouse populations have shown the existence of local infections in rural farm mice and in mice in feed mills. The virus recovered from wild New York mice was shown to be oncogenic in baby mice and hamsters; the oncogenic activity of rural polyoma virus strains is under study.

4. It has been found that the virus is frequently present in transplanted tumor lines.

5. Production stocks of laboratory mice freed of infection by rearing under conditions similar to specific pathogen-free methods.

6. Polyoma virus in baby mice was shown to be a potent interfering agent, producing interference against the lethal effects of vesicular stomatitis and Coxsackie A viruses.

7. The reaction of mucoprotein non-specific inhibitors with the virus has been further characterized. The reaction with the inhibitor was found to induce marked aggregation of the virus. The strains which have become highly sensitive to inhibitors were shown to have lost, to a great extent, their ability to induce tumors in mice.

B. Mouse leukemia viruses. Studies of the Gross, Schwartz, and Moloney mouse leukemia viruses have centered primarily on reproducing the disease in laboratory mice, in attempts to free these viruses of contaminating mouse viruses, and on developing simpler assay procedures for the leukemia viruses.

1. Moloney and Gross leukemia viruses have been propagated through several serial mouse passages, produced a high incidence of leukemia with relatively short incubation periods. By passage of the Gross virus in the presence of suitable antiserum, it has been freed of polyoma and K viruses for long periods, which are contaminants of the standard Gross passage A virus. This should make it possible to attempt the development of in vitro assay procedures which will be specific for the leukemia virus..

C. Lymphomatosis virus of chickens. The sero-epidemiologic studies of antibodies to the cytopathic virus of Sharpless and Burmester indicated definitely that this virus is a contaminating agent and not the etiologic agent of visceral lymphomatosis (see Project Report 68-A).

Significance to the Program of the Institute: The contributions which can be made to the knowledge of the biology of tumor viruses, such as polyoma Friend leukemia and rabbit papilloma, when studied with standard virologic techniques is now becoming well recognized. The advantages of rapid indirect procedures for assay of tumor viruses (and extraneous viruses as well) as compared with the slow unpredictable tumor response endpoints make it imperative to attempt to develop such procedures for additional animal tumor viruses in order for reliable progress to be made in clarifying the viral etiology of cancer.

Proposed Course of the Project: The emphasis in the coming year will be to develop in vitro systems for recognition of the mouse leukemia viruses, particularly the Gross and Moloney agents, with special reference to a propagation in tissue culture. The epidemiologic studies of polyoma virus will be continued.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Rowe, W.P., Huebner, R.J., and Hartley, J.W.: The ecology of a mouse tumor virus. Monograph in: PERSPECTIVES IN VIROLOGY II. In press.

Huebner, R.J.: Viruses in search of cancer. Monograph in: PERSPECTIVES IN VIROLOGY II. In press.

Rowe, W.P., Hartley, J.W., Estes, J.D., and Huebner, R.J.: Growth curves of polyoma virus in mice and hamsters. National Cancer Institute Monograph No. 4 - Symposium on Phenomena of the Tumor Viruses, New York City, N.Y., March 25 and 26, 1960.

Rowe, W.P.: The epidemiology of mouse polyoma virus infection. Bacteriological Reviews. In press.

Rowe, W.P., Hartley, J.W., and Huebner, R.J.: The natural history of polyoma virus infection - a summary. In: CANADIAN CANCER CONFERENCE. In press.

Dawe, C.J., Rowe, W.P., and Law, L.W.: Influence of age, species, and immune factors on the response of salivary gland to polyoma virus in tissue culture. Pathologie et Biologie. In press.

Law, L.W., Rowe, W.P., and Hartley, J.W.: Studies of mouse polyoma virus infection. V. Relation of virus infection to lymphocytic neoplasms of the mouse. The J. of Exp. Med., v:111, 517-523, 1960.

Honors and awards related to this project:

Eli Lilly Award - Meeting of the Society of American Bacteriologists, May, 1960, Philadelphia, Pa.

Invited to participate in Symposium On Perspectives in Virology II, and present paper entitled "The Ecology of a Mouse Tumor Virus" January 25 and 26, 1960, New York City, N.Y.

Invited to attend Symposium On Phenomena of the Tumor Viruses, and present paper entitled "Biologic Behavior of the Polyoma Virus in Tissue Culture" March 26, 1960, New York City, N.Y.

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Appointed to Research Advisory Committee on Etiology of Cancer of the American Cancer Society, September, 1960.

Serial No. NIAID - 71A
1. Infectious Diseases
2. Virus & Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Continuing studies of adenoviruses and salivary gland viruses as examples of latent viruses.

Principal Investigator: Dr. Wallace P. Rowe

Other Investigators: Dr. J.W. Hartley, Dr. R.J. Huebner

Cooperating Units: Dr. J.A. Kasel, LCI

Man Years (calendar year 1960):

Total:	5/12
Professional:	1/12
Other:	4/12

Project Description:

Objectives: To characterize the salivary gland viruses and adenoviruses, and to determine their epidemiology and clinical importance.

Methods Employed: Serologic epidemiology and virus isolation in tissue culture. Studies of the biology of adenoviruses and salivary gland viruses in lower animals.

Major Findings:

Complement fixation tests with the human salivary gland virus have been done on sera of a number of suspected or diagnosed cases of cytomegalic inclusion disease of the newborn submitted by workers in various parts of the world. The findings suggest that in the newborn and young infant, the test is of little or no value in diagnosis, in that most cases have not yet responded with antibody during the period when diagnosis is necessary.

The finding of high prevalence of salivary gland virus infection in wild mice has been extended by testing mice from a number of rural areas, and the same high frequency of infection has been found. It has also been found that the infection in the wild

Part B included: Yes

mice is of long duration, the mice excreting virus in the saliva for as long as eight months after capture.

A new virus has been isolated from Microtus mice which appears to represent the Microtus strain of salivary gland virus group as detailed in Project No. NIAID-7IB.

We have continued to work with Dr. Kasel of the LCI in characterization of the enzyme formed by certain adenovirus types which destroys hemagglutination receptors on human erythrocytes. It has been established that the factor is neutralized type specifically by homologous rabbit antiserum, that it is separable from the infectious virus, and that its activity shows many characteristics of an enzymatic reaction.

Significance to the Program of the Institute: This laboratory is one of the very few in the world which has competence in handling the salivary gland viruses. There is great interest in clinical fields in the infections produced by this virus, and a corresponding interest in laboratory diagnosis of cases. The existence of comparable viruses in laboratory animals provides an excellent opportunity to study model infections.

Proposed Course of the Project: The diagnostic studies and the evaluation of the serologic tests will be continued. Epidemiologic studies of the spread of the virus in laboratory mice will be carried out by exposing mice through infected contacts under varying conditions.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Hartley, J.W., and Rowe, W.P.: A new mouse virus apparently related to the adenovirus group. Virology, v:11, 645-647, 1960.

Thalhammer, O., and Rowe, W.P.: Gibt es im Raum von Wien cytomegalie? Wiener klinische Wochenschrift. Sonderabdruck aus 72. Nr. 36, S. 621-624, 1960.

Kasel, J.A., Rowe, W.P., and Nemes, J.L.: Modification of erythrocyte receptors by a factor in adenovirus suspensions. Virology, v:10, 388-391, 1960.

Rowe, W.P.: Adenovirus and salivary gland virus infections in children. In: VIRAL INFECTIONS OF INFANCY AND CHILDHOOD. Ed. Harry M. Rose. Hoeber-Harper book, 205-214, 1960.

Serial No. NIAID - 71B
1. Infectious Diseases
2. Virus & Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies of mouse viruses with special reference to their importance as extraneous viruses ("background noise") in mouse tumor systems.

Principal Investigator: Dr. Janet W. Hartley, Dr. Wallace P. Rowe

Other Investigators: Mr. L.W. Smith, Dr. R.J. Huebner

Cooperating Units: None

Man Years (calendar year 1960):
Total: 87/12
Professional: 30/12
Other: 57/12

Project Description:

Objectives: To characterize and develop methods for recognizing infection and determine the epidemiological patterns of the various mouse viruses, for the purpose of defining and eliminating the problem of extrinsic viruses as complicating factors in studies of mouse tumor viruses.

Methods Employed: Serologic, tissue culture, and animal pathogenicity studies; attempts to isolate agents from laboratory and wild mice and from mouse tumors. Studies of the epidemiology of the agents under natural and artificial conditions.

Major Findings:

A. Mouse adenovirus. The new virus recovered from General Purpose Swiss mice which produces myocarditis and adrenal necrosis, has been identified as a mouse representative of the adenovirus group. Infection with this agent is found in many laboratory mouse colonies, but, so far, has not been encountered in wild mouse populations.

Part B Included: Yes

Virus is found in urine of spontaneously and artificially infected mice for prolonged periods up to 8 months or longer, and it is concluded that the chief mode of spread of this virus is by urinary excretion.

B. Thymic agent (TA). A new virus has been recovered from the NIH General Purpose Swiss mice and from wild house mice. This virus induces a unique necrosis of the thymus when inoculated into infant mice. It produces a chronic infection with prolonged excretion of virus in saliva.

C. Reoviruses have been found to be very common agents of laboratory mice. Reovirus 3 has been found in the majority of mouse colonies, and suggestive evidence of its presence in germ-free mice has been obtained. The hepatoencephalitis virus of Stanley, which was isolated in 1953, and considered by some workers to be a mouse virus, has been identified as a Reo 3 strain.

D. A new virus, presumably of mouse origin, has been isolated from Ornithonyssus bacoti mites which were feeding on laboratory mice. This virus induces an encephalitis in baby mice.

E. In the course of studies of polyoma epidemiology in wild rodent populations, a new virus isolated from the Microtus mouse. This virus perhaps is the Microtus representative of the salivary gland virus group, but it differs from other species salivary gland viruses by its ability to grow in tissue cultures from many different species. A complement fixation test has been developed for this agent.

F. "K" virus. The K virus of mice has been found to be prevalent in almost all colonies tested. Suggestive evidence of its presence in germ-free mice has been obtained. K virus has also been isolated from wild mice, being found in saliva or urine. A complement fixation test has been developed which currently is being analyzed.

G. Mouse hepatitis virus. Studies have been made of the mouse hepatitis virus and its activation by Eperythrozoon coicoides as described by Gledhill. Hepatitis virus can be activated in specific pathogen-free mice with the same regularity as in conventional laboratory mice, suggesting that the virus is transplacentally acquired.

H. Mouse salivary gland virus has been found to be prevalent in virtually all wild Mus musculus colonies both urban and rural. Its natural history is currently under study.

Significance to the Program of the Institute: With the continual expansion of attempts to develop indirect assay procedures for mouse tumor viruses, it has become increasingly evident that the viral flora of the mouse is a major stumbling block to developing reliable study systems. In this and other laboratories, attempts to develop such assays for tumor viruses have repeatedly foundered because of the emergence of extraneous mouse viruses in the indicator systems. Only by a thorough understanding of the extraneous agents which are likely to be encountered, and the ability to recognize them can tumor virus studies proceed intelligently with the hope of obtaining reliable information of high order. Secondly, the increasing evidence of the importance of the interference phenomenon in determining the outcome of viral experiments in mice makes it important to evaluate the importance of extraneous viruses as interfering factors in tumorigenesis studies. Thirdly, the possibility must be considered that so-called "non-tumor viruses" may play a role in the etiology of neoplasms; the mouse viruses provide an excellent opportunity to test this hypothesis. Fourthly, the viruses of mice have long been recognized to be useful models of virus infections of humans (some of them are identical or similar to human areas) and on their own merit are deserving of study.

Proposed Course of the Project: Epidemiologic studies of the newer mouse viruses will be continued, and additional attempts will be made to develop and evaluate serologic tests useful for detection of infection.

Extension of current serological surveys are planned to determine if associations exist between certain mouse tumors and past infection with the known and newly discovered mouse viruses. Mice, hamsters, and other laboratory species will be inoculated with sublethal doses of the various mouse viruses and long-term observation will be carried out for possible tumor induction.

Studies will be made of the influence of various mouse breeding practices such as sterilization of food and rearing under specific pathogen-free and germ-free conditions as they influence the occurrence of mouse viruses in the hope that practices can be designed to eliminate mouse viruses as an uncontrolled variable in study systems.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Hartley, J.W., and Rowe, W.P.: A new mouse virus apparently related to the adenovirus group. Virology, v:11, 645-647, 1960.

Serial No. NIAID - 72
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Laboratory studies of enteroviruses.

Principal Investigator: Dr. Karl M. Johnson

Other Investigators: Dr. Robert M. Chanock, Dr. Robert J. Huebner

Cooperating Units: Dr. L. Rosen, Epidemiology Section, LID

Man Years (calendar year 1960):
Total: 14/12
Professional: 4/12
Other: 10/12

Project Description:

Objectives: To develop simple reproducible methods for laboratory manipulation of enteroviruses, particularly the large group of newly recognized agents which appear to be potential causes of respiratory disease. Studies are designed specifically to determine optimum methods of cultivation of the agents in either tissue culture or small laboratory animals. In addition, efforts are made to a) determine the optimum manner of preparing specific antisera, b) to evaluate various serologic procedures potentially of use in field studies, and c) since many new viruses are fastidious in their growth requirements, to study where indicated certain fundamental properties of these viruses.

Methods Employed: These will be described where pertinent under consideration of results so far obtained with certain of the viruses.

Major Findings:

1. Coe virus: Newly recovered strains of Coe virus have proven to have properties differing from those described for the original prototype agent in several important respects. The presence of hemagglutinin is demonstrable when human type O erythrocytes are employed and the test performed at 4° centigrade. The reaction is temperature dependent and can be reversed at 34° C. Elution, however, is not enzymatic since the erythrocyte receptors are not altered following

Part B Included: No

reversal of hemagglutination. The component of the virus which participates in the hemagglutination reaction contains specific Coe antigen as indicated by the inhibitory effect of specific antiserum. The hemagglutinin is quite stable at 4° and at -20° C., but is fairly rapidly inactivated at 34° C. Studies of virus antibody kinetics in the hemagglutination inhibition (HI) test indicate that optimal virus antibody reaction occurs following incubation at 24° for approximately 2 hours, or at 4° for 14 hours. Using the latter procedure, a rise in HI antibody was demonstrated for 15 of 19 men from whom the virus was recovered.

Comparative studies indicate that primary human embryonic kidney cells (HK) are more sensitive than serially passaged human cells to natural strains of Coe virus. In addition, HK cells produce a greater quantity of hemagglutinin than do continuous cell lines of human origin regardless of the nutrient medium employed. For optimum results with Coe virus it is necessary that the culture tube be rotated rather than incubated in the conventional stationary position.

Our strain of Coe virus, unlike the prototype, has been successfully adapted to the suckling mouse. Typical Coxsackie-like hind leg paralysis has been produced through 5 consecutive passages. This observation is currently being exploited in attempts to prepare specific viral antisera, as well as for experimental production of a complement fixing antigen.

2. Newly described enterovirus-like agents: In January of this year English workers described several strains of viruses with certain properties of enteroviruses, which they had recovered from nasal secretions of volunteers with clinical cold-like illnesses. They reported that an unusual set of laboratory conditions was required for a successful cultivation of these agents. In general, we have succeeded in confirming their observations and have made further progress in efforts to adapt and manipulate these viruses in the laboratory. Human embryonic kidney seems to be necessary for recovery of most of these agents. Preliminary studies suggest that there is an inverse relationship between the gestational age of the fetus from whom the cultures are derived and the occurrence of cytopathic effects produced by these agents. At least two of these viruses have been successfully adapted in this laboratory to continuous type cultures of human epithelial origin. Using these cells as a source of viral antigen, high-titered specific immune serum has been prepared in guinea pigs and a reproducible neutralization technique worked out. Current efforts are being directed toward a search for complement fixing antigens and hemagglutinins. Employing human embryonic kidney tissue cultures, numerous new virus strains have been recovered which seem to possess properties similar to those of the English viruses. Sorting out these new isolates, and the establishment of new serotypes, is currently under way.

Significance to the Program of the Institute: These studies provide new diagnostic tools and opportunities for epidemiologic investigation of a large group of viruses which are potentially significant in human respiratory disease.

Proposed Course of the Project: Efforts will be made to simplify the identification of the enteroviruses which grow predominantly in human tissue culture cells. This includes sorting out of the available isolates and the recognition of new serotypes. Fortunately, methods for CF and HI identification of enteroviruses developed by the laboratory unit of the Epidemiology Section and by the Serology Unit of LID, promises to reduce the time and work required to compare presumably "new" enteroviruses with the 58 serotypes already classified. Attempts will be made to develop simplified methods of antibody assay for use in epidemiologic studies. Until this is done, understanding of the role these agents play in human disease will proceed at a slow pace.

Serial No. NIAID - 72A
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study of viral respiratory disease in a military population.

Principal Investigator: Dr. Karl M. Johnson

Other Investigators: Dr. Robert M. Chanock, Dr. Robert J. Huebner

Cooperating Units: Dr. H.H. Bloom, Dr. M. Mufson, Naval Medical Field Research Laboratory, Camp Lejeune, North Carolina

Man Years (calendar year 1960):

Total:	24/12
Professional:	6/12
Other:	18/12

Project Description:

Objectives: To study on a continuing basis the role of newly recognized viruses in adult respiratory illness. This study was organized in order to compare viral experience of men fresh from recruit training with that of individuals forming the stable cadre personnel. An opportunity is also provided to assay the effectiveness of certain viral vaccines administered to personnel during their recruit training at another military center.

Methods Employed: Clinical records and appropriate specimens are collected routinely from individuals in various dispensaries at Camp Lejeune, North Carolina. Such materials are also obtained from individuals hospitalized at this base, and in certain instances, from children, wives and mothers seen in dependent outpatient clinics. Special emphasis has been placed upon the collection of specimens from matched control groups.

Clinical specimens are tested in monkey kidney and Hep-2 tissue cultures. Certain other tissue cultures are employed whenever indicated. Paired serum samples are available from well over 90% of individuals in the study and are assayed for development of antibody for various viral agents. Specimens for virus isolation are divided into two portions and stored in this laboratory and the Camp Lejeune

Part B Included: Yes

laboratory respectively.

Major Findings:

1. Adenoviruses: In February, March, and April of 1960 adenoviruses were responsible for a major outbreak of respiratory disease in the post recruit population (Infantry Training Regiment, ITR). Attack rates in this unit attained a level of nearly 200 per 1000 week. Infection and apparent illness were also disseminated into the cadre population, although there was a clearly diminishing incidence in correlation with length of time individuals had been in military service.

2. Myxoviruses (parainfluenzas): During February and March of 1960 sporadic isolations of para influenza viruses were also recorded. Although insufficient data were obtained to permit epidemiologic association of para influenza virus infection with illness, several new laboratory phenomena were observed which had not been previously encountered with these viruses in the study of pediatric populations. With only one exception, all 15 individuals from whom virus was recovered had significant neutralizing antibody against the infecting agents in their acute phase sera. Virus recovery proved difficult, and many of the isolates were not recognized until after two weeks of tissue culture incubation. Re-isolation of the agents proved exceedingly difficult. Appropriate controls were included in each test to insure that the isolates did not represent contamination from the laboratory environment. Serologic confirmation of infection was obtained in only 9 of the 15 cases despite the utilization of neutralization, hemagglutination-inhibition, and complement fixation techniques. The lack of serologic response to infection was most common with para influenza 1 virus. These observations suggest that the infections were mild and that very small amounts of virus were excreted by the infected individuals. Information derived from this experience, however, has allowed a better formulation of laboratory procedures designed to elucidate the role of these agents in adult respiratory illness in the future.

3. Coe virus outbreak: During October and November of 1960, a major outbreak of respiratory disease associated with Coe virus, a newly discovered enterovirus-like agent, was observed. Virus was recovered significantly more often from men with respiratory disease than from comparable control subjects free of such illness. Attack rates have not yet been calculated, but they seem to have been extremely high. Clinical illness consisted of mild upper respiratory infection with or without fever. Mild pharyngitis was frequently observed. Hospital surveillance indicated no significant increase in the number of admissions for pneumonia, nor were there any clinical cases of herpangina, pleurodynia, or aseptic meningitis. The occurrence of infection in children during this time has also been documented but the role of this virus in pediatric illness remains to be assessed.

The current outbreak represents the first documented association of an enterovirus with a large scale occurrence of mild respiratory illness in adults.

Significance to the Program of the Institute: This project represents a major continuing inquiry into the nature of respiratory disease in adult populations. It has already yielded evidence for the association of a new enterovirus with respiratory disease, as well as providing needed laboratory information concerning para influenza virus infection in adults. A well documented collection of case histories, specimens, and paired sera provides an excellent source of clinical material which is available for the evaluation of newly discovered agents, as well as those not yet known to us.

Proposed Course of the Project: Field surveillance is being continued. Extension of the program to incorporate dependents (both adults and children) is being undertaken. In this way a broader picture of the natural history of newly recognized respiratory viruses can be acquired.

A study designed to test the effectiveness of adenovirus vaccines is also being organized. Since all personnel at the Infantry Training Regiment represent immediate graduates of the recruit training base at Parris Island, South Carolina, and since all these men are currently being given adenovirus vaccine upon entry into the Marine Corps the following procedures will be employed: A large number of men undergoing training at Parris Island will be bled approximately 4 to 5 weeks following administration of adenovirus vaccine. Recruits are routinely bled when they enter service and this serum is available to us. These individuals will be observed during their time at the Infantry Training Regiment, and a certain percentage of them will also be available for study following this period of training. In this way the immunogenic potency of the vaccine as well as its effectiveness in preventing illness may be assessed.

In cooperation with personnel at the hospital at Camp Lejeune a study of the effectiveness of tetracyclines in the therapy of febrile respiratory disease with and without pneumonia is also being organized. Among the agents to be included in this study will be pneumonia caused by the Eaton virus which has recently been shown to respond to chemotherapy.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Bloom, H.H., Johnson, K.M., Chanock, R.M., Huebner, R.J., and Jacobsen, R.F.: Recovery of para influenza viruses from adults. J. of Clin. Investigation. In press.

Serial No. NIAID - 72B
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study of respiratory viruses in human volunteers.

Principal Investigator: Dr. Robert M. Chanock, Dr. Karl M. Johnson

Other Investigators: Dr. V.L. Knight, Dr. H.M. Kravetz, Dr. D.E. Rifkind, Dr. J.P. Utz

Cooperating Units: Laboratory of Clinical Investigations, NIAID

Man Years (calendar year 1960):
Total: 17/12
Professional: 5/12
Other: 12/12

Project Description:

Objectives: To determine whether newly recognized viruses of potential respiratory pathogenicity are capable of inducing mild illness in adults under controlled conditions. To examine the experimental pathogenesis of such viral infection, and to provide new information specifically designed to facilitate the study of naturally occurring mild respiratory illness in adults.

Methods Employed: Young adult male volunteers from federal prisons are selected on the basis of a) general good health, and b) neutralizing antibody status for the particular virus to be studied. Volunteers are maintained in groups of three in strict isolation on a clinical service of the NIAID. Histories and routine physical examinations, as well as certain basic laboratory procedures are performed upon admission. Known amounts of test viruses are administered intranasally, and daily clinical observations, as well as appropriate specimens for laboratory analysis, are obtained. Virus specimens are inoculated into appropriate tissue cultures and/or animals. Serologic tests are performed on suitably collected serum samples.

Part B Included: No

Major Findings:

Para influenza 4: In a single experiment, approximately 10,000 TCD_{50} of monkey kidney adapted para influenza 4 virus infected only two of six volunteers receiving the inoculum. No clinical illness was observed.

Respiratory Syncytial Virus: In four experiments, volunteers were successfully infected when given a small dose (160 to 640 TCD_{50}) of respiratory syncytial (RS) virus. One half of the volunteers developed typical cold-like illness without fever or pharyngitis. The occurrence of illness correlated with the length of time virus was shed and the onset of virus excretion was coincident with or preceded the appearance of clinical illness in every instance, providing evidence that the observed colds were actually produced by the inoculated virus. Development of RS virus complement fixing antibody correlated with the occurrence of illness. Since all volunteers in this study possessed RS neutralizing antibody prior to challenge, these experiments represent examples of mild illness produced by viral reinfection. The occurrence of illness was not related to the level of neutralizing antibody present prior to administration of virus. Studies designed to investigate the circumstances associated with this interesting and potentially significant finding are currently in progress.

Significance to the Program of the Institute: This project has resulted in the experimental demonstration of a significant new concept concerning the pathogenesis of mild respiratory illness in adults. It has also provided valuable information pertinent to the design and execution of future field studies of RS virus infection.

Proposed Course of the Project: Volunteer studies involving the Eaton agent (associated with all, or almost all, cold agglutinin positive atypical pneumonia and a significant proportion of cold agglutinin negative pneumonia) are in progress. This investigation should yield important information relating to the varying clinical consequences of infection with this agent. Future studies are planned to evaluate certain other potential respiratory viruses, including a para influenza virus, the family of Reoviruses, as well as certain newly described enteroviruses.

Influenza A and B viruses grown in monkey and human tissue cultures will be studied in volunteers as part of a search for attenuated variants suitable for inclusion in a live virus vaccine.

Serial No. NIAID - 73
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Application of fluorescent antibody staining technique to the study of respiratory viruses.

Principal Investigator: Dr. Alexander L. Kisch

Other Investigators: Dr. Robert M. Chanock, Dr. Karl M. Johnson

Cooperating Units: None

Man Years (calendar year 1960):
Total: 8/12
Professional: 6/12
Other: 2/12

Project Description:

Objectives: 1) To study the sequence of intracellular events leading to the production of viral antigen and the production of syncytia by respiratory syncytial (RS) virus, and to differentiate this virus from others, such as measles, also producing syncytia in tissue culture. 2) To develop, if possible, a rapid diagnostic test for certain viral agents causing respiratory disease in children and adults utilizing fluorescent antibody technique. 3) To determine the nature of the intracellular substance which is labelled by fluorescein-labelled antibody using cytochemical methods.

Methods Employed: The agents under study, specifically RS virus and Eaton agent, are grown in tissue culture using a variety of tissues. At timed intervals, infected coverslips are removed and stained by several methods: fluorescent antibody, Toluidine Blue-Molybdate (for RNA), Feulgen (for DNA), Hematoxylin-eosin (for inclusion bodies), Sudan Black and PAS (for definition of "golgi material"). RS virus is compared with measles virus,which also leads to syncytia production in the attempt to further characterize and classify RS virus.

Further, cell-bearing secretions from the respiratory tract of volunteers, some of whom have been infected with either RS or PAP virus, are stained by indirect fluorescent antibody technique utilizing

Part B Included: No

appropriate controls in the attempt to diagnose infection early and specifically. If this becomes possible, it would have significant therapeutic implications in one of the few virus diseases susceptible to broad spectrum antibiotics.

Major Findings:

It was possible to stain cells infected with RS virus specifically using human convalescent serum and fluorescein-labelled anti-human globulin. Fluorescent staining antigenic material is restricted to the cytoplasm, and is first demonstrable after approximately 12 hours. Individual infected cells show fluorescence 24 hours before the earliest characteristic CPE is recognizable by light microscopy. No intranuclear fluorescence was observed despite the appearance of nuclear changes occurring earlier and simultaneously in infected cells as demonstrated by Toluidine Blue-Molybdate staining.

Attempts to demonstrate specifically-staining antigen in cells from the nasopharynx of volunteers infected with RS virus have been unsuccessful to date.

Significance to the Program of the Institute: This project has resulted in a development in this laboratory of a technically difficult technique of wide applicability to the study of viral infection in tissue cultures as well as in infected patients. Should it become possible to diagnose human infections with RS virus and/or Eaton agent by this method, this will be of value in clinical and epidemiologic studies.

Proposed Course of the Project: Further studies to determine whether it is the viral or the soluble antigen of the RS virus which is stained by immunofluorescence, will be carried out. Studies on secretions obtained from volunteers infected with Eaton virus are under way, and further studies on subjects infected with RS virus will be carried out at a later date.

Serial No. NIAID-74
1. Infectious Diseases
2. Virus and Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Study of Bovine Respiratory Diseases.

Principal Investigator: F. R. Abinanti

Other Investigators: R. J. Huebner (LID); Robert Byrne (University of Maryland); Alvin Hoerlein (University of Illinois)

Cooperating Units: Veterinary Science Department, University of Maryland; College of Veterinary Medicine, University of Illinois

Man Years: (Calendar Year 1960)
Total: 6/12
Professional: 3/12
Other: 3/12

Project Description:

Objectives: This is a continuation of a project in effect during the preceeding fiscal year. Essentially these investigations include studies of outbreaks of respiratory virus disease in cattle, of experimental pathogenesis of the viruses injected into cattle, and conducting sero-epizootiologic surveys of their prevalence. Experimental vaccines are also prepared and their antigenic potency tested in cattle.

Specific Objectives: (1) The isolation of viral agents from the respiratory tract of normal cattle and cattle with respiratory disease; (2) to assess the role of myxovirus para-influenza 3 virus and other newly recognized viruses in respiratory disease of cattle; (3) to determine the relationships of the human and bovine strains of myxovirus para-influenza 3; and (4) to evaluate antiviral vaccines.

Part B included: Yes

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Methods Employed: Specimens of nasal secretions were taken from cattle and inoculated onto tissue cultures of bovine and monkey kidney. The viruses isolated are characterized and compared to known viral agents of man, cattle, and other animals. Serial samples of blood are taken from normal cattle and acute and convalescent specimens from diseased cattle, and hemagglutination inhibition, complement fixation, and neutralization tests are used to obtain information concerning the prevalence, spread and natural history of respiratory viruses. Groups of cattle are vaccinated with experimental vaccines and potency assessed by various serologic tests.

Major Findings: (1) Eighteen recoveries of myxovirus para-influenza 3 virus have been made from cattle suffering from respiratory disease and serologic evidence of a relationship of this virus to the outbreaks of respiratory disease was found. Extensive sero-epidemiologic studies provided evidence that infection with this virus is widespread. In a preliminary test, young cattle developed good levels of antibody to experimental vaccines prepared against both human and bovine strains of para-influenza 3; they appeared to be refractory to an aerosol challenge by live virus.

(2) Several large groups of cattle were vaccinated under field conditions with commercially produced para-influenza 3 (bovine strain) vaccines. These vaccines also produced good levels of circulating antibody. Unfortunately, in these field trials little or no respiratory disease occurred.

(3) Comparative studies of the human and bovine strains of the virus showed that they can be distinguished by means of prototype guinea pig sera in the HI test.

(4) Infectious bovine rhinotracheitis, a virus usually recovered from cattle with respiratory disease, was recovered from cattle involved in a large outbreak of bovine conjunctivitis - attended by little or no respiratory disease. The virus strain recovered produced conjuncitivtis and minimal respiratory illness in experimentally inoculated calves.

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Significance to the Program of the Institute: The discovery of viruses in domestic animals which are similar or identical to those responsible for large amounts of human respiratory disease is of obvious importance. Furthermore, the study of respiratory disease in domestic animals and the efficacy of antiviral vaccines in controlling such illnesses provide incomparable models of similar problems in man. In addition, solutions to some of the common viral diseases of food animals have great significance for human health in many areas of the world where animal protein is deficient. The prevalence of these domestic animal viruses suggests the possibility of a widespread zoonotic potential.

Proposed Course of Project: Work on this project has been temporarily discontinued. We do not have sufficient resources to mount adequate field studies, and so far we have found it impossible to find collaborators able to conduct studies designed to answer definitive questions on the etiology of bovine respiratory diseases.

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Abinanti, F.R., Byrne, R.J., Watson, R.L., Poelma, L.J., Lucas, F.R., and Huebner, R.J.: Observations on infection of cattle with myxovirus para-influenza 3. Amer. J. Hyg., 71:52-58, 1960.

Abinanti, F.R. and Plumer, G.J.: The isolation of infectious bovine rhinotracheitis (IBR) virus from cases of conjunctivitis and observations on the experimental infection. A.V.M.A. Research J. Accepted for publication.

Byrne, R.J., Abinanti, F.R., and Huebner, R.J.: Vaccination of cattle with myxovirus para-influenza 3. Cornell Vet. Accepted for publication.

Abinanti, F.R., Hoerlein, A.B., Watson, R.L., and Huebner, R.J.: Serological studies of myxovirus para-influenza 3 in cattle and prevalence of antibodies in bovines. J. Immunol. Accepted for publication.

Book: ADVANCES IN VETERINARY SCIENCE. Chapter on "Shipping Fever in Cattle", by S. K. Sinha and F.R. Abinanti. 100 pages. To be included in 1962 edition.

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Honors and Awards relating to this project:

Invited to participate in Annual Conference of Veterinarians. Talk: "Respiratory Diseases of Cattle" - University of Pennsylvania, Philadelphia, May, 1960.

Program Chairman and participant in Maryland Veterinary Medical Association Meeting - Ocean City, Maryland, June, 1960.

Participated in Conference on Zoonoses, University of Illinois, Urbana, September, 1960.

Invited to participate in CDC Biennial Public Health Conference. Talk: "Respiratory Diseases of Man and Animals" - Atlanta, Georgia, September, 1960.

Participated in Conference of Public Health Veterinarians, American Public Health Association, San Francisco, California, November, 1960.

Serial No. NIAID- 74A
1. Infectious Diseases
2. Virus and Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A Study of Squamous Cell Carcinoma (Cancer Eye) in Cattle.

Principal Investigators: Dr. F. R. Abinanti, Dr. W. Gay (DRS), Dr. M. Stanton (NCI)

Other Investigators: Dr. R. J. Huebner

Cooperating Units: Bay Manor Farms (owner Mr. Otis Smith), Lewes, Delaware; M.D. Anderson Hospital (Dr. Russell), Houston, Texas

Man Years: (Calendar Year 1960)

Total:	9/12
Professional:	6/12
Other:	3/12

Project Description:

Objectives: There are approximately 600 hereford cattle on the Bay Manor Farms, Lewes, Delaware, and about 30 per cent of these cattle have precancerous or cancerous lesions of the eyes and orbital appendages. The principal efforts are to determine if a virus is responsible for this condition and to study the natural history of the disease.

Methods Employed: Monthly inspections are made of the herd and a photographic record is being made of the progress of the lesions. At this time eye swabs are taken and blood for possible future serological studies. Dr. William Gay surgically removes affected tissues at intervals which are examined histologically by Dr. M. Stanton, and virologically by Dr. F. R. Abinanti. Further frozen sections will be examined by the fluorescent technique to explore the possibility of there being a specific antigenic material present in the cancers.

Part B Included: No

Serial No. NIAID- 74A

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Individual Project Report
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A sero-epizootiological study of cattle with and without "cancer eye" has been planned in cooperation with the M.D. Anderson Hospital "Cancer-Eye" research group under Dr. William Russell. Several hundred sera will be collected by this group from cattle with cancer eye and matched controls. The specimens will be tested for antibodies vs. bovine viruses and for some of the mouse, chicken and rabbit tumor viruses.

Major Findings: None. This is a new project.

Significance of the Program to the Institute: Provides an opportunity to explore the possible viral etiology of squamous cell carcinoma. The Bay Manor herd provides excellent nearby material for epidemiological and natural history studies of the cancer itself. Since the precancerous lesions are papillomatous, the possible relation of the lesions and papilloma viruses is currently under study.

Proposed Course of the Study: The continuation of this project will depend on whether practical leads concerning causative viruses can be found in the near future; in that event, the termination date of this project cannot at this time be predicted.

Serial No. NIAID- 75
1. Infectious Diseases
2. Virus and Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Laboratory and epidemiologic studies of viruses as possible etiologic agents of acute leukemia in the pediatric age group.

Principal Investigator: Dr. M. Katherine Cook

Other Investigators: Dr. Robert J. Huebner
Mr. Horace C. Turner

Cooperating Units: Dr. Charles Chany, Laboratoire des Virus, Hopital Saint-Vincent-de-Paul, Paris;
Dr. Jean Bernard, Hematology Service, le centre Claude-Bernard de l'Hopital Saint Louis, Paris

Man Years: (Calendar Year 1960)

Total:	14/12
Professional:	14/12
Other:	None

Project Description:

Objectives: (1) To search for viral agents, including unknown tumor viruses, in children with acute leukemia; (2) To study the role of these viruses in the etiology of acute childhood leukemia and to attempt to obtain some evidence to support or disprove the theory of the viral etiology of this disease; (3) To determine the effect of cortisone on the incidence of viral infections in children; (4) To study the possibility of lysogenic systems in herpes virus and enterovirus infections.

Part B Included: Yes

Serial No. NIAID- 75

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Methods Employed: The study population includes 110 children hospitalized for acute leukemia at l'Hopital Saint Louis, Paris; the control populations are (1) children hospitalized for other blood disturbances in the hematology wards with the acute leukemia patients; and (2) children of similar age, economic background, sex, and race hospitalized in the general pediatric wards at Hopital Saint-Vincent-de-Paul, Paris. The children hospitalized at St. Louis were admitted from all parts of France, and, on a consultation basis, from all over Europe. The only treatments currently being employed for acute leukemia at Saint Louis are X-radiation, cortisone, bone-marrow transplants, and transfusions with whole blood and/or packed red cells.

It is postulated that if the virus (or viruses) responsible for the leukemia were maintained in a "provirus" state in the cell of the individual that it should manifest itself by the occasional production of mature virus and that heavy cortisone treatment may enhance the possibility of recovering the agent, if present. It has been possible to study approximately 70 of the leukemic children from the day of diagnosis until their deaths.

Conventional virus isolation attempts in tissue cultures are used on throat and anal swabs taken weekly from every child for the duration of his hospitalization and at the time of his visits to the out-patient clinic. Bone-marrow specimens are obtained whenever possible. Blood specimens for serologic studies are obtained every two weeks during the period of hospitalization and also when the child reports to the out-patient clinic. In addition to the standard isolation techniques, the interference technique using para-influenza I (Sendai) as an indicator virus and the indirect fluorescent antibody technique are employed to study a number of the specimens. Serologic surveys with known viruses, including known animal tumor viruses, are being performed to obtain information on the incidence of past infection in the acute leukemia group as compared to the two control groups.

Laboratory data are correlated with clinical and epidemiologic information in an effort to determine virus-disease relationships.

Major Findings: This is a new project and present information is limited to evaluation of the interference technique and indirect fluorescent antibody techniques for demonstration of viruses which do not cause cytopathogenic effects or other changes in tissue culture, and to the incidence of known agents in the study population.

It can be stated, however, that herpes virus was extensively prevalent in the leukemic children and that enteroviruses were conspicuously absent as compared to the control group.

Significance to the Program of the Institute: This project, a controlled longitudinal epidemiologic and laboratory approach to the study of the viral etiology of acute lymphocytic leukemia in the pediatric age group, represents a rather unique and intensive effort to acquire a knowledge of the virologic experiences in children with acute leukemia. Such a study is needed to establish suitable baselines to interpret future laboratory studies of specimens from leukemia patients. At least one full year of epidemiologic and laboratory studies are essential to account for possible seasonal variations in the exacerbation of viruses. It has been reported earlier this year that two animal tumor viruses, Rous sarcoma virus (Prince, 1960) and polyoma virus (Vogt and Dulbecco, 1960) appear to undergo something suggestive of lysogeny. In view of these findings the potential importance of persistent and recrudescent viruses, such as recurrent herpes and varicella, and incomplete enteroviruses participating in a lysogenic system in the human leukemia patient needs investigation. The effect of cortisone on bacterial infection in man has been well documented but little information is available on its activity in viral infection. The extensive clinical use of this drug is sufficient justification for a controlled study to determine the effect of cortisone on viral infections in man.

Proposed Course of Project: During the next one-two months serologic studies with sera from the leukemia patients and the two control groups will be completed to determine the relative prevalence of viral infections in the leukemia group with viruses that are known to produce proliferative growth in tissue culture, with various animal tumor viruses and with the more common viral pathogens as compared with those in control populations.

In collaboration with Dr. Chany, anal specimens will continue to be collected from leukemia and control children at l'Hopital St. Louis to determine if the rate of enterovirus isolations in the study group continues to remain low when compared with the control group. Serologic studies of the prevalence of the prevalence of infection with the more common respiratory viruses in cortisone treated and non-cortisone treated children will be completed.

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Laboratory investigation of incomplete herpes virus and incomplete herpes virus and incomplete enteroviruses will be pursued at the Max-Planck Institute fur Virusforschung, Tubingen, Germany, where training received in nucleic acid work, high tension electrophoresis, ultracentrifugation and other physical-biological techniques will be applied to these problems.

PART B Honors, Awards, and Publications

Publications other than abstracts from this project:

Chany, C. and Cook, M.K.: Sur un facteur collulaire induit par le virus entrainant la formation de syncytium en culture de tissue. Ann. Inst. Pasteur. In press.

PHS - NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID-76-A
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A

A. Project Title: Ecologic studies of fungi pathogenic for man.

Principal Investigator: C. W. Emmons

Other Investigator: Willard Piggott

Cooperating Units: Individual studies within this project have been conducted in cooperation with Dr. Gordon Clark, Patuxent Wildlife Refuge and with Mr. Charles Hunt, U. S. Geologic Survey, Denver, Colorado.

Man Years (calendar year 1960):

Total:	20/12
Professional:	10/12
Other:	9/12

Project Description:

Objectives:

To find new environmental habitats of fungi which cause systemic mycoses; to investigate the factors which limit growth of these fungi to special habitats; to investigate methods of eliminating pathogenic fungi from specific habitats and to apply the information so obtained to studies of the epidemiology of the systemic mycoses.

Methods Employed:

Samples of soil were collected from sites suspected of being sources of infection in individual cases of mycoses, from types of habitats known to harbor fungi in order to extend experience in this study and repeatedly in certain selected sites known to harbor pathogens in intensive studies of their ecology and associated sap-

Part B included

rophytic microflora. The specimens were processed by conventional methods of mouse inoculation with soil suspensions.

A laboratory device for exposure of mice by inhalation of dry spores of fungi was designed in collaboration with Mr. Willard Piggott and has been used to simulate natural conditions of exposure in pursuing the objectives of this project.

Major Findings:

The association between Cryptococcus neoformans and pigeon excreta has been confirmed in many additional collections of specimens. Histoplasma is not found in this association. The actual occurrence of an acute pneumonic form of cryptococcosis has not yet been proved because there has been no opportunity to study such an outbreak. However, circumstantial evidence for the occurrence of this type of cryptococcosis continues to accumulate.

Significance to bio-medical research and the program of the Institute:

Environmental sources of infection unrelated to patients or infected animals are almost invariably associated with systemic human mycoses. Some of the diagnoses in reported epidemics or focal outbreaks of mycoses still remain equivocal until the type of information sought in this project adds to our knowledge about the epidemiology of the mycoses.

Proposed Course of Project:

This project will be continued indefinitely.

Part B. Honors, Awards and Publications.

Publications other than abstracts from this project:

Emmons, Chester W. Prevalence of Cryptococcus neoformans in Pigeon Habitats. Public Health Reports, 75:362-365, Apr., 1960.

Piggott, Willard R. and Emmons, Chester W. Device for Inhalation Exposure of Animals to Spores. Soc. Exp. Biol. & Med., 103:805-806, 1960.

Honors and Awards relating to this project:

Presidential Address before the Mycological Society of America at Stillwater, Oklahoma, August, 1960, entitled: "The Jeykll-Hydes of Mycology".

Presentation of a paper before the III National Congress of Microbiology, Mexico City, Oct. 12, 1960, entitled: "Inhalation Route of Infection in Experimental Cryptococcosis".

Presentation of a paper before the Washington Speleological Society, Dec. 6, 1960, entitled: "The Occurrence of Pathogenic Fungi in Caves".

Participation in "Fireside Conference", the American College of Chest Physicians, Washington, D. C., Nov. 27, 1960. Geographic Environment and the Systemic Mycoses.

President, Mycological Society of America, Aug. 1959-1960.

Elected Councilor-at-Large, Society of American Bacteriologists, July 1, 1960.

Chairman, Second Conference on Medical Mycology, New York Academy of Science, Jan. 11 and 12, 1960.

Appointed Board Member of the American Academy of Microbiology for a three-year term.

Chairman, Standards and Examination Committee, American Academy of Microbiology.

Convenor of Mycological Symposium, III National Congress of Microbiology, Mexico City, 1960.

Honors and Awards relating to this project (continued):

Member of Study Section on Mycology and Bacterial Diseases, NIH.

Invited lecture at Annual Meeting of the State Society of Bacteriologists, University of North Carolina, Chapel Hill, No. Carolina, Apr., 1960.

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Serial No. NIAID-76-B
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A

B. Project Title: In vivo tests of antimycotic drugs and antibiotics.

Principal Investigator: C. W. Emmons

Other Investigators: Prescott, Benjamin, Piggott, Willard, Utz, John P. and Andriole, Vincent - MPB Serial No. 64-A; LCI Serial No. 23-C.

Cooperating Units: Medical Physiological Bacteriology Section and Laboratory of Clinical Investigations.

Man Years (calendar year 1960):

Total:	22/12
Professional:	8/12
Other:	12/12

Project Description:

Objectives:

To test the efficacy and safety of new drugs and antibiotics in experimental mycoses in mice and cooperate with the Laboratory of Clinical Investigations in clinical trials.

Methods Employed:

Mice are infected intravenously with a dose of fungus cells determined in prior experimental work to be sufficient to kill untreated (control) mice in 14-21 days. Treated mice are given test drugs at doses up to tolerance and effectiveness of drug is measured by its ability to extend survival time of treated mice and to clear the animals of infection as determined by autopsy and culture of the surviving animals when experiment is terminated. Collaborator has provided some of drugs tested and has synthesized drugs on his initiative or of types suggested by principal investigator.

Part B included.

Major Findings:

A new antibiotic first received by the principal investigator four years ago has now been on clinical trials for 1 1/2 years and has been found to be superior to other antimycotic drugs in 4 mycoses.

Significance to bio-medical research and the program of the Institute:

Presently available antimycotic drugs are too toxic and too ineffective for safe and ideal clinical use. Experimental therapy in animals should precede clinical trial.

Proposed Course of Project:

This project will be continued indefinitely.

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Emmons, C. W. Failure of Griseofulvin to Control Experimental Systemic Mycoses in Mice. A.M.A. Arch. Derm. 81:700-702, May 1960.

Honors and Awards relating to this project:

Presentation of a paper entitled: "A New Antimycotic Antibiotic" at the Fifth Annual Meeting of VA-Armed Forces Coccidioidomycosis Cooperative Study", Los Angeles, Calif., Dec. 8 - 9, 1960.

Honorary Mention for an Exhibit on Chemotherapy of Systemic Mycoses at the American Medical Association Meeting in Miami Beach, Florida, held in June 1960.

Appointed to WHO Expert Advisory Panel on Parasitic Diseases for a term of 5 years.

Presided at a session on Antifungal Agents at the 1960 Conference on Antimicrobial Agents, sponsored by the Society for Industrial Microbiology, Oct. 26, 1960.

Appointed on Scientific Advisory Committee, Institute of Microbiology, Rutgers - The State University, New Brunswick, N. J. for a term of three years.

Member of Committee on National Index of Fungus Cultures, Quartermaster Research and Development, National Academy - Research Council, Natick, Massachusetts, for a term expiring June 1963.

Special Lecturer on Medical Mycology, George Washington University School of Medicine, for Fiscal Year beginning Aug. 1960.

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Serial No. NIAID-76-C
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A

C. Project Title: Identification and study of new and unusual fungi from mycoses.

Principal Investigator: C. W. Emmons

Other Investigators: William L. Jellison, RML, Montana, Lie-Kian-Joe, Univ. of Indonesia, Djakarta, and Charles Bridges, Agricultural and Mechanical College, Texas.

Cooperating Units: Rocky Mountain Laboratory and many hospitals, diagnostic laboratories and individual physicians.

Man Years (calendar year 1960):

Total:	14/12
Professional:	6/12
Other:	7/12

Project Description:

Objectives:

Studies of new mycoses and their etiologic agents, of unusual strains of pathogenic fungi, and of the geographic distribution of mycoses. Support and encouragement of medical mycology in laboratories which lack trained mycologists.

Methods Employed:

Routine methods of fungus identification, adaptation of such methods when necessary, tests of pathogenicity and virulence in experimentally infected animals.

Part B included.

Major Findings:

Additional studies of phycomycosis have been carried on. A new phycomycosis in horses has been studied and a paper submitted for publication.

Significance to bio-medical research and the program of the Institute:

This combines a useful diagnostic service with an important research function because it brings under scrutiny unusual, interesting and important pathogens.

Proposed Course of Project:

This project will be continued indefinitely.

Serial No. NIAID-76-C

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Joe, Lie-Kian, Eng, Njo-Injo Tjoei, Tjokronegoro, Sutomo, and Emmons, Chester W. Phycomycosis (Mucormycosis) in Indonesia -- Description of a Case Affecting the Subcutaneous Tissue. A. J. Trop. Med. & Hyg. 9:143-148, Mar., 1960.

Emmons, C. W. and Jellison, W. L. Emmonsia Crescens Sp. N. and Adiaspiromycosis (Haplomycosis) in Mammals. N. Y. Acad. Sci. 89:91-101, Aug. 1960.

Honors and Awards relating to this project:

Invited lecture at the Agricultural and Mechanical College, College Station, Texas, July, 1960.

Presentation of a paper entitled: "Emmonsia Crescens Sp. N. and Adiaspiromycosis (Haplomycosis) in Mammals" at the Second Conference on Medical Mycology, New York Academy of Science, Jan., 1960.

PHS - NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID-78-A
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A

A. Project Title: Biochemistry and Physiology of Pathogenic Fungi. (In vitro studies of the action of Antifungal agents on pathogenic fungi).

Principal Investigator: George W. Lones

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1 2/12
Professional: 9/12
Other: 5/12

Project Description:

Objectives:

To investigate the quantitative relationships of antifungal drugs with respect to fungicidal and fungistatic action; to study certain physical and chemical factors influencing this action; to study the effects of these drugs on certain metabolic activities of fungi; to study the emergence of strains resistant to these drugs; and ultimately to understand the mechanism of action of certain of the antifungal antibiotics.

Methods Employed:

Interest during the past year has centered on a new antibiotic, X-5079C. Conventional methods are used for determining the quantitative relationships of growth of Histoplasma capsulatum in the presence of varying concentrations of the antibiotic in suitable growth media under a variety of conditions. HeLa cell cultures are infected with H. capsulatum in the yeast phase and the effect of the antibiotic on the development and persistance of the fungus determined by

Part B not included.

microscopic examination of stained preparations and by subculture following trypsinization.

Major Findings:

Antibiotic X-5079C is fungistatic rather than fungicidal. Contrary to early impressions the agent is active against H. capsulatum in vitro as well as in animals. The sensitivity of the yeast phase of H. capsulatum to antibiotic X-5079C is substantially greater than that of the mycelial phase. An assay method for antibiotic X-5079C sensitive to 1 ug/ml has been developed with a strain of H. capsulatum as the test organism. Antibiotic X-5079C is active against H. capsulatum in HeLa cell culture, and factors influencing activity in this system have been investigated. It is of low toxicity for HeLa cells.

Significance to bio-medical research and the program of the Institute:

The need for new and more effective therapeutic agents for treatment of the systemic mycoses continues.

Antibiotic X-5079C has demonstrated impressive therapeutic effect in experimental mycoses in animals and encouraging results have been obtained in humans. The continued study of this and other antifungal agents is essential.

Proposed Course of Project:

It is planned to continue these studies. The investigation may well be extended to pathogenic fungi other than H. capsulatum. The effect, if any, of the antibiotic on respiration and assimilation will be determined. Attempts will be made to improve the assay procedure, since a sensitive assay method is essential for the determination of serum levels and excretion rates in man and animals.

PHS - NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID-78-B
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A

B. Project Title: Studies on the physiology of Coccidioides immitis.

Principal Investigator: George W. Lones

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1 2/12
Professional: 3/12
Other: 11/12

Project Description:

Objectives:

The biochemistry and metabolism of this dimorphic pathogen have been little studied. It is the purpose of this project to obtain information on the metabolic characteristics of this microorganism, and in particular to discover metabolic differences in the two forms.

Methods Employed:

Shake culture techniques are employed to produce the parasitic and saprophytic morphologic modifications of C. immitis in sizeable quantities for examination by standard chemical and biochemical techniques.

Major Findings:

A second strain of C. immitis has been successfully converted and maintained in the spherule form. Quantitative measurements have been obtained of the ability of a variety of potential carbon

Part B included

and nitrogen sources to support the growth of the spherule phase and the mycelial phase of strain M-11. No substrate has been found in this study which preferentially supports the growth of the spherule form. Only mannose is utilized as readily as glucose by the spherules. Mannose and fructose support growth of mycelium as well as glucose.

Significance to bio-medical research and the program of the Institute:

Progressive coccidioidomycosis is a disease with a high mortality. A better knowledge of the causative agent may favorably influence our diagnosis, prevention and treatment of the infection.

Proposed Course of Project:

Examination of the metabolism of the two morphological forms of the fungus will be extended. The current nutritional study will be completed.

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Lones, G. W. and Peacock, Carl L. Role of Carbon Dioxide in the Dimorphism of Coccidioides immitis. J. Bact. 79:308-309, 1960.

Lones, G. W. and Peacock, Carl L. Studies of the Growth and Metabolism of Coccidioides immitis. Annals N. Y. Acad. Sci. 89:102-108, 1960.

Honors and Awards relating to this Project:

Presentation of a paper entitled: "Studies of the Growth and Metabolism of Coccidioides immitis", Second Conference on Medical Mycology, New York Academy of Science, January, 1960.

PHS - NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID-79-A
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A

A. Project Title: Immunity Studies with Pathogenic Fungi.

Principal Investigator: H. F. Hasenclever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 5/12
Professional: 2/12
Other: 3/12

Project Description:

Objectives:

To obtain fundamental knowledge pertaining to the development of acquired resistance in laboratory animals to coccidioidomycosis.

Methods Employed:

Immunization procedures utilizing spherule cultures of Coccidioides immitis as vaccines are being studied. The vaccines are treated with formaldehyde to destroy viability. Mice immunized with this preparation are challenged with viable C. immitis arthrospores or spherules.

Major Findings:

Immunized mice show an increased survivor rate when compared to normal mice challenged with similar infecting doses of C. immitis. In experiments where sublethal infecting doses are utilized, the immunized mice are culturally negative sooner than normal control mice.

Part B not included.

Significance to bio-medical research and the program of the Institute:

We are utilizing for this investigation cultures of spherules grown *in vitro*. While mycelial preparations have been investigated, the "parasitic form" of *C. immitis*, as an immunizing agent, has been studied very little. This project has been under investigation for only a short time, but the results thus far are encouraging. Coccidioidomycosis is a disease for which, if a satisfactory vaccine were available, immunization would be of value and practical.

Proposed Course of Project:

This project is to be continued.

PHS - NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID-79-B
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A

B. Project Title: Antigenic Studies on Pathogenic Yeasts.

Principal Investigator: H. F. Hasenclever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)

Total:	9/12
Professional:	4/12
Other:	5/12

Project Description:

Objectives:

To learn more about the antigenic interrelationships of some of the provisional pathogens with particular emphasis upon the genus Candida.

Methods Employed:

Antisera to the desired species and strains of Candida are prepared in rabbits. Homologously and heterologously adsorbed antisera are utilized to show antigenic similarities or dissimilarities that exist within species or between species. Tube agglutination reactions have been used predominately and the applicability of complement fixation and agar gel diffusion has been tested.

Major Findings:

These studies have shown that two antigenic groups are present in the species Candida albicans. One of these groups is antigenically similar to C. tropicalis, while the other group is identical to C. stellatoidea.

Part B included.

Significance to bio-medical research and the program of the Institute:

These studies have contributed basic and fundamental information about the antigenic structure of these yeast-like fungi.

Proposed Course of Project:

To be continued with an effort to demonstrate qualitative antigenic differences between these two groups.

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Hasenclever, H. F. and Mitchell, William O. The observation of Two Antigenic Groups in Candida albicans. Bact. Proc. 1960.

Honors and Awards relating to this Project:

Convenor of Session on Medical Mycology at the 1960 Annual Meeting of the Society of American Bacteriologists.

Invitational lecture entitled: "Clinical and Mycological Aspects of Tinea pedis" before the Annual Scientific Convention of the American Podiatry Association, Nov., 1960.

PHS - NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID-79-C
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A

C. Project Title: Virulence and pathogenic studies with yeasts.

Principal Investigator: H. F. Hasenclever

Other Investigators: Vincent T. Andriole - LCI Serial #23-C.

Cooperating Units: Laboratory of Clinical Investigations.

Man Years (calendar year 1960):

Total:	18/12
Professional:	10/12
Other:	8/12

Project Description:

Objectives:

To learn more about the host-parasite relationship, virulence and pathogenicity, and the in vitro multiplication rates of several yeasts in normal and physiologically altered mice.

Methods Employed:

Virulence determinations are calculated by injecting graded doses of yeast cells into different groups of mice. The fifty per cent endpoint is utilized and death or survival of the test animals is the criterion employed. In vivo growth rates are obtained by determining the viable yeast cells in the various organs, at different stages of disease, in animals infected with a standard number of yeasts. Similar studies have been done using mice with alloxan induced diabetus mellitus.

Part B included.

Major Findings:

Studies with several strains of Cryptococcus neoformans have shown that several minutes after injection the largest number of yeast cells are found in lung tissue, regardless of whether the mice were injected intravenously or intracerebrally. Soon the population in the lungs decreases, and by 2-3 days after injection, multiplication in the brain is apparent. The time required to produce terminal cryptococcosis after injection in mice varies with the strain. In moribund mice, however, the number of viable yeast cells per 0.1 gm of brain tissue is about the same regardless of strain.

Studies with Candida tropicalis have shown that most strains of this yeast are quite pathogenic for mice but not for rabbits. Multiplication in vivo is quite similar to that of Candida albicans, i.e., the kidneys are the organs showing the greatest amount of tissue destruction, and the highest yeast cell population.

An effective dose of alloxan monohydrate which would produce constant glycosuria without a great number of fatalities was determined.

Lethality studies revealed that certain strains of Candida albicans and C. tropicalis caused death in alloxanized mice at a faster rate than in normal control animals. This was not true for certain strains of C. parapsilosis and C. guilliermondii.

Plating experiments revealed that the course of C. albicans infection in the mouse was more severe when the animal had been physiologically altered with alloxan monohydrate.

Significance to bio-medical research and the program of the Institute:

These investigations have contributed to knowledge about the parasite multiplication within host tissues. We have shown that mice are quite susceptible to infections due to C. tropicalis.

Proposed Course of Project:

To be continued with further study to be given to the effects of physiological alteration of the host in relation to increased or decreased susceptibility to fungus infections.

Serial No. NIAID-79-C
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part B. Honors, Awards and Publications.

Publications other than abstracts from this project:

Hasenclever, H. F. and Mitchell, William O. Virulence and Growth Rates of Cryptococcus neoformans in Mice. Annals N. Y. Acad. Sci. 89:156-162, 1960.

Hasenclever, H. F. and Mitchell, William O. Pathogenicity of Candida albicans and Candida tropicalis. Sabouraudia (In press).

Honors and Awards relating to this project:

Presentation of a paper entitled: "Comparative Pathogenicity of Candida albicans and Candida tropicalis," at the Annual Meeting of the Mycological Society of America, American Institute of Biological Sciences, 1960, Stillwater, Oklahoma.

Presentation of a paper entitled: "Virulence and Growth Rates of Cryptococcus neoformans in Mice", at the Second Conference on Medical Mycology, New York Academy of Science, Jan., 1960.

LABORATORY OF [illegible]

LABORATORY OF BIOLOGY OF VIRUSES

PHS-NIH
Summary Statement
Office of Chief
Laboratory of Biology of Viruses
Calendar Year 1960

This laboratory has been anticipating a physical shift in most of its activities to space in Building 5. Although scheduled to take place during the past calendar year, it is now obvious that it will not take place until well into 1961. Because of this delay, space problems have been severe. For instance, it has been impossible to establish our planned central tissue culture production unit although the subprofessional responsible for this program has been on duty since March 1960. She has become well acquainted with our technics, and equipment necessary for the operation has been collected. A second severe limitation of space involves the newly established Physical Biology Unit. Dr. Mattern has had to install his electron microscope in one-half of a basement room measuring 8 x 14 and this has been the total working space for a whole year for him and one professional assistant.

During this year one whole unit, The Rickettsial Biology and Metabolism Unit involving 3 professional and 2 subprofessional staff members, was transferred from this laboratory to the Division of Biologics Standards. No replacement personnel, budget or space was made available for this loss.

One new research associate joined the staff this year, making two such now a part of this laboratory. One of these associates has been recruited to become a part of the permanent staff at the end of this fiscal year.

The basic objectives of this laboratory continue to be the same as last year. It is obvious from this annual report that four out of our five units have projects with the same general objective--investigation of mechanisms and localization of animal virus synthesis within the infected cell. Each of these units is also interested in the infectious nucleic acid of viruses. In view of the complexity of this problem and the important implications of any information that is obtained, we feel that this "duplication" is quite justified. Actually, it is not duplication in so far as different approaches are being used and different virus-cell systems studied.

The electron microscope has been installed and is now being used not only by the Biophysical Unit but also in collaboration with other units of our laboratory and units in the Laboratory of Infectious Diseases. With studies on the structure of viruses and a project

concerned with the genetics of animal viruses added to our biochemical and biological studies, we feel that we now have fairly complete coverage of the important facets of basic virus biology.

By the use of radioautographs and staining with fluorescein tagged antiviral antibody, the intracellular location of poliovirus antigen--presumably viral protein--during the cycle of virus multiplication has been determined. Demonstrable antigen first appeared one hour after infection and was diffusely distributed through the cytoplasm. At 3 hours, just before the appearance of new virus, it was present throughout the nucleus with a tendency to be concentrated around the periphery of the nucleolus. At 5 to 7 hours, particulate accumulation of antigen in the cytoplasm was noted. Incorporation of radioactive-tagged amino acid into cell protein ceased shortly after the start of infection, whereas incorporation of cytidine into RNA continued until after 3 hours and tended to localize in the nucleoli.

Plaque type mutants of EMC virus have been found, segregated and characterized. The stability of the mutants has been determined and the plaque type has been shown to be a function of the viral RNA. It has been shown that the difference in the size of the plaques formed by these mutants is brought out by an inhibitor present in the agar overlay used on the plaque plates. This inhibitor resides in the agaropectin fraction of the agar and can be separated from the agarose fraction which then permits both plaque type mutants to form similar sized plaques.

By the use of a serum protection test in newborn hamsters, evidence was found that polyoma virus transforms normal cells to tumor cells quickly and directly without extensive virus multiplication being necessary. Furthermore, no evidence could be found to suggest a lysogenic relationship of virus to tumor cell. All attempts to show the presence of infectious or masked virus or of virus antigen in transplantable polyoma-induced tumors have been negative. It appears that once the virus initiates the tumor it is no longer required for tumor growth and maintenance.

The discovery has been made that when the antibiotic tetracycline stains tissues in such a way that they fluoresce under UV light, this fluorescence is localized in the mitochondria of the cells. This makes a convenient vital stain of these subcellular elements for further studies. There appears to be some similar localization of the antibiotic fluorescence in certain bacteria.

A complex model of tobacco mosaic virus has been constructed on theoretical grounds, and on checking this model against known biochemical and biophysical properties of the virus a remarkable consistency is found. Certain refinements of electron microscopic technics have produced photographs of this virus which reveal previously not seen fine structure also consistent with the theoretical model.

Serial No. NIAID-80
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Basic Studies of Virus-Host Cell Relationships

Principal Investigator: Dr. Karl Habel

Other Investigators: Rosalie Silverberg

Cooperating Units: None

Man Years:

Total:	1
Professional:	5/12
Other:	7/12

Project Description:

Objectives:

To determine mechanism of and factors influencing attachment, invasion, multiplication and release of viruses from susceptible and resistant cells.

Methods Employed:

Various strains of tissue culture cells either suspended or on glass are exposed to viruses and at different stages of infection the cells are studied for viability and virus content. All quantitation is by plaque methods.

Major Findings:

After rapid adsorption of poliovirus to HeLa cells the virus exists in 4 different relationships with the cell:

(1) Virus loosely bound that will wash off,
(2) Virus firmly bound but still available to extracellular antibody,
(3) Virus firmly bound but not available to antibody,
(4) Virus already eclipsed.

Part B included: Yes

3

Although all four states may exist at one time, the normal sequence of events seems to be (1), (2) and (4). The virus in (3) may go to (4) and thus initiate infection but this may be a relatively inefficient process compared to the other sequence.

Significance to Bio-medical Research and the Program of the Institute:

These findings suggest that effective initiation of the infectious cycle can occur rapidly but that under certain circumstances virus is firmly attached to the cell before being eclipsed. Understanding of these events occurring at the start of infections at the cell surface may provide a logical target for attempts at inhibition of infection without serious deleterious effects upon the cell.

Proposed Course of the Project:

These studies will be continued with attempts to determine morphological and biochemical cellular components responsible for the phenomena already demonstrated.

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Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Habel, Karl and Utz, John P. Mumps. Pediatric Clinics of North America, Vol. 7, No. 4, November, 1960.

Honors and Awards relating to this project:

None

Serial No. NIAID-80A
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Rabies Prophylaxis

Principal Investigator: Dr. Karl Habel

Other Investigators: Rosalie Silverberg

Cooperating Units: Expert Committee on Rabies, World Health Organization

Man Years:

Total:	1 6/12
Professional:	6/12
Other:	1

Project Description:

Objectives:

To improve methods of rabies prophylaxis in man and to reduce non-specific reactions caused by vaccines.

Methods Employed:

This year's activity has been chiefly aimed at developing a tissue culture source of virus for vaccine production.

Major Findings:

A practical tissue culture system consisting of normal chicken embryo cells in medium 199 has been infected with a fixed strain of rabies virus and propagated through 10 serial passages. Some passage fluids have had an infectious titer as high as 10^5 and with more concentrated inocula produced a cytopathic effect which was neutralizable with antirabies serum.

Part B included: No

Significance to Bio-medical Research and the Program of the Institute:

A practical tissue culture source of inactivated rabies vaccine would represent a tremendous improvement in the type of biological product used for rabies prophylaxis in man.

Proposed Course of the Project:

Tissue culture as a possible source of vaccine will be further explored.

Serial No. NIAID-81
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Mechanism of Oncogenic Activity of Polyoma Virus

Principal Investigator: Dr. Karl Habel

Other Investigators: Rosalie Silverberg and Dr. Lowell Glasgow

Cooperating Units: None

Man Years:

Total:	3 5/12
Professional:	2
Other:	1 5/12

Project Description:

Objectives:

To determine biological factors involved in the induction of tumors by oncogenic viruses.

Methods Employed:

By studying the virus-tumor cell relationships of polyoma virus in animal and tissue culture systems, the events occurring in newborn versus adult mice and hamsters on injection of virus are being compared. Attempts are being made to induce malignant characteristics in cells in tissue culture and in adult animals by varying the physiological state during infection with polyoma virus.

Major Findings:

Tumors produced in newborn hamsters by polyoma virus when passive antibody is present, occur only at the site of virus inoculation, contain no demonstrable virus and frequently do not result in an active anti-viral antibody response.

Part B included: Yes

Tumors can be produced in adult hamsters by applying virus to the granulation tissue resulting from scarification but not by simple intradermal inoculation of virus.

Attempts to "transform" normal mouse and hamster embryo tissue culture cells to tumor by establishing in them a continuing infection with polyoma virus have been negative.

Significance to Bio-medical Research and the Program of the Institute:

Any information concerning factors responsible for oncogenic properties of tumor viruses may well apply to the oncogenic effects of other agents and provide leads for understanding tumor development under natural conditions in man.

Proposed Course of the Project:

Attempts will be made to find factors that will enhance the ability of polyoma virus to induce tumors in adult animals and to inhibit this process in suckling animals.

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Habel, Karl and Silverberg, Rosalie J. Relationship of Polyoma Virus and Tumor in Vivo. Virology 12: 463-476, November 1960.

Honors and Awards relating to this project:

None

Serial No. NIAID-82
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Cell growth inhibiting substances

Principal Investigator: Dr. John W. Hornibrook

Other Investigators: None

Cooperating Units: Viral Biochemistry Unit, LBV.

Man Years:

Total:	2 7/12
Professional:	1
Other:	1 7/12

Project Description:

Objectives:

To find and investigate substances and mechanisms which prevent the growth of cells in the adult animal.

Methods Employed:

Isolation from serum and tissues of substances which will inhibit the growth of mammalian cells in tissue culture.

Major Findings:

An inhibitor from serum has been partially purified. It is apparently not a carbohydrate and does not adsorb U.V. radiation at 280 or 260 mμ. It is active at approximately 0.3 mg/ml.

Methods of isolating and purifying this material are being improved and this work will continue.

Part B included: No

Significance to Bio-medical Research and the Program of the Institute:

Information concerning the existence and mode of action of mitotic inhibitors in tissues or body fluids is of fundamental biological importance and of practical significance to those engaged in tissue culture and cancer research.

Proposed Course of the Project:

Attempts will continue to purify and identify these substances and study their mode of action.

Serial No. NIAID-83
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Mutation in Animal Viruses

Principal Investigator: Dr. K. K. Takemoto

Other Investigators: Dr. Harvey Liebhaber

Cooperating Units: None

Man Years:

Total:	3 6/12
Professional:	2 1/12
Other:	1 5/12

Project Description:

Objectives:

1. To isolate and characterize virus mutants and to investigate the chemistry and biology of these mutants.

2. To investigate various methods for concentration and purification of viral mutants in order to obtain sufficient quantities of virus for chemical as well as biological investigations.

Methods Employed:

Genetically pure lines of virus are isolated by plaque techniques. Mutants forming plaques which differ from the parental type are isolated and studied further.

Part B included: No

Major Findings:

1. Encephalomyocarditis (EMC) virus produces plaques which are small and ragged with diffuse boundaries. A mutant of this virus has been isolated which forms large plaques with sharply defined boundaries. Both virus types are immunologically identical and do not differ in their growth rates, thermal stability or mouse virulence. However, the large plaque mutant differs in its hemagglutinating properties, having a significantly higher HA/pfu ratio.

2. The basis for plaque size differences has been found to be due to an inhibitory factor in the agar used in the overlay medium. The primary effect of the inhibitor appears to be interference with adsorption of virus.

3. Infectious RNA extracted from both types of virus have yielded progeny which produce plaques identical to those from which the RNA was obtained, proving that genetic information determining plaque type is carried solely by the viral nucleic acid.

4. Preliminary experiments have indicated that large volumes of EMC virus can be concentrated and purified by a simple procedure which utilizes the partition coefficient of the virus between two phases of aqueous solutions of high molecular weight polymers.

Significance to Bio-medical Research and the Program of the Institute:

Studies on variations and mutations of animal viruses not only lead to further knowledge and understanding of the nature of viruses but have practical implications in the development of vaccines in virus diseases.

Proposed Course of the Project:

1. Utilizing the genetic markers of plaque type and hemagglutination, experiments are planned to demonstrate various types of genetic interaction such as recombination and reactivation.

2. Procedures are being developed for large scale purification of virus so that sufficient quantities of relatively pure virus will be available for studies on viral nucleic acid and protein.

Serial No. NIAID-84
1. Biology of Viruses
2. Virus Host Relationship
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Host-Parasite Relations

Principal Investigator: Victor H. Haas

Other Investigators: None

Cooperating Units: Miss Delta Uphoff (NCI)

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

(a) To continue studies on relationships between the virus of lymphocytic choriomeningitis (LCM) and the transmissible ascitic tumor P288 in mice, particularly in mice immune to the virus.

(b) To study the effect of various antigens, including tumor material and a virus strain derived from LCM-tumor passage, on immune response to the P288 tumor.

(c) To conclude studies on LCM immunity in X-rayed mice kept alive by marrow and spleen transplants.

Methods Employed:

A line of P288 tumor carrying LCM with it during passage was maintained in CDBA mice, some of them previously immunized against LCM. A strain of LCM virus derived from the tumor passage line was separated from the tumor and maintained by serial passage in non-immune mice.

Part B included: Yes

The P288 tumor was passaged in CDBA mice and from time to time in general purpose mice, the latter being treated with amethopterin. Detection of LCM in various passage mice was by demonstration of viremia or by immunity tests. Bone marrow and spleen transplants were done by Miss Uphoff in mice previously prepared by me in respect to immune status against LCM, and irradiation was obtained from Mr. Meyer of NCI.

Major Findings:

Two years ago, I established a passage strain of LCM virus that was carried with the ascites tumor P288 through mice immune to LCM. For the virus to survive, it was necessary to treat the mice with amethopterin. After nearly a year of such passage, I derived from it a strain of LCM which survived in immune mice, when passed with the tumor P288, even though no amethopterin was given. During the current year I have passed this latter strain of LCM through non-immune, non-tumor bearing mice and found that it produced a benign, transmissible ascites of a type which I have not encountered in my ordinary passage strains of LCM. White (general purpose) mice recovering from this ascitic infection frequently have been resistant to P288 tumor, when the tumor was given during amethopterin treatment, a combination generally fatal to these mice. No other instances of tumor-immunizing capability, and none of tumor-producing capability, of this virus strain have been detected, despite repeated efforts.

Earlier work in this laboratory demonstrated that general purpose mice are normally refractory to the P288 tumor, but that fatal ascitic tumors developed if they were treated with amethopterin after tumor injection. During this year, I have found that injections of tumor given without amethopterin treatment made the mice immune to later challenge with the same tumor plus amethopterin. Attempts to immunize with disrupted tumor cells (freezing-thawing) have indicated that such preparations may have an immunizing effect but these experiments are not as yet complete. A similar effect seemed to occur when normal tissues of the CDBA strain of mice (the strain in which tumor P288 originated and is maintained) were used in lieu of tumor preparations for immunizing the white mice.

Experiments on immunity to LCM in X-rayed mice kept alive by marrow and spleen transplants have been completed. The immune status of the X-rayed mouse before radiation and tissue transplant determined its response to later LCM challenge. Marrow and spleen from immune donors did not confer immunity on the recipients which had not been previously immunized.

Significance of the Program to the Institute:

A significant part of the Institute's program on basic research in virology concerns the alterations produced in the virus-infected animal, as an entity and on a cellular basis. One such alteration could be the induction of tumor growth, another could be the induction of resistance

to tumors. These experiments have yielded information on how a particular virus--LCM--and a transplantable tumor — P288--become inter-related during passage together. The alteration of the general purpose mouse's response to the P288 tumor by amethopterin provides an opportunity for studying the immune reaction to various antigens as measured by resistance to challenge with the tumor.

Proposed Course of the Project:

This project comes to an end this year because of my retirement.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Haas, Victor H. Serial passage of a lymphocytic tumor and choriomeningitis virus in immune mice. Jour. Natl. Cancer Inst. 25: 75-83 (1960).

Uphoff, Delta E. and Haas, Victor H. Immunologic response to lymphocytic choriomeningitis virus in lethally irradiated mice treated with bone marrow. Jour. Natl. Cancer Inst. 25: 779-786 (1960).

Honors and Awards relating to this project:

None

Serial No. NIAID-85
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Investigations of Animal Virus Reproduction

Principal Investigator: Dr. Hilton B. Levy

Other Investigators: Dr. Frank De Filippes

Cooperating Units: None

Man Years:

Total:	2 11/12
Professional:	11/12
Other:	2

Project Description:

Objectives:

To gain information relative to the relationship that exists between the infected cell and the virus reproducing therein. More particularly, to determine at a molecular level the mechanisms by which a virus is reproduced by the infected cell. Knowledge of either the details of altered cell metabolism or mechanisms of virus reproduction might be useful in the development of chemotherepeutic agents to inhibit virus growth and to aid the host. Comparison of oncogenic viruses such as Rous Sarcoma with cytocidal viruses such as poliovirus should give insight into growth controlling mechanisms.

Methods Employed:

The program for this year sought the intracellular localization of the infecting virus particle during the course of the infection, and also where the components of the new virus were made. High resolution autoradiography and fluorescent antibody techniques were used. Nucleic acid bases containing the weak beta particle emitter tritium were used to study nucleic acid metabolism and to prepare labelled poliovirus. Tritium labelled histidine was used for protein studies. Rabbit antiserum to highly purified poliovirus was prepared for the fluorescent antibody work.

Part B included: Yes

Major Findings:

The first detected change in poliovirus infected HeLa cells occurs about an hour after infection, when there is seen increased turnover of RNA, particularly and almost exclusively in the nucleoli. (It is about this time that our earlier work detected increased glycolytic energy production.) This increased RNA turnover continues for 3 1/2 to 4 hours after which it greatly decreases. At about 1 1/2 to 2 hours after infection, there appears a virus specific antigen in the cytoplasm, even though total cell protein metabolism, as measured by tritiated histidine, has declined markedly. By 3 to 3 1/2 hours after infection, viral antigen appears in the nucleus. This nuclear antigen does not occur in the nucleolus, which latter structure has a bright thin ring of stained antigen around it. It might be that this nuclear antigen is made at the periphery of the nucleolus, or is made in the nucleolus but is not susceptible to reaction with fluorescent antibody until released at its surface. By about 4 to 5 hours after infection there is a decline in the number of cells showing nuclear antigen and the appearance of brightly staining antigen in the cytoplasm, suggesting that the nuclear material has migrated there. Since parallel viral growth studies show the first appearance of new virus at this time, it would suggest that this nuclear to cytoplasmic migration is the step that forms new virus. Whatever nucleic acid or specific protein synthesis was implicated in the increased nucleolar RNA turnover decreases at this time. Shortly thereafter, the increased energy production stops.

Significance to Bio-medical Research and the Program of the Institute:

Increased research in biochemistry along the lines of protein and nucleic acid biosynthesis has resulted in the synthesis of at least two specific proteins in cell free systems. The way seems clear to do this with virus protein. The intracellular localization of where specific virus protein is made indicates which subcellular components to use in such an attempt.

Comparable studies with other cytocidal as well as with oncogenic viruses will add to the Institute's program directed to understanding mechanisms by which host cells are diverted from normal cell behavior to the production of new virus or to tumor characteristics.

Proposed Course of the Project:

Attempts will be made to utilize the information obtained this past year to synthesize some viral components in cell free systems. Comparison with other viruses will be made. Further studies on the nature of the increased nucleolar activity will be made to see if viral nucleic acid is being made.

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Levy, Hilton B. and Sober, Herbert. A Simple Chromatographic Method for Preparation of Gamma Globulin. Proc. Soc. Exptl. Biol.& Med. 103: 250-252, 1960.

Levy, Hilton B. and Snellbaker, LeRoy. Phosphorus Metabolism in Infection with Murine Leukemia Virus. Proc. Soc. Exptl. Biol. & Med. 103: 503-506, 1960.

Levy, Hilton B. and Lynt, R. K. Heterogeneity in Cytoplasmic RNAs of Mouse Spleen and Effect Thereon of a Leukemia Virus. Submitted for publication.

Honors and Awards relating to this project:

The work on the Friend leukemia virus was chosen to be published in the M. D. Anderson Hospital annual publication of significant reports in cancer research.

Serial No. NIAID-85A
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Animal Virus Synthesis

Principal Investigator: Dr. Frank M. DeFilippes

Other Investigators: Dr. Hilton B. Levy

Cooperating Units: None

Man Years:
Total: 1 1/12
Professional: 1 1/12
Other: 0

Project Description:

Objectives:

1. To investigate the synthesis of an animal virus in a tissue culture system by studying subcellular particles which are involved in protein synthesis in many animals.

2. To increase the efficiency of infection of monkey kidney cells with RNA extracted from purified poliovirus to a level similar to that obtainable with whole virus.

Methods Employed:

1. HeLa cells infected with poliovirus are grown in a radioactive medium. The cells are collected at different times and cell fractions are isolated with particular attention being given to the ribosomal material. The specific radioactivity of the ribosomes is followed during the increase of intracellular virus and compared to ribosomes isolated from uninfected cells. The ribosomal material is identified by its spectrum and also by electron microscopy. The variation of radioactivity of other cell fractions isolated during the purification of the ribosomes is also under investigation.

Part B included: No

2. Monkey kidney cell monolayers are infected with RNA extracted from purified poliovirus under a variety of ionic and pH conditions.

Major Findings:

1. A procedure has been worked out which consistently allows the isolation of at least 10% of the ribosomal material from HeLa cells in a relatively pure state. The ribosomal ribonucleoprotein particles may be separated from the viral ribonucleoprotein particles by passage through an ECTEOLA-cellulose column.

The specific activity of ribonucleoprotein particles from infected cells is less than that of the ribosomal material of uninfected cells 7 hours after the addition of virus under conditions of high and low multiplicity of infection. With a high multiplicity, the cellular protein sedimented at 15,000g for 10 minutes shows a dramatic and continuous decline in specific activity.

2. The efficiency of infection with extracted RNA has been brought to a level of about 0.5% that obtainable with whole virus.

Significance to Bio-medical Research and the Program of the Institute:

The program is designed to lead to a general picture of the synthesis of an animal virus by following events at a molecular level. It is hoped that the key steps involved in the conversion of the cellular machinery from normal metabolic activity to a virus producing system will be elucidated. Interference with these key steps may lead to new and general methods of arresting viral disease.

Proposed Course of the Project:

Investigation of cellular fractions and especially ribosomal material will be continued under conditions where the cells are infected with different virus multiplicities. It is hoped that infection with very high multiplicities may remove all remnants of normal activity and clarify the situation with respect to virus growth. Also ribosomes from cells actively synthesizing new protein during infection will be compared to cells which are infected while they are in a resting state.

Infection of monkey kidney cells with RNA extracted from poliovirus and the subsequent isolation and purification of ribosomal material will also be attempted to study the role of the viral genetic material.

Serial No. NIAID-86
1. Biology of Viruses
2. Virus-Host Relationship
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Investigations of interference with enzymatic functioning of mitochondria as a mechanism for carcinogenesis

Principal Investigator: Dr. Marie L. Hesselbach

Other Investigators: None

Cooperating Units: Laboratory of Pathology & Histochemistry, NIAMD
Laboratory of Pathology, NCI

Man Years:

Total:	10/12
Professional:	9/12
Other:	1/12

Project Description:

Objectives:

To determine whether interference with the functioning of mitochondrial enzymes leads to neoplasia. Specifically, it is desired to find a single dye which: 1) is adsorbed by mitochondria; 2) effects the functioning of mitochondrial enzymes, and 3) induces neoplasia. Then, perhaps, a connection between these two functions of a single agent can be demonstrated.

Methods Employed:

Conventional Warburg manometry for metabolic study of induced and transplanted tumors, also for analysis of interaction of the dyes under study, with mitochondrial enzymes.

Different methods of preparation of tissue for metabolic studies: slicing, homogenization, and differential centrifugation.

Part B included: No

Chemical analyses for total nitrogen, lactic acid and inorganic phosphorus.

Histological preparation and examination of treated areas, tumors, and organs.

A study of vehicles which would allow repeated injections of Janus green B over a long period has been made. Non-aqueous media were found to be necessary. Prolonged testing has made it possible to choose the best of these.

Major Findings:

Absence of glucolysis in most of the Fast Green- and Light Green-induced tumors and their early transplant generations was found to correlate with the large amounts of enzymatically inert collagen present in them. Age of tumor was found not to play a role in absence of glucolysis. In later generations glucolysis was more commonly seen and collagen decreased. Mitochondrial preparations which glucolyzed could be prepared from the most metabolically active tumors.

It has been demonstrated that Fast Green and Light Green can be added to total rat brain homogenates at concentrations which inhibit and at other concentrations which stimulate oxygen uptake with added glucose as substrate. The same concentrations which stimulate with glucose, fail to do so with fructose-diphosphate. The dyes do not appear to be uncouplers of oxidative phosphorylation.

Repeated injection of Janus Green B has induced gross changes suggestive of tumor formation, but these have not yet been checked histologically.

Significance to Bio-medical Research and the Program of the Institute:

Both viruses (exogenous) and altered subcellular particles (endogenous) have been implicated as the cause of cancer. This study is an effort to determine the relation of mitochondria to these possible oncogenic agents.

Proposed Course of the Project:

Further studies of the Fast Green-induced tumor transplant line will be made to see whether the homogenates acquire glucolysis "spontaneously," or by changes in preparative procedure, or the cofactors added. It will also be determined which of the 3 enzymes which convert glucose to fructose-diphosphate are destroyed by homogenization.

The study of the interaction of Fast Green and Light Green with mitochondrial enzymes will be extended to include all possible enzyme systems. The question of adsorption and physical interaction of these dyes with the mitochondrial substance will be taken up.

Now that it is possible to give repeated doses of Janus Green B, an experiment will be set up to see whether this dye is cancerogenic. The treated areas, and any tumors formed, will be analyzed histologically. Any induced tumors will be transplanted, and studied metabolically. The interaction of Janus Green B and mitochondrial enzymes will be examined in detail.

Serial No. NIAID-86A
1. Biology of Viruses
2. Virus-Host Relationship
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Demonstration of Glucose Metabolism and Peptide Bond Formation by Isolated Brain and Liver Mitochondria

Principal Investigator: Dr. Marie L. Hesselbach

Other Investigators: Dr. H. G. du Buy

Cooperating Units: Analytical Chemistry Section, NIAMD

Man Years:

Total:	4/12
Professional:	3/12
Other:	1/12

Project Description:

Objectives:

To demonstrate that isolated mitochondria participate in protein metabolism and that not only isolated brain mitochondria but also liver mitochondria contain the complete enzyme systems to metabolize glucose.

Methods Employed:

Conventional Warburg manometry, chemical lactic acid determinations, centrifugal separation of subcellular elements, biochemical and biophysical approach to choice of materials for a suspension medium which will keep mitochondria structurally and functionally intact, biochemical approach to determining materials to be added to isolated mitochondria to restore in vivo enzymatic activity. For demonstration of peptide-bond formation: one-dimensional paper chromatography.

Major Findings:

Work was continued on trying to demonstrate glucose utilization by isolated rat liver mitochondria. Many variations in medium composition

Part B included: No

were used. These involved physico-chemical agents such as methocel, salts, chelating agents, protein derivatives, ribonucleic acid, and phospholipid derivatives. The effects of the hormones epiniphrine and glucagon were studied, as well as the enzymes α- and β-amylase and hexokinase. The reducing agent and cofactor, glutathione, and the diabetogenic agent phlorizin, were also tried. Some of these substances were used in the isolation medium, while others were added to the reaction vessels.

At times hexokinase greatly increased glucose utilization, at others it had little or no effect. Some of the other materials, such as glutathione, seemed to increase glucose utilization very slightly, but not to a significant level.

Addition to liver mitochondria of the natural "fat," collected from the surface of these aqueous preparations, was more successful in increasing glucose utilization than anything except the specific enzyme, hexokinase.

Significance to Bio-medical Research and the Program of the Institute:

The relation of viruses to mitochondria (site of virus reproduction, origin of viruses, site of neoplastic change) can only be fully understood when the physical and chemical characteristics of mitochondria become known. It would be strange if liver tissue could not metabolize glucose, since it stores it (as glycogen), etc. It would also be strange if this essential, energy-producing metabolism were not located on the mitochondria in liver cells as it is in brain cells.

If isolated mitochondria can be shown to synthesize the peptide bond, it will add significantly to our knowledge of the role of mitochondria in the living cell.

Proposed Course of the Project:

It is desired to obtain liver mitochondria which will readily oxidize glucose in significant quantity, and will synthesize the peptide bond. Further studies will be made to obtain better media and to determine what other chemical or physical agents can be added to obtain sustained enzyme activity of a number of complete enzyme sequences on mitochondria in vitro.

Serial No. NIAID-87
1. Biology of Viruses
2. Virus-Host Relationship
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Cytopathogenic effect in single cells in tissue culture.

Principal Investigator: Jane Showacre

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 6/12
Professional: 4/12
Other: 2/12

Project Description:

Objectives:

To study the morphologic and metabolic effects of virus infection on individual virus infected cells in tissue culture.

Methods Employed:

Primary and established cell lines were cultured on coverslips and then mounted in a special tissue culture flow chamber which permits direct, phase and darkfield microscopy under conditions of cell growth. Cells growing in the chamber were then infected with virus and changes in morphology studied. Phase and fluorescent microscope observations were made in rapid succession on identical living cells immediately after mounting on regular slide mounts.

Part B included: No

Major Findings:

Studies with EMC, HA 1 and M 25 infected tissue cultures have continued. Marked changes in cell morphology have not been observed in initial stages of infection. Fluorescent antibodies against HA 1 and M 25 gave readily recognizable peripheral staining of living infected cells in late stages of infection, similar to those reported by O'Dea and Dineen with Herpes simplex. Antibody against EMC is being obtained to determine whether increased titer and purification will improve the efficiency of the technique in early stages of infection. Primary cultures of embryonic and adult mouse brain have been obtained for an in vivo study of the effects of neurotropic viruses on Nissl substance.

Significance to Bio-medical Research and the Program of the Institute:

Morphological evidence of early specific effects of virus invasion of a cell may indicate the intracellular locus of virus activity.

Proposed Course of the Project:

Further study of morphological and physiological changes of subcellular elements following the introduction of viruses and other pathogenic micro-organisms.

Serial No. NIAID-87A
1. Biology of Viruses
2. Virus-Host Relationship
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Characterization of mitochondria

Principal Investigator : Jane Showacre

Other Investigators: Dr. H. G. du Buy

Cooperating Units: None

Man Years:

Total:	8/12
Professional:	7/12
Other:	1/12

Project Description:

Objectives:

To study the localization of non-toxic fluorophors in living cells.

Methods Employed:

Living, unfixed tissues were examined by phase contrast and fluorescence microscopy following exposure to fluorescent compounds. Preparations were made from animal organs, a number of tissue cultures including monkey kidney, HeLa and strain L and from cultures of micro-organisms. The results were recorded photographically.

Major Findings:

Of the fluorescent compounds studied the most promising have been the tetracyclines. These antibiotics, tetracycline, oxytetracycline, and chlortetracycline, were found to specifically combine with mitochondria of living cells in tissue cultures or in fresh preparations from various organs of mice, and in bacteria such as Salmonella typhosa.

Part B included: Yes

Significance to Bio-medical Research and the Program of the Institute:

Tetracyclines can now serve as an additional vital stain for the characterization of mitochondria. As such these compounds may aid in the determination of subcellular changes under different conditions, e.g., viral infection. In this connection the specific localization suggests that mitochondria are implicated in the fatty degeneration occurring in liver following prolonged tetracycline therapy. Further, the fluorescent properties may serve to identify the site of antibiotic action in bacteria and elucidate differences in antibiotic effectiveness under different environmental conditions.

Proposed Course of the Project:

Studies are continuing on factors influencing the retention of tetracyclines by mitochondria as a preliminary to investigations of possible change in the staining properties of mitochondria during infection of cells with viruses, during cell division and following cell fractionation. Bacteria under different environmental conditions are also being examined.

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

duBuy, H. G. and Showacre, J. L. Selective localization of tetracycline in the mitochondria of living cells. Accepted for publication in Science.

Honors and Awards relating to this project:

None

Serial No. NIAID-88
1. Biology of Viruses
2. Virus-Host Relationship
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Biology of mitochondria and its relation to endogenous and viral diseases.

Principal Investigator: Dr. H. G. duBuy

Other Investigators: Dr. M. L. Hesselbach and J. L. Showacre

Cooperating Units: Analytical Services Unit, Mr. H. G. McCann

Man Years:

Total:	1 4/12
Professional:	10/12
Other:	6/12

Project Description:

Objectives:

Biological definition of normal versus pathogenic or virus-altered mitochondria.

Methods Employed:

The methods encompass the applications of the cytochemical findings reported under project No. NIAID-87A. They also include Warburg metabolic techniques and determinations of oxidative phosphorylation in order to define different mitochondria metabolically. The results are applied to in vitro cultivation of isolated mitochondria.

Major Findings:

A manuscript is in preparation on evidence that the enzyme behavior of mitochondria obtained by the sucrose gradient is mainly due to the unavoidable dilution of mitochondria, when this technique of separation is used. The results are applied specifically to the loss of enzymes by melanized mitochondria of the Cloudman S 91 mouse melanoma.

Part B included: No

Significance to Bio-medical Research and the Program of the Institute:

The maintenance or cultivation of mitochondria *in vitro* when accomplished should facilitate the investigation of many metabolic activities of normal cells as compared to tumor cells or those infected with various types of viruses.

Proposed Course of the Project:

Further studies of mitochondria from different sources will be carried out in order to learn more about the characteristics of these elements. Additional experiments will be done to explore further the complete enzymatic complement of mitochondria, especially as this relates to synthetic activities. All information obtained will be applied to continued attempts at *in vitro* cultivation of mitochondria.

Serial No. NIAID-88A
1. Biology of Viruses
2. Virus-Host Relationship
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Comparison of the properties of crude and crystallized Coxsackie A-10 virus, of Cloudman S 91, mouse melanoma and of mouse muscle nucleoprotein.

Principal Investigator: Dr. H. G. duBuy

Other Investigators: H. Sasame

Cooperating Units: None

Man Years:

Total:	1 4/12
Professional:	3/12
Other:	1 1/12

Project Description:

Objectives:

To compare preparations of purified virus with those of mouse muscle and melanoma nucleoprotein, chemically and immunologically.

Methods Employed:

For virus purification: Coxsackie A-10 virus of suckling mouse origin is purified and concentrated by chemical, physical and ultracentrifugal means. Purified virus is analyzed for protein and nucleic acid content.

For muscle and S 91 nucleoprotein preparation available methods were not applicable to the materials under study. Some steps, used for the preparation of so-called ribosomes, followed by modifications of existing purification procedures, have given promising results.

Part B included: No

Major Findings:

Melanin granules, isolated by selective centrifugation, contained 30 to 40 percent of the total ribose nucleo-protein of the melanoma cell. This supports the view that the granules are modified mitochondria. The antigenic activity of this material is determined by the number of "takes" of transplanted melanoma cells in mice which have previously been injected with immunizing doses of the melanoma nucleoprotein, as compared with non-immunized mice.

Purified Coxsackie A-10 virus has been introduced into normally resistant cells by cellular uptake of glass-adsorbed virus.

Significance to Bio-medical Research and the program of the Institute:

The introduction of virus into cells which are normally not susceptible to this virus, except in its nucleic acid stage, might throw further light on the conditions which govern virus multiplication in host cells.

The introduction of a self-duplicating portion of mitochondria from cancer cells into susceptible hosts might lead to formation of this specific neoplasm, and thus bridge the virus and the mitochondrial theories of carcinogenesis.

Proposed Course of the Project: .

To obtain sufficient quantities of the three nucleoproteins, each with a standard nucleic acid-protein ratio as an index of purity, to allow quantitative antigenic studies. At this time, purified muscle nucleoprotein to be used for control studies has not yet been obtained.

Serial No. NIAID-89
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Kinetics and Sites of Coxsackie Virus Multiplication in the Monkey Kidney Cell

Principal Investigator: Dr. C. F. T. Mattern

Other Investigators: Lotta Chi

Cooperating Units: None

Man Years:
Total: 1
Professional: 1
Other: 0

Project Description:

Objectives:

To establish the intracellular site or sites of viral RNA and protein synthesis.

Methods Employed:

Coxsackie A-9 virus is cultivated in monkey kidney cells for various time periods. Cells are fractionated by the Dounce Citric Acid Procedure and by conventional homogenization. Two major fractions are now being investigated for mature virus content, namely the nuclear fraction and the remainder of the cell, called the "cytoplasmic" fraction. It has been previously shown by this group that cold phenol will not extract RNA from purified Coxsackie. Also the suckling mouse inoculated I.M. is an excellent assay system for the RNA, whereas tissue culture is poor. These cell fractions are assayed for mature virus and extracted with cold phenol for a virus precursor, whether free RNA or a RNP other than mature virus.

Part B included: No

Major Findings:

1. Mature virus, that is virus refractory to cold phenol extraction of its RNA, is clearly associated with the cytoplasmic fraction at all time periods from 2 to 12 hours post-inoculation.

2. There is evidence of a cold phenol extractable precursor. This precursor has been extracted from whole cells and cell homogenates and appears maximally produced by 6 hours, remaining constant in quantity thereafter. The titer of mature virus, on the other hand appears to continue to increase until 9-12 hours post-inoculation. Most of this "precursor" appears associated with the "nuclear" fraction, thus showing a distribution within the cell that is different from that of "mature" virus.

Significance to Bio-medical Research and the Program of the Institute:

This study is intended to contribute to our knowledge of the nature of the processes by which viruses multiply. It has been proposed by others that viral RNA is synthesized in the nucleus on the basis of indirect evidence. The studies herein described would be the first direct evidence of viral RNA synthesis in the nucleus.

Proposed Course of the Project:

It is proposed to continue this project in order to evaluate the significance of our findings to date, especially with respect to whether they are representative of actual intracellular events or artifacts of cell fractionation. Other cell fractionation techniques will be employed. Our first "control" experiments indicated, for example, that this distribution of "precursor" is not the result of selective absorption of "cytoplasm precursor" by nuclei. In addition a collaborative project with Dr. Hilton Levy is planned in which these events will be followed by autoradiography in the Coxsackie-monkey cell system. Dr. Levy has been utilizing a poliovirus-HeLa cell system and has made observations which seem compatible with our interpretation of our infectivity data.

Since there is reason to believe that this "precursor" is a RN protein, we plan to attempt to isolate and characterize this component.

Serial No. NIAID-89A
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Virus Structure

Principal Investigator: Dr. C. F. T. Mattern

Other Investigators: Lotta Chi

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives:

This project involves the construction of biological and related biochemical models of viruses by a new model approach. The immediate objective is to test the "reality" of the virus models by electron microscopy and X-ray diffraction.

Methods Employed:

1. Electron Microscopy. The substructure of viruses is being examined by several techniques: conventional shadowing, negative staining with phosphotungstic acid, and positive staining with uranium salts. In addition, a new shadowing procedure has been developed and its potentialities in revealing substructure are being studied.

2. X-ray diffraction. This involves an indirect approach in which theoretical diffraction patterns may be calculated and compared with patterns obtained from viruses by others.

Part B included: No

Major Findings:

1. A new model for Tobacco Mosaic Virus has been constructed which contains a substantial number of those structural elements known from a wide variety of biochemical and biophysical data of others.

2. The model has predicted several gross features which differ from currently accepted models and which should be demonstrable by electron microscopy. Our electron micrographs taken with metal shadowing and negative staining are remarkably compatible with the model.

Significance to Bio-medical Research and the Program of the Institute:

This project is an effort to elucidate the three dimensional structure of viruses, beyond the subunit organization. The selection of TMV for constructing a detailed model was necessary because it is the most thoroughly studied virus and the only one about which there is sufficient structural data to attempt to construct a detailed model. The structure of other viruses, in particular animal viruses, will also be investigated by this approach as additional data accumulates.

Proposed Course of the Project:

Because of the controversial nature of this project, it is felt advisable to proceed to accumulate a substantial amount of experimental data before challenging the currently held views on virus structure, and in particular TMV. A purely hypothetical paper describing the model building system, without detailed reference to specific structures, is in the process of being written.

LABORATORY OF TROPICAL VIROLOGY
Middle America Research Unit
Arthropod-Borne Virus Section

PHS-NIH
Summary Statement
Office of Chief
Laboratory of Tropical Virology
Calendar Year 1960

I. ADMINISTRATIVE ASPECTS

The second year of the Laboratory's administrative existence was eventful and stormy. Activities of the Arthropod Borne Virus Section (ABVS) in Bethesda have been affected by the uncertainties in connection with Dr. William Pond's resignation from the position of Section Head and Assistant Chief, LTV. Difficulties were compounded by the resignation of Dr. Herbert T. Dalmat, who temporarily was Acting Head of the Section. Mr. Clarence J. Gibbs, Jr. then became Acting Head under difficult administrative circumstances aggravated by loss of several technicians and the usual problems in communications with Office of the Chief physically located in Panama. The Section has weathered the storms, carried on the active research program and is now anticipating assignment of replacements for Drs. Pond and Dalmat.

As all other NIH components in Bethesda, the ABVS is plagued with a shortage of space. Besides a modest area allotment in square feet, the laboratory is located in a somewhat uninviting basement of Building 5. Scheduled expansion into several rooms finally vacated by the Division of Biologic Standards will alleviate the work space shortage. However, such plans as the creation of a third section of LTV to be located in Bethesda (proposed a year ago) are hardly realistic.

The Panama Laboratory has also had space problems - but in reverse: for the past ten months the headaches were due to planning and execution of reconstruction to convert some of the ample space into a functional research laboratory and supporting service areas. Although the job is not finished, sometime early in 1961 the virus research area will be more than doubled. Especially needed are the several isolation cubicles constructed on the second floor and provision of air-conditioned desk space for investigators near their own working areas.

With clarification of the MARU mission and commitment to several important projects there has been a commensurate gradual increase in personnel, particularly at the sub-professional level.

The next calendar year will bring many more changes in the Laboratory of Tropical Virology. At MARU there will be a new Director, a new Head of Virus Section and a new Head of Mycology Section; in Bethesda, a new Head of the Section and physically relocated Laboratory Chief from Panama. Undoubtedly, these factors will affect administration as well as the research program of the laboratory.

II. RESEARCH HIGHLIGHTS

MARU, C. Z. - VIRUS SECTION

1. Virus Isolates from Panamanian Mosquitos and Sandflies

During the first 12 months of a 3-year project on the ecology of arthropod borne viruses in the tropical rain forest, which is being conducted by GML with the collaboration of MARU, major emphasis has been on virus isolation in suckling mice and hamster kidney cell cultures. Fourteen virus strains were isolated at MARU from 412 pools and 63,000 specimens provided by GML. Virus isolation rates were for Phlebotomus 1:700 and for mosquitoes 1:7000, although the rates varied greatly with species. Of the five Phlebotomus isolates, two of broad host range (including cell culture) and short incubation period are serologically identical. These viruses have now been identified as the Indiana type of vesicular stomatitis virus. The other three phlebotomus and nine mosquito viruses are being related to each other, to known virus groups and to human and/or animal infection and disease.

2. Eastern Equine Encephalomyelitis Virus Infection in Panama

The prevalence of EEE antibodies in horses and man in two areas of suggested EEE virus endemicity has been determined, allowing an evaluation of the relative usefulness of several serological methods applicable to studies of this type. It was found that the incidence of EEE antibodies in 460 humans tested increased with advancing age (0.8% under 10 years with progressive increase to 9% in the 41-50 year group). Complement fixation results on the same sera indicated the probable presence of other group A viruses.

Lizards of species common to this part of Panama were examined as a possible virus reservoir. Specific EEE virus hemagglutination-inhibitors were found in some of their sera. The occurrence of viremia and HI antibody response following virus inoculation were experimentally confirmed by inoculation of lizards.

3. Etiology of "Jungle Fever"

About 200 paired specimens were collected from military students participating in jungle warfare training courses in the Canal Zone. From one of the specimens obtained during an episode of fever following known exposure to jungle environment, a virus was isolated. This agent as yet has not been related to viruses known to be active on the Isthmus of Panama.

4. Encephalomyocarditis Virus Infection

Previously this laboratory described an outbreak of a fatal disease of swine caused by the EMC virus. The outstanding lesion in pigs dying during the outbreak was acute myocarditis. Since epidemiological obser-

vations suggested that natural infection resulted from ingestion of contaminated food, experiments were undertaken to reproduce the disease by feeding virus to young pigs. Viremia and virus excretion from the gastrointestinal tract were found to occur following the administration of brain from EMC inoculated mice. Infected pigs developed high titers of HI and neutralizing antibody during convalescence and had myocardial fibrosis at autopsy. Other studies included demonstration of EMC antibodies in a small number of city rats and rats caught on the affected farm, although wild rodents were found to be negative. Human sera were examined with interesting differences in the results depending on the donors' age: while a substantial proportion of the Panamanian population has been infected with EMC virus, the antibodies were found to be more common in persons of younger age.

5. Enterovirus Flora in Children of Central America

For a period of 12 months the enterovirus flora of infants at an outpatient clinic in Panama City was systematically explored establishing a base line of enterovirus fluctuation. The majority of viruses isolated belonged to the ECHO group, although in late 1959 and early 1960 poliovirus type 2 had become very prevalent. This was reflected in an uncommon occurrence of a small outbreak of paralytic disease due to type 2 poliovirus.

Other enterovirus studies have included 1) surveillance for the presence of type 1 poliovirus in Panama in late 1960 as a check on dissemination and threatened spread of this commonly epidemic type, 2) studies on a major epidemic of Echo-9 virus which swept through the Republic of Panama and the Canal Zone and 3) initiation of a collaborative project on possible relation of enterovirus flora of Guatemalan children to their dietary status.

6. Mite Virus Project

Drs. J. M. Brennan and C. E. Yunker of Rocky Mountain Laboratory staff have been assigned to LTV component in Panama to conduct a two-year study on the possible role of chiggers and other Acarina of the American tropics in the maintenance and transmission of animal viruses. To our knowledge this is the first serious attempt to explore this important area.

MYCOLOGY SECTION, MARU

The research program of the Section has markedly increased local awareness of histoplasmosis in all of its clinical forms, as evidenced by recognition of three disseminated cases (2 fatal and one successfully treated) within a period of 18 months (until then only one fatal case had been described since Darling's original cases in 1906). Ecological and epidemiological studies led to isolation of H. capsulatum from eight additional soil samples bringing up to sixteen the total number of recent isolations from Panamanian soil (while only a single

positive soil sample was recorded until this Section was established). The fungus has been repeatedly recovered from the organs of trapped ground mammals confirming its wide dissemination in nature.

Histoplasmin skin test continues to be a major tool for the study of epidemiology of histoplasmosis. Data on 9,200 children between 6 and 19 years of age have been obtained indicating, as expected, that the percentage of reactors increases progressively with age. The rate of histoplasmin sensitivity varies from 13 to 58% among six-year olds and from 68 to 92% among 19 year olds, depending on location of their residence. A survey of 631 pre-school children (6 months to 6 years) in the Canal Zone demonstrated an increase in hypersensitivity beginning with three years of age. A continuing similar study of Panamanian children in a city hospital is now in progress with information/over 800 already available.

Projects on other mycotic diseases have included diagnostic study and therapy of moniliasis, found to be a major superficial mycosis among both indigenous and transient population in the tropics.

LTV, BETHESDA - ABV SECTION

In spite of the difficult administrative and working conditions, the research staff pursued the several important projects initiated during the preceding calendar year. New projects involved an interesting application of the technique of antiserum pool combinations to typing of arthropod borne viruses, a wealth of data evaluating experimentally produced EEE virus infection in horses and a promising attempt to develop an inactivated EEE virus vaccine for human use. The infected horses yielded specific antiserum which is being processed for prophylactic use in cases of human exposure under laboratory or natural conditions.

Accidental laboratory infection of a staff member with an arthropod borne group C (Apeu) virus led to the first clinical-virological study of a syndrome produced by this important and common group of viruses of the western hemisphere.

III. PERSONNEL

ABVS, Bethesda - Dr. W. L. Pond and Dr. H.T. Dalmat resigned during second half of the year; not replaced at the end of Calendar Year.

Virus Section, MARU:

Dr. J. E. Craighead and Dr. C. G. Dobrovolny resigned in midyear. Dr. Craighead's position at MARU is now occupied by Dr. E. A. Bruckner transferred from Bethesda to the Canal Zone. Dr. J. V. Ordonez, staff member of Bacteriology Section of Instituto de Nutricion de Centro America y Panama (Guatemala) began a one year fellowship in virology (under the sponshorship of Parke, Davis & Co.) in July 1960. Drs. J. M. Brennan and C. E. Yunker transferred from RML to LTV-MARU for a period

of 2 years to initiate a project on the role of Acarina in transmission of infectious diseases.

Mycology Section, MARU:

No professional personnel changes. The Research and Development Command of US Army has accepted Mrs. M. Shacklette and Mr. J. Fuentes by transfer from NIH to US Army Caribbean payroll.

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SERIAL NO. NIAID - 93
1. TROPICAL VIROLOGY
2. ARTHROPOD-BORNE VIRUS
3. BETHESDA, MARYLAND

PHS-NIH
INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: STUDIES ON ARTHROPOD-BORNE VIRUSES IN THE SUB-TROPICAL AREAS OF THE UNITED STATES

PRINCIPAL INVESTIGATOR: ROBERT M. PENNINGTON

OTHER INVESTIGATORS: CLARENCE J. GIBBS, JR.
WILLIAM L. POND

COOPERATING UNITS: UNIVERSITY OF MIAMI SCHOOL OF MEDICINE
SOUTHWEST BLOOD BANKS, INC.

MAN YEARS (CALENDAR YEAR 1960)
TOTAL: 2-3/4
PROFESSIONAL: 1-1/4
OTHER: 1/2

PROJECT DESCRIPTION:

OBJECTIVES:

TESTING OF SERA FROM HUMAN BEINGS RESIDING IN THE SOUTHERN UNITED STATES HAS BEEN IN PROGRESS TO ESTABLISH A BASELINE FOR THE INTERPRETATION OF TESTS USED IN INVESTIGATIONS OF VIRAL DISEASES IN NON-TEMPERATE CLIMATES. MOREOVER, THESE TESTS CAN BE EXPECTED TO GIVE AN INDICATION AS TO WHICH ARTHROPOD-BORNE VIRUSES ARE PRESENT AND WHICH VIRUSES ARE PROBABLY ABSENT FROM THIS AREA.

METHODS EMPLOYED:

NEUTRALIZATION, HEMAGGLUTINATION INHIBITION, AND COMPLEMENT FIXATION TESTS OF THE ARTHROPOD-BORNE VIRUSES ARE BEING CARRIED OUT ON SERA OBTAINED FROM RESIDENTS OF SUBTROPICAL AREAS OF THE UNITED STATES.

MAJOR FINDINGS:

NEUTRALIZATION AND HEMAGGLUTINATION INHIBITION TESTS ON 125 SERA FROM MIAMI RESIDENTS INDICATE ACTIVITY OF ST. LOUIS ENCEPHALITIS, ENCEPHALOMYOCARDITIS, AND POSSIBLY ILHEUS VIRUS. THERE

ARE ALSO STRONG INDICATIONS THAT ANOTHER GROUP "B" VIRUS IS ACTIVE OR HAS BEEN ACTIVE IN THE MIAMI AREA. THE IDENTITY OF THIS AGENT IS BEING INVESTIGATED.

SIGNIFICANCE TO PROGRAM OF THE INSTITUTE:

POTENTIAL AND ACTUAL PUBLIC HEALTH IMPORTANCE OF ARTHROPOD-BORNE VIRUSES FOR RESIDENTS OF THE SOUTHERN UNITED STATES SHOULD BE DETERMINED AS PART OF INVESTIGATIONS OF TROPICAL VIRAL DISEASES.

PROPOSED COURSE OF PROJECT:

NEUTRALIZATION, HEMAGGLUTINATION INHIBITION, AND COMPLEMENT FIXATION TESTS WILL BE CARRIED OUT ON SERA NOT ALREADY TESTED AND THE SERUM COLLECTIONS ON HAND WILL BE AUGMENTED WITH ADDITIONAL SERA FROM OTHER SUBTROPICAL AREAS OF THE UNITED STATES.

PART B INCLUDED - NO

SERIAL NO. NIAID - 94-A
1. TROPICAL VIROLOGY
2. ARTHROPOD-BORNE VIRUS
3. BETHESDA, MARYLAND

PHS-NIH
INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: STUDIES OF ARTHROPOD-BORNE VIRUSES IN TISSUE CULTURE. PART 1. EVALUATION OF TISSUE CULTURE SYSTEMS FOR USE IN VIRAL ISOLATION, IDENTIFICATION, AND IN SEROLOGICAL TESTS FOR VIRAL ANTIBODIES.

PRINCIPAL INVESTIGATOR: CHARLES R. ROSENBERGER

OTHER INVESTIGATORS: WALTER L. NEWTON

COOPERATING UNITS: LABORATORY OF GERMFREE ANIMAL RESEARCH, NIAID-118

MAN YEARS (CALENDAR YEAR 1960)

TOTAL: 1-7/8

PROFESSIONAL: 7/8

OTHER: 1

PROJECT DESCRIPTION:

OBJECTIVES:

TISSUE CULTURE STUDIES ARE BEING APPLIED TO ARTHROPOD-BORNE VIRUSES TO (A) DEVELOP A MORE EFFICIENT SYSTEM FOR THE SUCCESSFUL ISOLATION OF VIRUSES FROM VECTORS AND NATURALLY INFECTED HOSTS, (B) AID IN CLASSIFICATION, IDENTIFICATION, AND CHARACTERIZATION OF THESE VIRUSES THROUGH DEVELOPMENT OF NEW TECHNIQUES UTILIZING CELL CULTURES, AND (C) PROVIDE A SOURCE OF VIRUS MATERIAL SUITABLE FOR USE IN VACCINE DEVELOPMENT.

METHODS EMPLOYED:

PROPAGATION AND CYTOPATHOGENICITY OF VIRUSES ARE STUDIED IN A VARIETY OF CELL CULTURES PREPARED FROM SELECTED ANIMAL AND HUMAN TISSUES. MAXIMUM SENSITIVITY OF CELL LINES TO VIRUS INFECTION AND PROLIFERATION IS DETERMINED BY TYPE AND DEGREE OF CYTOPATHIC CHANGES AS WELL AS BY TITERS OF VIRUS OBTAINED IN CELL CULTURES AND IN MICE. VIRUSES ARE IDENTIFIED AND CLASSIFIED INTO SEROLOGICAL GROUPS BY

NEUTRALIZING, WITH SPECIFIC ANTISERUM, THE ABILITY OF THE VIRUS TO CAUSE CYTOPATHIC CHANGES, FORM PLAQUES, OR PRODUCE HEMADSORPTION. STRAINS OF VIRUS WHICH SHOW REDUCED PATHOGENICITY IN ANIMALS, GREATER GROWTH OR CYTOPATHOGENICITY IN CELL CULTURES, OR OTHER DESIRED CHARACTERISTICS, ARE SELECTED BY SERIAL PASSAGE IN CELL CULTURES OR BY SELECTING INDIVIDUAL VIRUS PARTICLES THROUGH PLAQUE TECHNIQUES.

MAJOR FINDINGS:

HAMSTER KIDNEY CELL CULTURES (HKTC) HAVE BEEN FOUND TO BE PARTICULARLY USEFUL IN PROPAGATING ARTHROPOD-BORNE VIRUSES. PRESENTLY, MORE THAN 20 OF THESE VIRUSES HAVE BEEN GROWN AND OBSERVED TO PRODUCE CYTOPATHIC CHANGES IN THIS TYPE CELL LINE.

A CONTAMINATING NON-VIRAL ORGANISM WAS ISOLATED FROM APPARENTLY NORMAL HKTC. THIS ORGANISM APPEARS TO BE A BACTERIAL L FORM. IT PRODUCES A "HEMONUCLEAR ADSORPTION REACTION" IN CELL CULTURES WHICH RESULTS IN DISSOLUTION OF THE CYTOPLASM OF NUCLEATED CHICK ERYTHROCYTES LEAVING NUCLEI ADSORBED ONTO CELLS OF INFECTED CULTURES. THE ORGANISM WILL PROPAGATE IN A SUSPENSION OF CHICK ERYTHROCYTES IN BALANCED SALT SOLUTION. THE "HEMONUCLEAR ADSORPTION" PHENOMENON HAS PROVEN TO BE RELIABLE AND IS USED ROUTINELY IN OUR LABORATORY AS A TEST TO DETECT THE PRESENCE OF THIS ORGANISM IN CELL CULTURES.

IN COLLABORATION WITH DR. WALTER L. NEWTON, LABORATORY OF GERM-FREE ANIMAL RESEARCH, MANY CELL LOTS HAVE BEEN PREPARED FROM GERM-FREE ANIMALS. COMPARATIVE TESTS, WITH KIDNEY CELL CULTURES PREPARED FROM GERMFREE AND CONVENTIONAL MICE, HAVE NOT SHOWN DIFFERENCES IN GROWTH OR CYTOPATHOGENICITY WITH THE FOLLOWING VIRUSES: YELLOW FEVER (FRENCH NEUROTROPIC STRAIN), ANOPHELES A, MURRAY VALLEY ENCEPHALITIS, AND ORIBOCA. PREVIOUSLY REPORTED PRELIMINARY RESULTS OF GREATER CYTOPATHOGENICITY WITH DENGUE TYPE 1 VIRUS (MOCHIZUKI STRAIN) IN KIDNEY CELLS PREPARED FROM GERMFREE MICE THAN IN CELLS FROM CONVENTIONAL MICE HAVE NOT BEEN CONSISTENT ON ADDITIONAL INVESTIGATIONS.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE:

THESE STUDIES SHALL PROVIDE ADDITIONAL METHODS FOR MORE SUCCESSFUL ISOLATION, IDENTIFICATION, AND CLASSIFICATION OF ARBOR VIRUSES. THEY WILL FACILITATE INVESTIGATIONS INTO THE MECHANISM OF VIRUS-CELL INTERRELATIONSHIPS.

PROPOSED COURSE OF THE PROJECT:

THE STUDY OF ARBOR VIRUSES IN TISSUE CULTURE SYSTEMS WILL CONTINUE WITH GREATER UTILIZATION OF PLAQUE TECHNIQUES.

A STUDY OF THE BUNYAMWERA GROUP OF VIRUSES HAS BEEN INITIATED. CELL LINE SUSEPTIBILITY, GROWTH CHARACTERISTICS, PLAQUE PRODUCTION, AND ANTIGENIC RELATIONSHIP BETWEEN THE VIRUSES OF THIS GROUP WILL BE INVESTIGATED. THESE STUDIES WILL PROVIDE DEFINITIVE TESTS FOR THE IDENTIFICATION AND CLASSIFICATION OF THESE VIRUSES.

PART B INCLUDED - No

SERIAL NO. NIAID - 94-B
1. TROPICAL VIROLOGY
2. ARTHROPOD-BORNE VIRUS
3. BETHESDA, MARYLAND

PHS-NIH
INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: STUDIES OF ARTHROPOD-BORNE VIRUSES IN TISSUE CULTURE. PART 2. DEVELOPMENT OF A CELL CULTURE SYSTEM UTILIZING ARTHROPOD TISSUE.

PRINCIPAL INVESTIGATOR: CHARLES R. ROSENBERGER

OTHER INVESTIGATORS: NONE
COOPERATING UNITS: NONE
MAN YEARS (CALENDAR YEAR 1960)

TOTAL: 1/8
PROFESSIONAL: 1/8
OTHER: NONE

PROJECT DESCRIPTION:

OBJECTIVES:

TO DEVELOP METHODS OF PREPARING CELL CULTURES FROM ARTHROPOD TISSUES. TO PROVIDE A SENSITIVE AND MORE SUITABLE CELL CULTURE SYSTEM FOR PHYSIOLOGICAL STUDIES OF ARTHROPOD-BORNE VIRUS CELL-HOST INTERACTIONS.

METHODS EMPLOYED:

SPECIFIC TISSUES ARE DISSECTED FROM ARTHROPODS AND PREPARED AS CELL CULTURES. THE BLOOD OF THE ARTHROPOD IS SOMETIMES COLLECTED AND ADDED TO THE CULTURE MEDIA. VARIOUS CULTURE MEDIA AND CULTURE TECHNIQUES ARE USED IN ATTEMPTS TO PROVIDE A SYSTEM SUITABLE FOR A PARTICULAR TISSUE. CERTAIN ARTHROPODS ARE REARED UNDER STERILE CONDITIONS TO ELIMINATE CONTAMINATING ORGANISMS WHEN CELL CULTURES ARE PREPARED.

MAJOR FINDINGS:

TREATMENT OF SILKWORM OVARIAN TISSUE WITH A BALANCED SALT SOLUTION EXTRACT OF THE CROP OF THE BLUE CRAB CAUSES DISASSOCIATION OF CELLS. THIS TREATMENT PROVIDES A SUSPENSION OF CELLS RATHER THAN TISSUE FRAGMENTS OR CLUMPS OF CELLS. IN CULTURE, CELLS ATTACH TO THE GLASS SUBSTRATUM BUT FAIL TO PROLIFERATE IN WYATT'S MEDIUM WHEN HELD AT A VARIETY OF TEMPERATURES OF INCUBATION.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE:

CELL CULTURES OF ARTHROPOD TISSUES WOULD PROVIDE A COMPLETELY NEW APPROACH FOR STUDIES OF VIRUSES, HOST CELLS, AND THEIR INTERACTIONS. IN THE FIELD OF ARTHROPOD-BORNE VIRUS RESEARCH, INSECT CELL CULTURE MAY PROVIDE A SENSITIVE AND MORE SUITABLE CULTURE MEDIUM.

PROPOSED COURSE OF THE PROJECT:

BASIC STUDIES IN THE FIELD OF CELL CULTURE OF ARTHROPOD TISSUES WILL BE CONTINUED. THE POSSIBILITY OF MAINTAINING TISSUE AND CELL SUSPENSION TYPE CULTURES PREPARED FROM MOSQUITOES REARED UNDER STERILE CONDITIONS WILL BE INVESTIGATED.

PART B INCLUDED - NO

SERIAL NO. NIAID - 95-A
1. TROPICAL VIROLOGY
2. ARTHROPOD-BORNE VIRUS
3. BETHESDA, MARYLAND

PHS-NIH
INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: STUDIES ON ANTIGEN-ANTIBODY REACTIONS OF ARTHROPOD-BORNE VIRUSES. PART 1. KINETIC STUDIES OF THE SERUM NEUTRALIZATION OF ARTHROPOD-BORNE VIRUSES.

PRINCIPAL INVESTIGATOR: ROBERT M. PENNINGTON

OTHER INVESTIGATORS: WILLIAM L. POND

COOPERATING UNITS: NONE

MAN YEARS (CALENDAR YEAR 1960)

TOTAL:	1
PROFESSIONAL:	1/2
OTHER:	1/2

PROJECT DESCRIPTION:

OBJECTIVES:

TO INVESTIGATE THE VARIABLES OF THE NEUTRALIZATION TEST. SPECIFICALLY TIME (AS AN INDEPENDENT VARIABLE) AND THE RELATIVE DEGREE OF REACTION (AMOUNT OF VIRUS NEUTRALIZATION) DUE TO PRIOR INCUBATION OF THE VIRUS-SERUM REACTANTS. SUCH INFORMATION IS EXPECTED TO GIVE INSIGHT INTO THE KINETICS OF VIRUS-ANTIBODY COMBINATION. THE RATE OF REACTIVITY OF THE VIRUS-ANTIBODY SYSTEMS MAY INDICATE IF THE INSTANTANEOUS REACTION OF HOMOLOGOUS BACTERIAL ANTIGEN-ANTIBODY SYSTEMS IS ALSO APPLICABLE TO HOMOLOGOUS VIRUS-ANTIBODY SYSTEMS.

METHODS EMPLOYED:

THE RAPID IN VITRO COMBINATION OF VIRUS WITH HOMOLOGOUS ANTIBODY AND THE COMPARATIVELY SLOW SPEED OF REACTION (AVIDITY) WITH HETEROLOGOUS ANTIBODY HAS BEEN SUCCESSFULLY APPLIED TO MARKEDLY INCREASE THE SPECIFICITY OF THE ARTHROPOD-BORNE VIRUS NEUTRALIZATION TESTS. PROJECT TYPE SEROLOGICAL TESTS, RESULTS OF WHICH ARE UNINTERPRETABLE BECAUSE OF BROAD CROSS-REACTIVE ANTIGENIC RELATIONSHIPS, WERE STUDIED BY KINETIC NEUTRALIZATION TESTS.

MAJOR FINDINGS:

THE CONCEPT OF "INSTANTANEOUS" NEUTRALIZATION OF VIRUSES WITH HOMOLOGOUS ANTIBODY WAS TESTED USING GROUP "B" MODERATELY REACTIVE SERA OBTAINED FROM RESIDENTS OF GUATEMALA AND GROUP "B" BROADLY REACTIVE SERA OBTAINED FROM INDIGENOUS RESIDENTS OF SOUTHEAST ASIA.

THE DATA INDICATE THAT THE NEUTRALIZATION OF VIRUS BY HOMOLOGOUS ANTIBODY IS AN INSTANTANEOUS PHENOMENON. THE GUATEMALAN SERA, AS EXPECTED, DUE TO THEIR MODERATE CROSS REACTIVITY, AS DEMONSTRATED IN THE CONVENTIONAL NEUTRALIZATION TEST, WERE DIFFERENTIATED BY NEUTRALIZATION INDEX USING THE "MODIFIED" NEUTRALIZATION TEST (NO INCUBATION OF THE VIRUS-SERUM REACTANTS) /DISCUSSED IN THIS REPORT/. THE SOUTHEAST ASIAN SERA, HOWEVER, REMAINED BROADLY CROSS REACTIVE, ALTHOUGH TO A LESSER DEGREE IN THE MODIFIED NEUTRALIZATION TEST THAN IN THE CONVENTIONAL NEUTRALIZATION TEST.

SIGNIFICANCE TO PROGRAM OF THE INSTITUTE:

THE KINETIC NEUTRALIZATION STUDIES HAVE SHOWN THAT THE SPECIFICITY OF THE MOUSE NEUTRALIZATION TEST MAY BE INCREASED BY ELIMINATING THE PRIOR INCUBATION OF VIRUS-SERUM REACTANTS. WITH CROSS REACTIVITY AT A MINIMUM, A SPECIFIC VIRUS MAY BE IDENTIFIED AS CAUSING A ONCE BROADLY CROSS REACTIVE SERUM ANTIBODY. THIS STUDY DEMONSTRATES THAT THE BASIC PHENOMENON OF INSTANTANEOUS HOMOLOGOUS ANTIGEN-ANTIBODY COMBINATION EXISTING IN BACTERIAL ANTIBODY SYSTEMS FUNCTIONS IN THE SAME MANNER WITH VIRUS ANTIBODY SYSTEMS.

PROPOSED COURSE OF PROJECT:

THE INSTANTANEOUS NEUTRALIZATION OF VIRUSES WITH HOMOLOGOUS AND HETEROLOGOUS ANTIBODY IS TO BE STUDIED USING SERA ON HAND COLLECTED FROM HUMAN BEINGS RESIDING IN THE MIAMI AREA. THESE STUDIES WILL BE ENLARGED TO INCLUDE ADDITIONAL SEROLOGICAL TESTS DESIGNED TO ELICIT SEROLOGICAL SURVEY SPECIFICITY. IN ADDITION, ANIMAL SERA, PREPARED BY SINGLE AND DUAL INFECTIONS, WILL BE STUDIED TO DETERMINE THE CORRELATION OF SEROLOGICAL TEST RESULTS OBTAINED WITH THE MIAMI SERA AND THE LABORATORY ANIMAL SERA.

PART B INCLUDED - NO

SERIAL NO. NIAID - 95-B
1. TROPICAL VIROLOGY
2. ARTHROPOD-BORNE VIRUS
3. BETHESDA, MARYLAND

PHS-NIH
INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: STUDIES ON ANTIGEN-ANTIBODY REACTIONS OF ARTHROPOD-BORNE VIRUSES. PART 2. DEVELOPMENT OF A PRACTICAL AND SPECIFIC FLOCCULATION TEST FOR THE DEMONSTRATION OF ARTHROPOD-BORNE VIRUS ANTIBODIES.

PRINCIPAL INVESTIGATOR: ROBERT M. PENNINGTON

OTHER INVESTIGATORS: NONE

COOPERATING UNITS: NONE

MAN YEARS (CALENDAR YEAR 1960)
TOTAL: 1/2
PROFESSIONAL: 1/2
OTHER: NONE

PROJECT DESCRIPTION:

OBJECTIVES:

DEVELOPMENT OF A RAPID FLOCCULATION TEST FOR THE DETECTION OF ARTHROPOD-BORNE VIRUS ANTIBODIES AND ITS EVALUATION AS A WORTHWHILE LABORATORY PROCEDURE.

METHODS EMPLOYED:

ARTHROPOD-BORNE VIRUSES, CONTAINED IN CELL CULTURE SUPERNATANT FLUIDS, ARE ADSORBED ONTO CLAY PARTICLES IN THE MANNER PREVIOUSLY DESCRIBED BY BOZICEVICH OF THE LABORATORY OF CLINICAL INVESTIGATIONS FOR THE BENTONITE FLOCCULATION TEST FOR TRICHINOSIS AND FOR OTHER DISEASES. FLOCCULATION OF THE VIRAL ANTIGEN-COATED BENTONITE PARTICLES IS DEMONSTRATED IN THE PRESENCE OF THE SPECIFIC VIRAL ANTIBODY.

MAJOR FINDINGS:

THE BENTONITE FLOCCULATION TEST USING EASTERN EQUINE ENCEPHALOMYELITIS (EEE), AND ST. LOUIS ENCEPHALITIS (SLE) VIRUSES AS PROTOTYPES AGAINST HOMOLOGOUS ANTISERA HAS GIVEN SPECIFIC HIGH TITERED REACTIONS. NO CROSS REACTIVITY WAS DEMONSTRABLE IN FLOCCULATION

TESTS WITH RELATED GROUP "A" SERA OR RELATED GROUP "B" SERA. HOWEVER, ANTIBODY TITERS, USING THE SAME IMMUNE SERA AND DIFFERENT LOTS OF INFECTIVE HKTC FLUID, HAVE BEEN VARIABLE. THIS VARIABILITY IS MOST LIKELY A FUNCTION OF VIRUS ANTIGEN CONCENTRATION AND/OR THE AMOUNT OF ADSORPTION OF VIRUS TO THE BENTONITE CLAY PARTICLES.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE:

THE BENTONITE FLOCCULATION TEST, AS APPLIED TO THE ARTHROPOD-BORNE VIRUSES, MAY CONSTITUTE ANOTHER POSSIBLY VALUABLE SEROLOGICAL TEST TO BE USED IN CONJUNCTION WITH THE STANDARD COMPLEMENT FIXATION, HEMAGGLUTINATION INHIBITION, AND NEUTRALIZATION TESTS. MOREOVER, PRELIMINARY OBSERVATIONS INDICATE THAT THE ANTIGEN-BENTONITE COMBINATION MAY BE OF VALUE IN SUBSEQUENT ADSORPTION OF SELECTED ANTIBODY FROM SERUM.

PROPOSED COURSE OF THE PROJECT:

EVALUATION OF THE BENTONITE FLOCCULATION TEST (AS APPLIED TO ARTHROPOD-BORNE VIRUS SYSTEMS) IN TERMS OF (1) IMPROVED ANTIGEN ADSORPTION TO BENTONITE, (2) REPRODUCIBLE RESULTS, (3) STABILITY OF THE TEST REAGENTS, (4) IMPROVEMENTS IN THE METHODS FOR PREPARING TEST REAGENTS AND TECHNIQUES OF THE TEST.

PART B INCLUDED - NO

SERIAL NO. NIAID - 95-C
1. TROPICAL VIROLOGY
2. ARTHROPOD-BORNE VIRUS
3. BETHESDA, MARYLAND

PHS-NIH
INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: TYPING OF VIRUSES BY COMBINATIONS OF ANTISERUM POOLS. APPLICATION TO TYPING OF ARTHROPOD-BORNE VIRUSES.

PRINCIPAL INVESTIGATOR: CLARENCE J. GIBBS, JR.

OTHER INVESTIGATORS: NONE

COOPERATING UNITS: NONE

MAN YEARS: (CALENDAR YEAR 1960)
TOTAL: 1
PROFESSIONAL: 1/2
OTHER: 1/2

PROJECT DESCRIPTION:

OBJECTIVES:

THE PURPOSE OF THIS INVESTIGATION IS TO DEVELOP METHODS BY WHICH INDIVIDUAL TESTS OF AN UNKNOWN ARBOR VIRUS AGAINST A NUMBER OF TYPING SERA CAN BE REPLACED BY TESTS AGAINST A SMALL NUMBER OF POOLS OF THESE SERA. THE SERUM POOLS MUST YIELD COMBINATIONS OF RESULTS SPECIFIC FOR EACH TYPE ACCORDING TO THE DISTRIBUTION OF THE SERA IN THE POOLS.

METHODS EMPLOYED:

VIRUSES THUS FAR EMPLOYED IN THESE STUDIES ARE PROTOTYPE STRAINS OF REPRESENTATIVE ARTHROPOD-BORNE VIRUSES OF SEROLOGICAL GROUPS A, B, AND C. THESE ARE EASTERN EQUINE ENCEPHALOMYELITIS, JAPANESE B ENCEPHALITIS, AND APEU, CARAPARU, ORIBOCA, MARITUBA, MURUTUCU, AND ITAQUI VIRUSES. IN ADDITION, AN UNKNOWN VIRUS ISOLATED FROM A LABORATORY INVESTIGATOR AND SUSPECTED OF BELONGING TO GROUP C, WAS EMPLOYED. WITHOUT EXCEPTION, THE ANTISERA EMPLOYED IN THESE STUDIES WERE PREPARED BY HYPERIMMUNIZATION OF RABBITS WITH MOUSE ADAPTED VIRUS STRAINS. NEUTRALIZATION TESTS WERE CARRIED OUT BY INTRACEREBRAL INOCULATION OF 5-7 DAY OLD SUCKLING MICE. IN ALL TESTS EQUAL VOLUMES OF INACTIVATED UNDILUTED SERUM, OR SOMETIMES SERA, WERE MIXED WITH EQUAL VOLUMES OF VARYING DILUTIONS OF VIRUSES. COMBINATION SERUM

POOLS WERE PREPARED BY MIXING KNOWN SPECIFIC ANTISERA ON A 1:1 RATIO WITHOUT REGARD TO QUANTITATIVE LEVELS OF NEUTRALIZING ANTIBODY. COMBINATION POOLS OF GROUP C ANTISERA WERE PREPARED ON THE BASIS OF SEROLOGICAL SUB-GROUPS. CONTROLS CONSISTED OF MIXTURES OF EQUAL VOLUMES OF VIRUS WITH UNDILUTED NORMAL RABBIT SERUM OR 10% NORMAL RABBIT SERUM IN BORATE-KCL BUFFER SOLUTION AT PH 9.0.

MAJOR FINDINGS:

PROTOTYPE VIRUSES WERE NEUTRALIZED TO A GREATER EXTENT WHEN TESTED AGAINST THEIR HOMOLOGOUS ANTISERUM ALONE OR AGAINST POOLS WHICH CONTAINED SUCH ANTISERUM. HETEROLOGOUS NEUTRALIZATION OF PROTOTYPE VIRUSES OCCURRED ONLY BETWEEN MEMBERS OF THE SAME SEROLOGICAL GROUP. THERE WAS NO SEROLOGICAL CROSSING OVER BETWEEN GROUPS A, B, OR C. AN UNKNOWN VIRAL ISOLATE, SUSPECTED OF BEING APEU, A MEMBER OF SEROLOGICAL GROUP C, WHEN TESTED AGAINST COMBINATION SERUM POOLS WAS NEUTRALIZED BY COMBINATION OF GROUP C ANTISERA BUT NOT BY POOLS OF GROUPS A OR B. FURTHERMORE, POOLS CONTAINING APEU ANTISERUM SHOWED GREATER DEGREES OF NEUTRALIZATION THAN DID POOLS THAT DID NOT CONTAIN SPECIFIC APEU ANTISERA. THUS, OUR DATA SHOW THAT THIS METHOD CAN BE APPLIED SUCCESSFULLY IN GROUP TYPING OF AN UNKNOWN VIRUS IF THE VIRUS DOES NOT CROSS REACT TO ANY GREAT EXTENT WITH HETEROTYPIC ANTISERA AND IF THE POTENCY OF THE ANTISERA PERMITS THEIR MUTUAL DILUTION WHEN MIXED TOGETHER.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE:

THE METHOD DESCRIBED IN THIS REPORT REDUCES THE NUMBER OF TESTS THAT HAVE TO BE DONE IN IDENTIFYING AND CLASSIFYING AN UNKNOWN VIRUS. WITH FURTHER REFINEMENTS, IT SHOULD ALLOW A CENTRAL LABORATORY TO SUPPLY OTHER LABORATORIES WITH POOLS OF ANTISERA REPRESENTING ALL SEROLOGICAL GROUPS OF ARBOR VIRUSES MADE AND TESTED IN BULK. WITHOUT THE NECESSITY OF HAVING TO CARRY STOCKS OF SPECIFIC ANTISERA TO ALL TYPES, ANY FIELD LABORATORY CAN TYPE AN UNKNOWN VIRUS AS A MEMBER OF A SEROLOGICAL GROUP. IT WILL FACILITATE HANDLING OF EPIDEMICS DUE TO ARBOR VIRUSES AS THE GROUP REACTIVITY OF SEVERAL ISOLATES SHOULD POINT TO WHERE EMPHASIS MUST BE PLACED.

PROPOSED COURSE OF THE PROJECT:

THESE STUDIES ARE TO BE CONTINUED ALONG THE LINES DESCRIBED AND WILL BE BROADENED TO INCLUDE ALL OF THE KNOWN SEROLOGICAL GROUPS OF ARTHROPOD-BORNE VIRUSES. METHODS OF COMBINING SPECIFIC ANTISERA IN ORDER TO PROVIDE BROADLY REACTIVE BUT GROUP SPECIFIC POOLS OF ANTISERA WILL BE DEVELOPED. THIS TECHNIQUE WILL BE EMPLOYED AS AN AID IN CLASSIFYING AND FURTHER IDENTIFYING UNKNOWN VIRUSES SUBMITTED TO THIS SECTION FOR STUDY.

PART B INCLUDED - NO

SERIAL NO. NIAID - 96-C
1. TROPICAL VIROLOGY
2. ARTHROPOD-BORNE VIRUS
3. BETHESDA, MARYLAND

PHS-NIH
INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: SURVIVAL POTENTIAL OF THE ADULT OF *HAEMAGOGUS EQUINUS*, A SYLVAN VECTOR OF YELLOW FEVER.

PRINCIPAL INVESTIGATOR: PAUL A. WOKE

OTHER INVESTIGATORS: NONE

COOPERATING UNITS: NONE

MAN YEARS (CALENDAR YEAR 1960)

TOTAL:	1
PROFESSIONAL:	1
OTHER:	0

PROJECT DESCRIPTION:

OBJECTIVES:

TO IMPROVE THE OPPORTUNITIES FOR MORE INDIVIDUALS IN CAGED POPULATIONS OF ADULTS TO LIVE OUT THEIR POTENTIAL LIFE SPANS, THUS PROVIDING HEALTHIER AND LONGER LIVED EXPERIMENTAL INSECTS FOR USE IN STUDIES ON THE INTERRELATIONSHIPS BETWEEN VIRUSES, VECTORS, AND THE ENVIRONMENT, FOR STUDIES ON THE SPREAD OF VIRUS DISEASES AMONG VERTEBRATE HOSTS IN NORMAL SITUATIONS AND IN STUDIES DIRECTED TOWARD THE CONTROL OF ARTHROPOD-BORNE VIRUS DISEASES.

METHODS EMPLOYED:

HAEMAGOGUS EQUINUS IS USED AS THE EXPERIMENTAL SPECIES FOR WHICH IT IS SUITED BY REASON OF ITS IMPORTANCE AS A VECTOR AND ITS ADAPTABILITY TO LABORATORY EXPERIMENTAL CONDITIONS. POPULATIONS WERE SET UP UNDER CONDITIONS FOR SURVIVAL THAT WERE THE BEST POSSIBLE AT THE TIME. THE MOSQUITOES WERE CLOSELY OBSERVED THROUGHOUT THEIR LIFESPANS IN ORDER TO LEARN ALL PREVENTABLE CAUSES OF DEATHS. CORRECTIONS WERE APPLIED AND MEANS DEVISED BY WHICH TO REDUCE AND/OR ELIMINATE THE CURRENT CAUSES OF MORTALITY. THE INFLUENCE OF PARENTAL AGE, AGE OF EGGS AT THE TIME OF HATCHING, AND CONDITIONS UNDER WHICH THE LARVAE WERE REARED WERE DETERMINED BY TRIAL. SURVIVAL SERVED AS A CRITERION OF SUITABILITY.

PROPOSED COURSE OF THE PROJECT:

CONTINUE TO IMPROVE CONDITIONS FOR THE MAINTENANCE OF EXPERIMENTAL STOCKS OF HAEMAGOGUS EQUINUS AND TO APPLY THE FINDINGS TO OTHER SPECIES OF VECTORS AND POTENTIAL VECTORS OF ARTHROPOD-BORNE DISEASES.

UTILIZE THE METHODS AND FINDINGS IN STUDIES ON THE INTERRELATIONSHIPS OF VIRUSES, VECTORS, AND THE ENVIRONMENT.

APPLY THE FINDINGS IN STUDIES DIRECTED TOWARD THE DEVELOPMENT OF CONTROL MEASURES FOR ARTHROPOD VECTORS OF VIRUS DISEASES.

PART B INCLUDED - NO

MAJOR FINDINGS:

SURVIVAL OF HAEMAGOGUS EQUINUS ADULTS IS GREATEST IN THOSE POPULATIONS THAT ARISE FROM EGGS LAID BY YOUNG FEMALES FERTILIZED BY YOUNG MALES, FROM SURVIVING EGGS OF BATCHES THAT HAVE BEEN HELD FOR PERIODS OF TIME UP TO NEAR THE MAXIMUM PERIODS OF SURVIVAL UNDER ADVERSE CONDITIONS, AND FROM LARVAE THAT DEVELOPED AT LOWER TEMPERATURES, AND IS GREATEST IN THOSE POPULATIONS OF ADULTS THAT ARE MAINTAINED IN VARIED RATHER THAN CONSTANT TEMPERATURES AND HUMIDITIES. SURVIVAL IS REDUCED BY HIGHER TEMPERATURES, BY EXCESSIVE ACTIVITY INDUCED BY HIGH INTENSITIES OF WHITE LIGHT, AND BY AIR-BORNE VAPORIZED OIL OF EXCEEDINGLY LOW CONCENTRATION.

UNDER PRESENT LABORATORY CONDITIONS THE 70% SURVIVAL POINT FOR HAEMAGOGUS EQUINUS ADULTS IS 50 DAYS FOR MALES AND 64 DAYS FOR FEMALES; THE 90% SURVIVAL POINT IS 33 DAYS FOR MALES AND 44 DAYS FOR FEMALES. THE MAXIMUM LIFE SPAN HAS BEEN INCREASED FROM 11 DAYS FOR MALES AND 39 DAYS FOR FEMALES TO 107 DAYS FOR MALES AND 108 DAYS FOR FEMALES. KNOWN CIRCUMSTANCES INDICATE THAT THE SURVIVAL POTENTIALS AND POTENTIAL LIFE SPANS ARE STILL ABOVE THESE VALUES.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE:

HAEMAGOGUS EQUINUS ADULT MOSQUITOES CAN NOW BE CHOSEN FOR LABORATORY EXPERIMENTS WITH REASONABLE CERTAINTY OF SURVIVAL ACCORDING TO A PREDICTABLE DISTRIBUTION WHEN MAINTAINED WITHIN A CERTAIN GENERALLY USEFUL SET OF CONDITIONS. THE LIFE EXPECTANCY IS SUCH AS TO PERMIT THE COMPLETION OF EXPERIMENTS INVOLVING ALL NORMAL EVENTS; THE SURVIVORSHIP DISTRIBUTION PROBABLY COMES NEAR TO THE POTENTIAL FOR THE SPECIES. LONG SURVIVAL IS ACCOMPANIED BY HEALTH AND VIGOR. NEEDED LABORATORY INVESTIGATIONS CAN NOW BE PLANNED INTELLIGENTLY IN NUMEROUS AREAS ON THE BASIS OF THE KNOWN LIFESPAN OF THE SPECIES WHEN MAINTAINED BY THE METHODS THAT HAVE BEEN DEVELOPED. THUS, INSECTS CULTURED ACCORDING TO THE METHODS NOW AVAILABLE ARE SUITABLE FOR LABORATORY INVESTIGATIONS OF VIRUS-VECTOR-ENVIRONMENT INTERRELATIONSHIPS AND OF FACTORS IMPORTANT IN THE TRANSMISSION OF THE VIRUS AND SPREAD OF THE VIRAL DISEASE, AND FOR INVESTIGATIONS DIRECTED TOWARD THE DEVELOPMENT OF CONTROL MEASURES AGAINST THE VECTOR. ANALYTICAL STUDIES OF THE EFFECTS ON SURVIVAL OF SPECIFIC ENVIRONMENTAL FACTORS ARE NOW POSSIBLE. INFORMATION THAT HAS BEEN AND CAN BE DERIVED FROM LABORATORY EXPERIMENTATION CAN BE USED IN FIELD STUDIES ON THE SPREAD OF ARTHROPOD-BORNE VIRUS DISEASES AND ON MEANS BY WHICH TO CONTROL THE VECTORS. THE SPREAD OF VIRUSES AND CONTROL OF VECTORS IN THE NATURAL HABITAT ARE INFLUENCED BY THE ENVIRONMENT, AND EFFICIENCY AS A VECTOR DEPENDS IN PART ON LONGEVITY OF THE VECTOR. METHODS, EXPERIENCE, AND INFORMATION WHICH HAS BEEN GAINED IN THIS STUDY OF HAEMAGOGUS EQUINUS WILL BE USEFUL IN SIMILAR WORK WITH OTHER VECTORS OF ARTHROPOD-BORNE VIRAL DISEASES.

SERIAL NO. NIAID - 97-A
1. TROPICAL VIROLOGY
2. ARTHROPOD-BORNE VIRUS
3. BETHESDA, MARYLAND

PHS-NIH
INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: A QUALITATIVE EVALUATION OF EXPERIMENTALLY INDUCED EASTERN EQUINE ENCEPHALOMYELITIS (EEE) VIRUS INFECTION IN HORSES

PRINCIPAL INVESTIGATOR: CLARENCE J. GIBBS, JR.

OTHER INVESTIGATORS: WILLIAM L. POND
ROBERT J. BYRNE (UNIVERSITY OF MARYLAND)
CHARLES R. ROSENBERGER

COOPERATING UNITS: GRAYSON LABORATORY
UNIVERSITY OF MARYLAND

MAN YEARS (CALENDAR YEAR 1960)
TOTAL: 3
PROFESSIONAL: 1-1/2
OTHER: 1-1/2

PROJECT DESCRIPTION:

OBJECTIVES:

AN IMMUNOLOGICAL INVESTIGATION OF EXPERIMENTALLY INDUCED EEE VIRUS INFECTION IN HORSES DESIGNED TO (1) ELICIT THE FORMATION, DEVELOPMENT, AND PERSISTENCE OF VIREMIA AND HEMAGGLUTINATION-INHIBITING (HAI), COMPLEMENT FIXING (CF) AND NEUTRALIZING (NEUT) ANTIBODIES; (2) TO ESTABLISH A RESERVOIR OF STANDARDIZED REFERENCE EEE ANTISERUM FOR USE IN IMMUNOLOGICAL AND SEROLOGICAL INVESTIGATIONS OF EEE AND RELATED ARTHROPOD-BORNE VIRUSES.

METHODS EMPLOYED:

EACH OF THREE HORSES FREE OF SEROLOGICALLY DETECTABLE EEE ANTIBODIES WAS PRE-BLED AND THEN INJECTED WITH 10,000 MOUSE INTRACEREBRAL (IC) LD_{50} DOSES OF INFECTIOUS EEE VIRUS. FOLLOWING INOCULATION, SMALL VOLUME BLEEDINGS (50 ML.) WERE TAKEN ON THE FIRST 6 DAYS AND LARGE VOLUME (500 ML.) BLEEDINGS APPROXIMATELY EVERY 5 DAYS THEREAFTER FOR A TOTAL OF 50 DAYS. AFTER DAY 50, BLEEDINGS WERE PERFORMED AT 90 DAY INTERVALS. BLEEDINGS ON EACH OF THE FIRST 6 DAYS WERE PROCESSED

IMMEDIATELY FOR VIREMIA STUDIES. SUBSEQUENT BLEEDINGS WERE PROCESSED FOR SERUM 24 HOURS AFTER STORAGE ON THE CLOT AT 4-8°C. VIREMIA DETERMINATIONS WERE PERFORMED ON A COMPARATIVE BASIS IN ONE DAY OLD CHICKS, HAMSTER KIDNEY CELL CULTURES, AND IC IN 3 DAY OLD AND 21 DAY OLD MICE TO ELICIT THE BEST SYSTEM TO BE USED IN FIELD ISOLATIONS OF EEE VIRUS. HAI, CF, AND NEUT TESTS ARE BEING CARRIED OUT ON ALL SERUM SAMPLES COLLECTED.

MAJOR FINDINGS:

FOLLOWING THE EXPOSURE OF HORSES TO EEE VIRUS, VIREMIA IS DETECTABLE WITHIN 24 HOURS AND PERSISTS AT A SIGNIFICANT LEVEL FOR 72 HOURS. THE 1 DAY OLD CHICK IS THE MOST EFFICIENT SYSTEM FOR THE DETECTION OF EEE VIRUS IN THE HORSE BLOOD. SIGNIFICANT LEVELS OF HAI ANTIBODY ARE PRESENT ON THE 10TH DAY AFTER INOCULATION (1:160, 1:80, 1:80) AND ARE MAINTAINED AT A HIGH LEVEL 30 DAYS POST-INOCULATION (1:1280, 1:640, 1:320). COMPLEMENT-FIXING ANTIBODIES ARE DETECTABLE 10 DAYS AFTER INOCULATION (1:4, 1:256, 1:8) AND REACH A MAXIMUM LEVEL BETWEEN DAYS 30 AND 35 (1:1024, 1:2048, 1:1024). HIGH LEVELS OF CF ANTIBODIES ARE DETECTABLE THROUGH DAY 50. NEUTRALIZING ANTIBODIES ALSO ARE DETECTABLE AT A SIGNIFICANT LEVEL 10 DAYS AFTER INOCULATION (LOGS PROTECTION 1.3, 2.0, 1.7), REACH A HIGH LEVEL BETWEEN THE 21ST AND 30TH DAYS (2.5, 3.8, 3.0) AND PERSIST THROUGH THE 204TH DAY AFTER INOCULATION(3.4, 3.6, 2.7).

SIGNIFICANCE TO PROGRAM OF THE INSTITUTE:

THIS STUDY IS PROVIDING DETAILED SEROLOGICAL AND IMMUNOLOGICAL DATA OF EEE VIRUS INFECTION IN HORSES. THE NORTH AMERICAN ENCEPHALITIDES HAVE BEEN CAPABLE OF PRODUCING EXPLOSIVE EPIDEMICS IN ANIMALS AND HUMAN BEINGS; E.G., MASSACHUSETTS, NEW JERSEY, AND PANAMA, WITH HIGH MORTALITY IN ANIMALS AND MEN INFECTED WITH EEE VIRUS. AFTER BEING PROPERLY CHECKED FOR SAFETY AND STERILITY, THE SERUM OBTAINED DURING THIS STUDY WILL PROVIDE A POST-EXPOSURE PROPHYLACTIC SERUM FOR INOCULATION OF PERSONNEL EXPOSED TO THE VIRUS. IT ALSO PROVIDES THE PUBLIC HEALTH SERVICE WITH STANDARDIZED REFERENCE ANTISERUM AS PART OF ARBOR VIROLOGY INVESTIGATIONS THROUGHOUT THE WORLD.

PROPOSED COURSE OF PROJECT:

IT IS INTENDED THAT HAI, CF, AND NEUTRALIZATING ANTIBODY PERSISTENCE WILL BE FOLLOWED ON A CONTINUING BASIS IN AT LEAST ONE OF THE THREE HORSES. IN ADDITION, THE CRITICAL NEED FOR STANDARDIZED REFERENCE POLYVALENT ANTISERUM NECESSITATES INOCULATION OF EEE IMMUNE HORSES WITH OTHER GROUP A VIRUSES IN ATTEMPTS TO BROADEN THE SPECTRUM OF SEROLOGICAL REACTIVITY.

PART B INCLUDED - NO

SERIAL NO. NIAID - 97-B
1. TROPICAL VIROLOGY
2. ARTHROPOD-BORNE VIRUS
3. BETHESDA, MARYLAND

PHS-NIH
INDIVIDUAL PROJECT REPORT
CALENDAR YEAR 1960

PART A

PROJECT TITLE: THE DEVELOPMENT OF AN INACTIVATED VACCINE AGAINST EASTERN EQUINE ENCEPHALOMYELITIS (EEE) VIRUS.

PRINCIPAL INVESTIGATOR: CLARENCE J. GIBBS, JR.

OTHER INVESTIGATORS: CHARLES R. ROSENBERGER

COOPERATING UNITS: NONE

MAN YEARS (CALENDAR YEAR 1960)
TOTAL: 1-1/2
PROFESSIONAL: 1
OTHER: 1/2

PROJECT DESCRIPTION:

OBJECTIVES:

THE PURPOSE OF THIS STUDY IS TO DEVELOP AN ANTIGENICALLY POTENT AND SAFE INACTIVATED VACCINE SUITABLE FOR IMMUNIZING ANIMALS AND HUMAN BEINGS AGAINST EEE VIRUS INFECTION.

METHODS EMPLOYED:

HAMSTER KIDNEY CELL CULTURES (HKC) ARE INOCULATED WITH CELL CULTURE PROPAGATED EEE VIRUS. INFECTED CULTURES ARE MAINTAINED IN MEDIUM 199 FREE OF SERUM AT 35°C. UNTIL A VIRAL CYTOPATHIC EFFECT ON THE CELLS OF 3+ OR GREATER (4+ = 100% CELLULAR DESTRUCTION) HAS BEEN OBSERVED MICROSCOPICALLY. THE SUPERNATANT MATERIAL, CONTAINING VIRUS AND CELLULAR DEBRIS, IS ASEPTICALLY HARVESTED, POOLED, AND CLARIFIED BY CENTRIFUGATION AT AN R.C.F. OF 1070XG. IN AN INTERNATIONAL REFRIGERATED CENTRIFUGE. THE SUPERNATANT MATERIAL IS SEPARATED AND ALIQUOTS REMOVED FOR VIRUS INFECTIVITY TITRATION IN HKC AND SUCKLING MICE AS WELL AS FOR BACTERIOLOGICAL STERILITY CHECKS ON BLOOD AGAR PLATES AND IN THIOGLYCOLLATE BROTH. THE REMAINDER OF THE SUPERNATANT MATERIAL IS FORMALINIZED TO A FINAL CONCENTRATION OF 0.1 PERCENT NEUTRAL FORMALIN BY VOLUME. THE FORMALINIZED PREPARATION IS HELD AT 37°C. FOR 72 HOURS AND AT 4-6°C. FOR AN ADDITIONAL 6 DAYS PRIOR TO TESTING FOR VIABLE VIRUS.

Safety tests consist of inoculating 400 gram guinea pigs intracerebrally (IC) with 0.1 ml. of undiluted vaccine. Our safety requirements also include IC inoculation of 8-10 gram mice with 0.03 ml. of vaccine undiluted and diluted 1:10, 1:100, 1:1000 with survival of all animals as the criterion of safety.

Antigenic potency of the vaccine is determined by injecting 600 gram guinea pigs intradermally with two 0.1 ml. doses at 7 day intervals. Fourteen days after the last dose of vaccine, animals are challenged IC with 100-1000 LD_{50} doses (per 0.1 ml.) of an EEE strain different from that used to prepare the vaccine. In order to meet the minimum requirements of the Bureau of Animal Industry, Department of Agriculture, at least 2/3 of the vaccinated guinea pigs must survive the challenge. There are no prescribed minimum requirements for use in human beings.

Pre and post-vaccination bleedings are done on all test animals to determine serologically detectable response to the vaccine.

Major Findings:

So far, two lots of EEE-HKC vaccine have been prepared and assayed. Our data show that neutral formalin, in the concentration employed, is capable of completely inactivating detectable viable virus without destroying the antigenicity of the product. Vaccinated animals have been able to survive IC challenge with as many as 1000 IC guinea pig LD_{50} doses of virus. Serological data show the vaccine to be capable of eliciting neutralizing antibodies (log. of neut. indices 1.5 - 2.2), but that hemagglutination-inhibiting antibodies are not detectable. Tests to determine complement fixing antibody response have not been done.

Significance to the Program of the Institute:

EEE virus constitutes a serious veterinary and human public health problem in many areas of the world. The virus is also hazardous to handle in the laboratory. No licensed EEE vaccine is available for immunizing human beings at risk in the laboratory or in an epidemic area because a safe and potent vaccine has not been developed.

PROPOSED COURSE OF THE PROJECT:

ADDITIONAL LOTS OF VACCINE WILL BE PREPARED EMPLOYING EEE INFECTED HKC CULTURES AS THE SOURCE OF ANTIGEN. INASMUCH AS FORMALIN MAY REDUCE SOME OF THE ANTIGENICITY OF THE VACCINE, OTHER METHODS OF INACTIVATION WILL BE STUDIED; E.G., ETHYLENE OXIDE VAPORS AND INACTIVATION BY USE OF HIGH INTENSITY LIGHT SOURCES (PHOTOINACTIVATION) OTHER THAN ULTRA-VIOLET. STUDIES TO DETERMINE THE EFFECTS OF FILTRATION ON THE VACCINE WILL BE CARRIED OUT. ADDITIONAL SAFETY AND STERILITY STUDIES ARE PLANNED.

PART B INCLUDED - NO

offer viral diagnostic services to the community and public health agencies.

Methods Employed:

Clinical case specimens are either submitted by practicing physicians or public health officials in Panama, the Canal Zone and the neighboring countries, or are collected by MARU medical personnel. In the case of epidemic outbreaks already in progress (or when leads of epidemic importance are uncovered in the course of testing clinical case specimens) arrangements for field activities are initiated either by appropriate officials or by Director of MARU. In the Republic of Panama such arrangements are made through Director of Gorgas Memorial Laboratory and in the Canal Zone through Health Director's office. For other Middle America countries two-way communications between MARU and the National Governments are always through Office of the Representative, Zone III of Pan-American Sanitary Bureau, Pan-American Health Organization, WHO.

Laboratory procedures with specimens from either individual clinical cases or from epidemic outbreaks are generally the same; appropriate cell cultures (as available) and laboratory animals are inoculated for virus isolation, while serological testing of blood specimens for the presence of specific antibodies is performed by standard techniques.

Major Findings:

1. Clinical Cases. A variety of viral agents has been recovered with the predominance of enteroviruses (polioviruses, Coxsackie A & B, ECHO) from throat and rectal swabs, cerebrospinal fluid and patients' sera. Strains of myxoviruses, adenoviruses and Herpes simplex have also been recovered. In many cases the virus isolates were related to the clinical illness by serological tests.

2. CA Virus Infection in Adults. Croup-Associated (parainfluenza 2) virus infections were demonstrated in two young adults. The virus was recovered from throat swabs taken at the time of acute illness, utilizing human amnion cultures and the hemadsorption test; significant rises in HI and neutralizing antibodies during convalescence were demonstrated. The findings indicate that CA virus can cause clinical illness in adults.

3. Epidemic Influenza, 1959. Last year's report referred to etiological studies on an epidemic of influenza in Panama due to influenza B-virus and a major epidemic in British Guiana during the same months of mid-1959 due to the Asian influenza virus (A_2). A report describing these findings has been accepted for publication in the Am. J. Trop. Med. & Hyg.

4. Viral CNS Disease in a Guatemala Nursery. Last year's report referred to a tragic occurrence of fatal paralytic poliomyelitis cases and many aseptic meningitis cases due to polio and non-polioviruses in a Guatemala City charity nursery. The laboratory findings could not be fully reported at the time. A total of 44 enterovirus strains were

offer viral diagnostic services to the community and public health agencies.

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isolated from 103 individuals tested during five months of the study. Two or more specimens were available from the majority of affected infants. Twenty-two attendant nursing personnel were found to be negative for any enterovirus isolations.

From the two initial paralytic fatal cases Type 1 poliovirus strains were isolated from several sources (in one - from the throat and rectal swabs, cerebrospinal fluid and brain sections, and in the other from several areas of the brain). Two nonparalytic cases occurring during the early part of the outbreak also yielded T1 poliovirus. The only other T1 isolate was from an infant, undoubtedly suffering from non-polio viral meningitis, who was infected with T1 after many days in a hospital ward. No strains of poliovirus T2 were found, but 2 strains of poliovirus T3 and 3 strains of Coxsackie A were isolated from asymptomatic infants. The remaining 34 viruses apparently belonged to the ECHO group: 9 ECHO types 2, 11 and 12 and 17 which were either untypable or were not typed. Work on the remaining 8 strains, recovered during post-epidemic spread, was abandoned.

5. Poliovirus Type 2, Panama. A small outbreak of paralytic poliomyelitis caused by T2 virus was investigated. Of the 15 clinical cases, 11 were under 24 months of age. Type 2 viruses were recovered from 12 cases. During the epidemic (October 1959 to March 1960) a survey of enterovirus flora in Panamanian children was conducted as part of another study. It indicated a wide dissemination of the virus in the community. The emergence of poliovirus T2 during the past two years as an epidemic virus in Central America will be closely followed by MARU staff.

6. ECHO-9 Virus Epidemic, Panama. A major epidemic of ECHO-9 virus disease swept through Panama and the Canal Zone from July to October 1960. To date isolations have been made from 20 patients. In these clinical cases ECHO 9 virus was isolated most commonly from cerebrospinal fluid, then throat swabs and least often from rectal swabs.

During the epidemic (but apparently after its peak) 189 Panamanian children at the Hospital del Nino Outpatient Clinic were surveyed with throat swabs and rectal swabs taken from all and 98 venous blood specimens drawn at the same time as finger puncture blood was absorbed on filter paper discs. The purpose was to document occurrence of ECHO 9 virus (and possibly polioviruses) and to study the practical applications of filter paper discs as a serological tool. In contrast to clinical cases, the virus was much more frequently recovered from rectal swabs than from throat swabs. Relevant serological results with filter paper disc eluates are discussed under a separate project heading.

7. Measles Epidemic, Panama. In June 1960 the Ministry of Health of Panama requested MARU assistance. A staff medical officer accompanied two Panamanian physicians in a U.S. Air Force helicopter to Las Barretas village in the interior of Panama where a serious epidemic of measles had occurred: between late April and early June 35 deaths were reported in a population of 1500. During the 2 days' stay the sick were treated and specimens collected.

The area is not endemic for measles and the last epidemic occurred in 1952. Of 33 fatal cases whose age was known, 27 were seven years old and less. Forty-seven sera were tested for complement fixing antibodies: The majority of individuals over seven years of age with no history of a recent characteristic rash had the CF titer of less than 1:4. Studies by others in areas where measles is endemic revealed that CF titer persists in a fashion similar to neutralizing antibody titers. By using the neutralization test on sera from communities where measles has been absent for known intervals, we hope to clarify the immunological status of populations in non-endemic areas.

8. Epidemic Poliomyelitis, Nicaragua. A severe epidemic of poliomyelitis occurred in Nicaragua in late December 1959 through March 1960. Nicaraguan public health authorities and the Pan-American Sanitary Bureau invited the laboratory participation of MARU. Specimens for virus isolation were sent to both MARU and the Lederle laboratories. The epidemic was of special interest since Nicaragua had suffered an epidemic of unprecedented severity due to poliovirus Type 2 in 1958 and most of the children had received the Lederle live poliovirus vaccine. Unfortunately, the initial campaign concentrated on manovalent T2 vaccine with much sparser coverage by T3 and T1 vaccine strains.

Of 66 paralytic cases from whom specimens were received, 49 virus isolates were made - 47 of T1 and 2 of T2. The findings were reported to the Government of Nicaragua and the Pan-American Sanitary Bureau. Representatives of both of these agencies made a report on the joint findings at the Second International Conference on Live Poliovirus Vaccines held in Washington, D. C. in June 1960.

9. Community Spread of Poliovirus Type 1, Panama. Following the isolation of poliovirus T1 from two paralytic cases during the first week of November 1960, a survey of enterovirus flora was conducted in Panama and the Canal Zone. From children at four Canal Zone locations on the Pacific Coast 112 rectal swab specimens were collected and 90 rectal swabs were procured from the out-patient clinic of Hospital del Nino.

Testing of these in monkey kidney cell cultures failed to support the suspicion of wide dissemination and spread of T1 poliovirus strains in the community. A most striking finding was the abundance of enteroviruses among children of the poorer families in Panama and the sparsity of isolations from the Canal Zone, regardless of the community, ethnic background or relative income.

10. Another Outbreak of Fatal Swine Disease, Panama. An epizootic of fatal disease at a major commercial pig farm, similarly affected two years ago, was investigated in October jointly with other agencies. The clinical picture was different from the EMC syndrome described by our group for the 1958 outbreak (Science 131: 498-499, 1960). Fever, lethargy, difficulty in standing and walking, coughing and nasal discharge, labored breathing and diarrhea were now common. Miscellaneous specimens were collected from 17 live pigs; organs from two autopsied animals. Serum, rectal swabs and a few urine and nasal swabs were tested for virus isola-

lation in suckling mice, monkey kidney and hamster kidney cell culture tubes, and chick embryo cells in bottles. No virus isolations were accomplished in these systems. Subsequently, it was established by pathological and bacteriological studies at Laboratorio Veterinario and Gorgas Memorial Laboratory that this was an outbreak of hog cholera (swine fever) complicated by Pasteurella suis infection. Unfortunately, the outbreak continued to spread involving many animals. Our study was terminated early.

Significance to Bio-medical Research and the Program of the Institute:

Besides providing viral diagnostic services to the medical community, this project represents in essence epidemic intelligence indispensable to proper functioning of this field station. With but a few exceptions, such as Bocas del Toro study, the virus projects have been an outgrowth of careful consideration of leads discovered in the course of our willing cooperation with the health agencies and individual physicians in Panama and nearby countries.

Proposed Course of the Project:

Individual subprojects will be either terminated or developed into planned major projects as indicated. Participation in work-up of epidemic outbreaks and of promising individual cases will continue.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Craighead, J.E., Shelokov, A., Vogel J.E., Peralta, P.H. An Outbreak of Influenza B in Panama. Accepted for publication in Amer. J. Trop. Med. & Hyg.

2. Craighead, J. E., Shelokov, A., Peralta, P.H., Vogel, J.E. Croup Associated Virus Infection in Adults: Report of Two Cases. Accepted for publication in New Eng. J. Med.

3. Chi, L., Vogel, J.E., Shelokov, A., Selective Phagocytosis of Nucleated Erythrocytes by Cytotoxic Amebae in Cell Culture. Science, 130: 1763 (Dec.) 1959 (LISTED LAST YEAR AS ACCEPTED FOR PUBLICATION)

Honors and Awards relating to this project:

None

Serial No. NIAID-100-B
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: A Clinical and Virological Study of Oropharyngeal Lesions in Panamanian Children

Principal Investigator: Dr. J. V. Ordonez

Other Investigators: Dr. S.de Leon (H.del N.), Dr. J. A. Brody, Dr. E. Bruckner

Cooperating Units: Hospital del Nino, Panama City

Man Years (calendar year 1960)
Total: 3/12
Professional: 2/12
Other: 1/12

Project Description

Objectives:

Clinical and virological evaluation of oropharyngeal lesions in Panamanian children.

Methods Employed:

Swabs are collected in skim milk medium from oropharyngeal lesions in children examined in a pediatric outpatient clinic. Each group of specimens (collected over a half-day period) is kept frozen until inoculation into the test systems (monkey kidney, hamster kidney and chicken fibroblast cell cultures, as well as suckling and weanling mice).

Patient Material:

Children with observed oropharyngeal lesions attending the outpatient clinic of Hospital del Nino in Panama.

Major Findings:

This study is still in its initial stages and consequently no data are as yet available for presentation.

Part B Included No

Significance to Bio-medical Research and the Program of the Institute:

Distressing and even painful lesions of the oropharynx are not uncommon among children seen in the outpatient clinics of Panama. To our knowledge, no virological investigation regarding the etiology of such lesions has ever been carried out in the American tropics.

Proposed Course of the Project:

Work has been initiated as proposed and will be carried out for at least the next six months.

Serial No. NIAID-101-A
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Virological Aspects of a Cooperative Investigation on the Ecology of Arthropod Borne Viruses.

Principal Investigator: Dr. A. Shelokov

Other Investigators GML: Dr. P. Galindo, Dr. E. de Rodaniche and Dr. C. M. Johnson
MARU: Dr. P.H. Peralta, Dr. C.G. Dobrovolny, D.. Longfellow, J. Vogel, Dr. E.A. Bruckner, Dr. J.V. Ordonez

Cooperating Units: Gorgas Memorial Laboratory, Panama City
Hospital Div., Chiriqui Land Co., Almirante, R.de P.
Arthropod Borne Virus Section, LTV (Bethesda)

Man Years: (calendar year 1960)

Total:	5-9/12
Professional:	3-1/12
Other:	2-8/12

Project Description

Objectives:

1) To isolate and identify arthropod-borne viruses occurring in the tropical rain forest area near Almirante, Bocas del Toro Province, R.de P.; 2) To investigate the role of the virus isolates in human and domestic animal disease; 3) To study the role of arthropod vectors and animal reservoirs in the epidemiology of infections due to the arthropod borne viruses in the study area and other parts of the Isthmus.

Methods Employed:

In accordance with the original plan for the joint project, Gorgas Memorial Laboratory (GML) has provided MARU with arthropods collected by standard techniques and identified as to genus and in most cases species. MARU has also received aliquots of all human sera jointly collected at Almirante in October 1960 and from the small mammals bled by GML collectors earlier in the year. An ex-

perimental method for the collection of bird blood by saturation of filter paper discs has been utilized for virus isolation attempts at MARU. All birds caught or shot by the GML team beginning October are being thus sampled.

Standard methods have been used for: 1) isolation attempts in suckling mice (SM) and hamster kidney cell cultures (HKTC); 2) passages of isolates in these and other host systems for characterization and adaptation; 3) desoxycholate and ether sensitivity tests; 4) preparation of viral antigens and specific antisera and 5) serological tests - complement fixation (CFT), hemagglutination (HA), hemagglutination-inhibition (HI), and neutralization (NT). Preparation of "immune" ascitic fluid in laboratory animals has been initiated on a small scale.

Major Findings:

1. Isolation. The results of virus isolation from mosquito and phlebotomus pools during the first year at MARU and GML are shown by species in the attached table. In the new year of operations a few additional isolations have been made at MARU, including three from Psorophora ferox and 1 from Aedes (Ochlerotatus) spp. Isolation attempts with bird bloods eluted from discs were begun late in the year, without positive results so far.

2. Characterization and Identification. a) Phlebotomus Isolates: Three of these are similar in that they infect SM i.c. only, with a somewhat irregular incubation period (3-5 days) even after repeated passage; titers of SM brain pools have been low, making them difficult to work with. A sufficiently high titer has been obtained with one of these to permit testing by NT against antisera to the two types of Sandfly fever virus with negative results.

The other two sandfly agents appear indistinguishable in their biological characteristics and serological cross-reactivity (both CFT and NT). BT-78 (chosen as prototype), a DCA and ether-sensitive agent, was not neutralized in HKTC by antisera to the two types of Sandfly Fever, Herpes-B, EMC or the New Jersey type of Vesicular Stomatitis virus (VSV). It was neutralized by an antiserum for the Indiana type VSV, with which it also was reactive by CF test.

b) Mosquito Isolates: The twelve mosquito isolates fall into several groups according to their behavior in mice and MKTC. The only definite serological cross-relationship so far demonstrated (both CFT and NT) was between the two virus strains from pools of C.vomerifer. None of the several isolates tested has been cross-reactive with Eastern or St. Louis encephalitis, Ilheus or Mayaro (known or suspected in the area) or the viruses isolated from Panamanian mosquitoes collected in 1958-59.

3. Antibody Survey. Preliminary testing of human sera on hand from several areas in Panama against phlebotomus virus isolate BT-78 indicated that 20-35% of the population in certain areas possess neutralizing antibodies. Convalescent sera from 27 Panama City and Canal Zone patients

with influenza-like illnesses, suspected CNS disease or FUO were negative by NT.

Among domestic animals, 4/28 pigs (from two areas) and 19/63 horses and mules (from 5 localities on the isthmus) were shown to have neutralizing antibodies, with 65% of the equines positive in one area.

Significance to Bio-medical Research and the Program of the Institute:

The stated objective of the Virus Section of MARU has been "to evaluate the significance of viral agents found among the inhabitants of Middle America as related to causation of human and animal disease. Special emphasis is placed on arthropod borne viruses, their natural reservoirs and vectors as well as other agents which may be of potential danger to the population of other American countries, including the United States". The Bocas collaborative project is a major endeavor to fulfill this mission in a most thorough, efficient and economical way by combining and supplementing the efforts and skills of Gorgas Memorial Laboratory and Middle America Research Unit.

Proposed Course of the Project:

This field and laboratory project is now entering the second of its three scheduled years. Isolation attempts will be continued as the GML field team proceeds with arthropod collection. Culicoides midges will be collected during the second year. Bird and small animal blood and organs will be obtained in increasing numbers for virus isolation and serological testing. Sentinel suckling mouse techniques are being adapted for field use in Panama. Characterization and identification procedures will be accelerated with the increasing availability of immune sera to project isolates, type specific and groupings sera (obtained with the collaboration of ABVS-LTV). Plans include the use of additional techniques (such as TC plaques, agar-gel diffusion) as they become feasible and desirable for the execution of the project.

The second and third objectives of the project will be actively pursued with arthropod borne virus isolates for which the vector-reservoir relationships and public health significance should be sought.

BOCAS DEL TORO PROJECT

First Year's Summary of Mosquito and Sandfly Collections* and Virus Isolations at GML and MARU
1 Sept. 1959 - 31 Aug. 1960

/Data from Dr. P. Galindo, GML/

Species	Pools Inoc.		Specimens Inoculated		Virus Isol.	
	GML	MARU	GML	MARU	GML	MARU
Haemagogus spp.	15	12	884	787		
Aedes (Ochlerotatus) spp.[1]	69	59	9,670	8,199		1
leucocelaenus clarki	4	3	168	116		
leucotaeniatus	1		22			
(Finlaya) spp.	1		6			
(Howardina) spp.	3	2	85	40		
Psorophora albipes	13	15	1,651	2,032	1	
ferox	12	8	1,401	1,079	3	2
lutzii	1	1	101	97	1	
cingulata	6	4	543	420		
spp.[2]	36	38	4,932	5,142	1	1
Mansonia venezuelensis	67	67	9,849	9,291		
titillans	20	19	2,400	2,257		
arribalzagae	4	2	248	227		
indubitans	3		54			
nigricans	1		34			
Culex nigripalpus[3]	223	139	23,296	20,058	1	1
declarator	5	4	432	410		
coronator	8	5	552	598		
corniger	4	1	234	105		
vomerifer	15	15	1,782	1,749		2
taeniopus	9	7	816	627		
elevator	6	6	558	555		
chrysonotum	5	4	418	351		
spp.	9	8	704	653		
Uranotaenia spp.	3	4	95	89		
Trichoprosopon spp.	7	7	609	585		
Wyeomyia spp.	15	14	1,531	1,513		
Sabethes chloropterus	17	13	1,344	1,311	1	
Sabethes spp	5	5	283	175		
Anopheles neivai	3	1	68	27		
Anopheles spp.	14	13	1,408	1,366	1	1
Phlebotomus spp.	18	18	2,721	3,391	2	5
Totals:	622	494	68,899	63,160	12	13

* BY GORGAS MEMORIAL LABORATORY STAFF

1. Includes: A. serratus, A. angustivittatus, A. hastatus, A. tormentor, A. oligopistus, A. fulvus.
2. Includes: P. ferox, P. albipes and P. lutzii
3. Includes: C. inflictus.

Serial No. NIAID-101-B
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: The Role of Chiggers and Other Acarina of the American Tropics in the Maintenance and Transmission of Animal Infectious Agents: I. Viral Aspects.

Principal Investigator: Dr. J. M. Brennan

Other Investigators: Dr. C. E. Yunker

Cooperating Units: Rocky Mountain Laboratory, NIAID
Gorgas Memorial Laboratory, R.de P.
U.S. Army Malaria Control & Survey Branch, C.Z.
Veterinary Division, Health Bureau, C.Z.

Man Years (calendar year 1960):
Total: 1-2/12
Professional: 1
Other: 2/12

Project Description:

Objectives:

To explore the role of chiggers and possibly other Acarina, such as mites and ticks, in the maintenance and transmission of viral agents of actual or potential importance in Panama and nearby tropical areas.

Methods Employed:

The problem will be approached by trapping, bleeding, preparing and identifying hosts of parasitic Acarina, collecting their mites and processing for inoculation (by the staff of Virus Section, MARU) into laboratory animals and tissue culture. If viral agents are isolated, they will be identified, related to possible occurrence of viremia or the presence of antibodies in the donor host by standard virological procedures.

Part B included No

Major Findings (during the calendar year):

This is a new collaborative project in a field setting which has taken several months to initiate. The many anticipated and some unexpected problems have been largely resolved and the project should be in full operation by the beginning of the new calendar year. In the meantime, approximately 400 animals, including bats, snakes, birds, and rodents, have been examined for parasitic mites.

In the course of initial exploratory studies a discovery was made of two new genera and species of chiggers parasitic in the nasal passages of Panamanian bats. Although intranasal chiggers have been recovered from rodents in Africa and Malaysia, to our knowledge no chiggers utilizing an intranasal habitat have ever been recorded for Chiroptera.

Significance to Bio-medical Research and the Program of the Institute:

The role of chiggers and other Acarina in transmission of certain diseases is well known, (e.g., scrub typhus, rickettsialpox, Russian spring-summer encephalitis, etc.). However, no serious attempt has been made so far to define their role in transmission of classical and the newly recognized arthropod borne viruses. Hence positive or negative information will constitute a distinct contribution to our limited understanding of the ecology, epidemiology and epizootiology of this important group of pathogens.

Proposed Course of the Project:

The field project will continue as proposed until leads indicate the desirability of faunal or geographical specialization. In the event of bona fide isolation of viral agents, emphasis will be placed on a particular species relative to its abundance, distribution, host relationships, disease transmission potential, including field ecological studies and laboratory colonization attempts. To determine the potential public health significance of the viral isolates attempts will be made to relate them serologically to human and animal infection.

As opportunities arise, it is planned to explore further the role of Acarina in maintenance and transmission of other pathogenic agents, including rickettsiae, protozoa, bacteria and fungi.

Serial No. NIAID-101-C
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Viral "Jungle Fever" in U.S. Military Personnel

Principal Investigator: Dr. J. A. Brody

Other Investigators: D. Longfellow, Dr. J. V. Ordonez, Dr. E. Bruckner and Dr. A. Shelokov

Cooperating Units: Lt. Col. J. E. Goldoni, CO, Jungle Warfare Training Center (JWTC) U.S. Army
Col. H. B. Leach, MC, Chief Surgeon, USARCARIB

Man Years (calendar year 1960)

Total:	8/12
Professional:	3/12
Other:	5/12

Project Description

Objectives:

1. Isolation of viral agents from the blood of men with minor illness following known exposure to jungle environment.

2. To determine the rate of human infection with the newly isolated as well as viruses known to be active on the Isthmus.

Methods Employed:

Blood samples are drawn from military students participating in a 3-week course at JWTC. Specimens are secured by corpsmen from men reporting to the aid station with other than traumatic complaints.

Specimens are tested for virus isolation in suckling mice (SM) and hamster kidney cell cultures. Three successive cycles of classes at JWTC were bled before and after the 3-week course. Some 200 paired specimens were collected.

Part B included No

Major Findings:

Because of reticence to be bled only 18 specimens for isolation have been secured from the first 900 candidates. These have yielded one definite virus isolate (JW-10). The agent was isolated and re-isolated from a soldier with fever of 101°F and malaise. The virus produces illness in SM inoculated intracerebrally in 3 days with 100% mortality in 5 days. When inoculated intraperitoneally the illness and death are delayed 1 to 2 days. It is pathogenic for weanling mice i.c. but not i.p. Virus pools have been prepared and an immune mouse serum is available. Adequate CF and HA antigens are being worked out. Preliminary results with an HA antigen at low pH (5.2 - 5.7) are promising. To date attempts to adapt this agent to various cell culture lines have not been successful.

Significance to Bio-medical Research and the Program of the Institute:

The likelihood that isolates from human blood are of pathogenic significance makes information from this study important in defining not only the local health problems but viral infections of importance to the military training program of the area.

Proposed Course of the Project:

Although the numbers of specimens for isolation are small, each isolate is significant. Therefore, attempts at isolation will continue. Paired bloods will be tested for antibody rises against known isolates by suitable means and will also be tested against other MARU virus isolates as indicated.

Serial No. NIAID-102-A
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Enterovirus Infections of Rural Guatemalan Children in Relation to Nutrition

Principal Investigators: Dr. A. Shelokov & Dr. W. Ascoli (INCAP)

Other Investigators MARU: Dr. J. V. Ordonez, Dr. J. A. Brody, Dr. P. H. Peralta, Dr. E. Bruckner
INCAP: Miss V. Pierce, Mr. H. Bruch, Dr. N. Scrimshaw & Dr. J. Gordon

Cooperating Units: Instituto de Nutricion de Centro America y Panama (INCAP), Guatemala

Man Years (calendar year 1960)

Total:	1/12
Professional:	1/12
Other:	None

Project Description:

Objectives:

1. To compare the incidence and nature of viral flora among the children in three carefully controlled villages under constant surveillance.

2. To explore, if possible, the relationship of certain enteroviruses to the occurrence of clinical cases of diarrhea.

Methods Employed:

Each village comprises a population of approximately 1,000 with 200 children under age 5. Socio-economic, climatic, and topographical aspects are comparable. One village serves as control, in the other all children under age 5 receive liberal diet supplements, while in the third the diet is unchanged but sanitary, prophylactic, and therapeutic measures to prevent and ameliorate cases of diarrhea are enforced.

Part B included No

The entire study group population of each village will be surveyed every six weeks by rectal swabs for isolation of cytopathogenic enteroviruses. These will be identified and grouped as far as possible. Virus isolates will be related to clinical cases of diarrhea by epidemiological and serological techniques.

Major Findings (during the calendar year):

This is a new collaborative project between INCAP and MARU which has been planned during the calendar year. The INCAP group is engaged in a 3-year epidemiological study of the relationship between infectious diseases and nutrition in children under 5 years of age in these three rural Guatemalan communities. This has presented an unusual opportunity for a concurrent investigation of the viral flora during the second year of the INCAP study. Dr. J. V. Ordonez, staff member of INCAP's Bacteriology section was assigned to MARU in August 1960 on a 1-year fellowship (Parke, Davis & Company) to participate in the laboratory work and to provide liaison.

The first group of specimens should be received before the end of the calendar year, but no findings can be listed as yet.

Significance to Bio-medical Research and the Program of the Institute:

Diarrhea in young children ranks high among serious public health problems and causes of death in Guatemala and all other countries of Central America. Any and all information resulting from this study will provide much needed background. The unusual opportunity to explore the epidemiological aspects of enterovirus infections in relation to the nutritional status of controlled population groups would in itself justify this laboratory project.

Proposed course of Project:

It is anticipated that collection of appropriate specimens from children in the study groups and their testing will continue for one year. Another year will be devoted to identification of virus isolates, serological studies (if possible) and correlation of laboratory and epidemiological information.

Serial No. NIAID-102-B
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Laboratory Support of Phase 1, National Program for Poliovirus Vaccine Administration in Costa Rica, 1959.

Principal Investigator: Dr. A. Shelokov

Other Investigators: Dr. J. A. Brody, D. Longfellow & J. E. Vogel

Cooperating Units: Ministry of Public Health, Costa Rica
Pan-American Sanitary Burea/PAHO/WHO
San Juan de Dios Hospital, San Jose, Costa Rica
PASB-TC Lab. at Univ. del Valle, Cali, Colombia
Division of Biological Standards, NIH
Epidemiology Branch, CDC, BSS

Man Years (calendar year 1960):

Total:	2/12
Professional:	1/12
Other:	1/12

Project Description:

Objectives:

1. To support surveillance of neurological illnesses during the administration of live poliovirus vaccine in San Jose, Costa Rica.

2. To provide virological information on a series of well studied cases of neurological illness in a Central American country.

Methods Employed:

All cases of neurological illness during the oral vaccination program in the capital city were investigated by Dr. Brody on duty in Costa Rica. From almost every patient throat and rectal swabs and paired blood samples were obtained.

Specimens for isolation were tested at MARU in monkey kidney cell cultures and suckling mice. Duplicate specimens were tested by Dr. H. Doany (PASB-TC Laboratory, Cali) in HeLa cells and suckling mice. Serological testing of acute and convalescent blood

specimens was performed at the Cali laboratory, Division of Biological Standards in Bethesda and to a limited extent at MARU.

Major Findings:

Most of the work was accomplished during the preceding calendar year. In summary, 51 cases or siblings were examined. Six of these had clinical poliomyelitis and in 6 more the final diagnosis of poliomyelitis could not be ruled out. There were no cases in which the vaccine could be clearly related to the clinical illness.

Eleven viral agents from 10 individuals were isolated by testing 84 specimens. The isolations included one strain of poliovirus T1, two poliovirus T2, two poliovirus T3, two Coxsackie B4, two ECHO 14, one ECHO 8 and one unidentified virus. Serological studies against homologous virus isolates supported the isolation findings. Determinations of antibodies for the 3 types of poliovirus revealed occasional vaccine failures.

Among the cases of clinical poliomyelitis only 2 were seen early enough in the course of illness to make specimens meaningful. Poliovirus T2 was isolated from one and no virus was isolated from the other. Neither patient had been vaccinated. Of the 6 cases in which the diagnosis of poliomyelitis could not be ruled out, the following poliovirus isolations were made: T3 - 2 cases, T2 - 1 case, T1 - 1 case. Coxsackie B4 was recovered from two cases: from one T1 poliovirus was also recovered and the other had a serological rise to T3 poliovirus. The latter patient had not been vaccinated, while the others had received at least one dose of oral vaccine.

Significance to Bio-medical Research and the Program of the Institute:

This study failed to raise serious doubts as to the safety of the Lederle oral live poliovirus vaccine. It emphasized the difficulties in diagnosis and interpretation of laboratory results on hospitalized patients with neurological symptoms, especially when fed live virus vaccine. It also revealed that in the absence of a clear cut syndrome the virus yield in this hospitalized population was small.

Proposed Course of Project:

The project has been terminated and the findings were reported to the Government of Costa Rica and the Pan-American Sanitary Bureau.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B:

Publications other than abstracts from this project:

1. Quirce, J. M., Vargas Mendez, O., Nunez J., Montoya, J. A., Brody J., Henderson, D. A., and Martins Da Silva, M. "Vaccination with Attenuated Polioviruses in Costa Rica" in Live Poliovirus Vaccines: Papers Presented and Discussions Held at the First International Conference on Live Poliovirus Vaccines, Washington, D. C., Pan American Sanitary Bureau/WHO, 1959, 713 pp.

2. Quirce, J. M., Nunez, J., Guevara, E. C., Montoya, J. A., Doany, H., and Shelokov A. "Vaccination with Attenuated Polioviruses in Costa Rica. Second Progress Report, Section II: Surveillance Program" in Live Poliovirus Vaccines: Papers Presented and Discussions Held at the Second International Conference on Live Poliovirus Vaccines. Washington, D. C., Pan American Health Organization/PASB/WHO, 1960, 634 pp.

Honors and Awards relating to this project:

None

Serial No. NIAID-102-C
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Enterovirus Flora of Panamanian Children: A Twelve-Month Survey.

Principal Investigator: Dr. J. E. Craighead

Other Investigators: Dr. A. Shelokov

Cooperating Units: Hospital del Nino

Man Years (calendar year 1960):

Total:	6/12
Professional:	2/12
Others	4/12

Project Description:

Objectives:

This study was undertaken with the purpose of gaining information on the enteric virus flora of infants and young children residing in a tropical environment. Surveying the population at regular intervals for a year should reveal fluctuations in the incidence and type of virus infections and contribute to a better understanding of community outbreaks of clinical disease due to enteroviruses.

Methods Employed:

During the 12 months of the study, 30-35 rectal swab specimens were obtained every two weeks by the same MARU investigator from children under the age of three years in the out patient clinic of Hospital del Nino. Specimens were tested for virus isolation in MKTC. Identification of cytopathogenic agents was accomplished by neutralization tests employing antiserum for the three types of poliovirus, five Coxsackie B types and Coxsackie Ag. No attempt was made to identify agents not neutralized by one of these specific antisera.

Part B included No

Major Findings:

A total of 805 specimens was collected during the 12 months of the study; of these 256 were obtained in the months of January, February, March and April of 1960. The results during 1960 followed the trends established in 1959. Forty six cytopathogenic agents were recovered during the four months of 1960 (if contaminated with bacteria specimens were excluded from the final computations). Twenty per cent of rectal swabs satisfactorily tested in 1960 and 25% of specimens tested over the 12 months of the study were positive for enterovirus isolation. In January 1960, 19% yielded viruses, in February 20%, in March 16% and in April 25%.

Throughout the survey surprisingly few Coxsackie B, Coxsackie A9 and poliovirus type 1 and 3 strains were isolated. The majority of agents recovered were not identified by the techniques used (presumably they are miscellaneous ECHO viruses). In late 1959 and the early months of 1960, a substantial proportion of children tested yielded poliovirus Type 2. The appearance of this virus in the survey was associated with an outbreak of paralytic poliomyelitis due to the same virus type in Panama City. The outbreak is reported under another heading. In this study intestinal viral infection was not associated with a recognizable clinical syndrome. Viruses were recovered as frequently from well children as from children with diarrhea, fever and respiratory symptoms. No significant seasonal variations in the incidence of enterovirus infection were demonstrated.

Significance to Bio-medical Research and the Program of the Institute:

Infantile diarrhea and severe respiratory infections have been among the most serious health problems in Central and South America. Viruses are often blamed but while in North America and Europe enterovirus flora has been carefully investigated, practically no information is available on the frequency and nature of enterovirus infection in the American tropics. The recognized role of non-polio enteroviruses in the causation of aseptic meningitis (and occasionally muscle paresis) underscores the need for long-range evaluation of local enterovirus flora.

Proposed Course of Project:

Specimen collections for this 12-month survey (which developed from an earlier 6-week exploratory study in 1958) were completed in May 1960. Interesting and significant information is emerging from analysis of the data, providing background for subsequent studies. One such investigation on the community spread of poliovirus type 1 is already in progress.

Serial No. NIAID-103-A
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Use of Filter Paper Discs for Virus Isolation and Serological Testing.

Principal Investigator: Dr. J. A. Brody

Other Investigators: Dr. J. V. Ordonez and D. Longfellow

Cooperating Units: Staff of Gorgas Memorial Laboratory and Hospital del Nino

Man Years (calendar year 1960)

Total:	6/12
Professional:	3/12
Other:	3/12

Project Description

Objectives:

To develop a field tool for virus isolation and for serological testing.

Methods Employed:

Discs produced by Carl Schleicher and Schnell Co. (cat. No. 740-E) are used in all experiments. Humans are bled by finger puncture and small animals, particularly suckling mice (SM), by opening the chest and absorbing the blood onto the discs.

For virus isolation, Eastern equine encephalitis (EEE) virus has served as a model. Virus is inoculated into SM which are bled in 36 hours. Whole blood and discs are collected and treated under varying conditions. At definite intervals the dried discs are resuspended in diluent and titered. Virus determinations are conducted in hamster kidney cell cultures and occasionally in SM. In addition St. Louis encephalitis (SLE), Ilheus and yellow fever viruses have been studied to a limited extent.

Serological experiments have employed standard neutralization procedures in monkey kidney cell cultures using ECHO-9 and polioviruses. Discs are resuspended overnight before use and compared

with sera obtained by venipuncture from same subjects.

Major Findings:

EEE virus, titering 10^7 in whole SM blood, was absorbed on discs and left in the refrigerator and at ambient temperatures. After 12 days in the refrigerator the titer on the disc was 10^6 and after 32 days $10^{2.5}$. The environmental temperature disc titered $10^{2.5}$ at 4 days and the virus was recoverable (in SM) at 8 days. Crude attempts to dehumidify the discs did not seem to enhance preservation of the virus. Various methods of resuspension have not improved on the basic method of 0.6cc of diluent per disc left overnight at 4°C. Freezing wet and dry discs did not alter titers appreciably. SLE virus is fairly stable on dried discs. When viremia titered $10^{5.5}$ in SM, the discs left at 26°C contained $10^{1.5}$ logs of virus at 21 days and the discs left 4 days at ambient temperatures contained $10^{1.5}$ logs of virus. Ilheus and yellow fever viruses are more fragile on discs. Both were recoverable from discs after 4 days at 4°, but not from discs at environmental temperatures.

Because of the practical advantages of the disc method for isolation of viruses from small wild mammals and birds, Gorgas Memorial Laboratory is cooperating in a field trial. Birds shot at Bocas del Toro are being bled by cutting the jugular vein to saturate the discs then sent to MARU under refrigeration. Three discs are combined, resuspended and inoculated into SM. To date no virus isolations have been made. (GML reports that no viruses have been isolated by standard methods from sera of the same birds).

Serological studies have been performed using finger puncture discs and venous blood sera from the same individuals. We have established that blood resuspended from discs can not be titered out and can not be stored at room temperature for more than 3-4 weeks.

Significance to Bio-medical Research and the Program of the Institute:

The original work by Karstad and Hanson at University of Wisconsin showed that EEE could be recovered from discs. We are amplifying this finding by exploring the stability of viruses dried on discs. The potential value of discs as a field tool is considerable: increasing emphasis is being placed on cycles involving nestling birds and small mammals in the ecology of arthropod borne viruses; study of these cycles is limited because their bloods are difficult to collect; the discs may offer a partial solution to the problem of virus isolations in the field.

The possibilities of a reliable serological screening tool which does not require a syringe and vein are enormous. In tropical areas, where conversion rates are high in the young children (who are difficult to bleed) this procedure should be especially helpful.

Proposed Course of Project:

1) To continue field trials for virus isolations; 2) To explore fur-

ther the use of discs as a reliable serological method for human surveys; 3) To experiment with CF and HAI antibodies on disc-eluted bloods; 4) To attempt serological procedures for arthropod borne viruses on disc-absorbed blood specimens from wild life.

Serial No. NIAID-103-B
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Eastern Equine Encephalomyelitis (EEE) Virus Infection in Panama

Principal Investigator: Dr. J. E. Craighead

Other Investigators: Dr. P. H. Peralta, D. Longfellow J. E. Vogel and Dr. A. Shelokov

Cooperating Units: Gorgas Memorial Laboratory (GML)
Laboratorio Veterinario, Panama
U.S. Army Mission to Panama
Vet. Div., WRAIR, Washington, D. C.

Man Years (calendar year 1960)

Total:	9/12
Professional:	5/12
Other:	4/12

Project Description:

Objectives:

The 1960 objectives were an outgrowth of our investigation of a 1958 EEE outbreak in horses: 1) To determine the prevalence of EEE antibody in horses and man in two areas of probable EEE virus endemicity; 2) To evaluate the possible role of lizards as reservoirs of EEE virus in Panama; 3) To evaluate the relative usefulness of serological methods applicable to studies of this type.

Methods Employed:

Neutralization (NT) in mice, complement fixation (CFT) and hemagglutination-inhibition (HI) were used as classical techniques or with recently described but widely accepted modifications. EEE virus isolate (1958) from a Panamanian horse brain was used both as infectious agent and antigen.

Major Findings:

1. Human Population: Four hundred sixty serum specimens collected by GML and MARU in Darien and Panama provinces of the Republic

were surveyed for EEE antibodies by HI test. Reactive sera were tested in mouse NT. Thirty-two sera (7.6%) were reactive by HI test at $\geq$ 1:20; 12(3%) neutralized $\geq$ 1.7 logs of virus in the adult mouse test and 4 additional sera neutralized $\geq$ 1.7 logs of virus when tested I.P. in suckling mice. The incidence of EEE antibodies in humans increased with advancing age (0.8% under 10 years with progressive increase to 9% in the 41-50 year group). The mean percentage of positive sera was 3.7%. Antibodies were equally common in both sexes. Complement fixation tests were carried out on all 460 sera, but the results were difficult to interpret, suggesting heterologous antibodies to other group A viruses.

2. The Virus Reservoir: Many lizards of several species were observed on the ranches where the outbreak had occurred. Other investigators (RML) have indicated the possible role of reptiles in the ecology of ARBOR viruses. Because of these considerations, 246 wild lizards caught in the suspect area were examined by the HI test. Specific EEE virus hemagglutination inhibitors were found in a small proportion of these sera.

Several experiments were carried out with lizards of one of the three species common in Panama (Ameria festiva) confirming the occurrence of viremia and HI antibody response following virus inoculation.

3. Evaluation of Methods: The horse serum HI results were compared with CFT and NT results on same sera. Kaolin and acetone procedures for removal of non-specific serum hemagglutination inhibitors were also compared. It was concluded that the HI test is useful for rapid evaluation of sera for antibodies, since HI negative sera were consistently negative by NT and CFT. Further, large numbers of specimens can be quickly tested by surveying sera at a single dilution. However, a titer can be considered only suggestive of specific EEE antibodies and the NT is needed for confirmation, particularly in areas of likely activity of other group A viruses (6 of the 13 sera tested were shown to have VEE neutralizing antibodies by a cooperating laboratory). Our studies suggest that kaolin treatment of sera is not as efficient as acetone extraction for routine removal of inhibitors, but acetone may also remove much heterologous antibody, which can be disadvantageous in some survey situations.

Significance to Bio-medical Research and the Program of the Institute:

EEE virus continues to cause not only epizootics, but occasional epidemics associated with human fatalities and permanent sequelae. Studies on the ecology and epidemiology of the disease utilizing improved sero-diagnostic procedures should contribute to control of this public health problem common to both temperate and tropical areas.

Proposed Course of Project:

Experimental work on this project was concluded in June 1960 and the results are being prepared for publication.

Serial No.NIAID-103-C
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Encephalomyocarditis (EMC) Virus Infection. Studies on pathogenesis in swine, virus reservoirs in rodents and antibody status of human and animal populations.

Principal Investigator: Dr. J. E. Craighead

Other Investigators: Maj. T. G. Murnane, VC, USA, Dr. P. H. Peralta
Dr. A. Shelokov

Cooperating Units: Laboratorio Veterinario, R.de P.
U. S. Army Mission to Panama

Man Years (calendar year 1960)
Total: 8/12
Professional: 4/12
Other: 4/12

Project Description:

Objectives:

1) To conclude a basic investigation on the pathogenesis of EMC infection in swine; 2) To examine small wild rodents for EMC antibodies as possible reservoir hosts; 3) To determine the prevalence of EMC infection in the Panamanian population by testing randomly collected human sera for antibodies.

Methods Employed:

Laboratory methods developed during the preceeding calendar year were employed in these studies. Relevant details are mentioned under Major Findings.

Major Findings:

1. Biology and Pathogenesis of EMC Infections. Following the feeding of virus, 3 pigs without pre-existing antibodies developed viremia for 2-4 days and excreted virus in the feces for as long as 10 days. During the period of active infection the pigs exhibited no evidence of illness. All 3 developed substantial increase in antibody by hemagglutination inhibition (HI) and neutral-

Part B included Yes

ization (NT) tests. The 2 autopsied pigs had histological evidence of myocardial fibrosis compatible with earlier myocarditis. A fourth pig, with pre-existing antibody, developed viremia transiently, excreted virus for only two days and exhibited an antibody rise for only a brief period. A fifth pig was fed a large amount of virus after only one mouse passage. This pig died (in arrythmia?) 5 days later with histologic evidence of acute myocarditis. The virus titer of the heart tissue was greater than 10^6; other organs yielded lesser amounts.

2. Virus Reservoirs: In an attempt to find possible virus reservoirs in nature, wild rodents were trapped, bled and tested for EMC antibodies. Two of 53 rats (Rattus rattus) caught in or in the vicinity of Panama City were found to have antibodies, but all sera from 42 other small rodents of several species were negative.

3. Human Serological Studies: Eighteen percent of 158 sera from 4 localities in Panama were found to contain significant levels of EMC antibodies by NT in HeLa cell cultures (TC). There were interesting differences in the results with age. Thus, in the group under 10 years 23% were positive, 10-19 years - 23%, 20-29 years - 17%, 30-49 years - 17%, while only 7% of persons over the age of 50 possessed antibodies. These studies were carried out employing diluted serum and varying dosages of virus. Sera considered positive neutralized 2.5 logs or more of virus. Many of the positive sera were evaluated in the diluted state against approximately 100 TCD_{50} doses of virus. More than half of the sera tested in this way had demonstrable antibodies at a dilution of 1:4 or greater. A large number of NT negative and positive sera were tested by HI. Twenty-nine of 40 NT positive sera were also positive at a dilution of 1:4 or greater in the HI test, whereas only 4 of 36 NT negative were positive. Many of the HI positive sera had demonstrable antibodies at high dilutions. Limited studies were carried out in an attempt to compare the results of HeLa TC and mouse NT tests. While many sera were positive by both tests, TC positive sera frequently were negative in mouse NT. These data suggest that a substantial proportion of the Panamanian population has been infected with EMC virus.

The explanation for the relatively high incidence of antibodies in the younger persons is obscure. Possible explanations are: a) the virus has been recently introduced, b) infection occurs early in life and antibodies disappear with time, c) the virus infection ultimately contributes to mortality at a young age, d) the "antibodies" are actually non-specific neutralizing substances (this is highly unlikely).

Significance to Bio-medical Research and the Program of the Institute:

This project was based on our demonstration of natural infection of swine with EMC virus causing the most extensive outbreak of EMC infection in man or animals in which the virus was recovered. Our subsequent studies suggest that rats may constitute a natural source of infection, while pigs contract the disease by ingestion of rats and/or droppings. Improved laboratory diagnostic procedures developed during this study allowed ready demonstration of specific antibodies in many Panamanians, especially in

the younger age groups. These findings may contribute to a definition of not only another veterinary problem, but of possibly an important zoonosis, especially in the Caribbean area and Southern United States.

Proposed Course of the Project:

The project has been terminated and the findings are being reported in medical and veterinary research journals.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Murnane, T. G., Craighead, J. E., Mondragon, H., Shelokov, A. Fatal Disease of Swine Due to Encephalomyocarditis Virus, Science, 131:498-499 (Feb.) 1960 /reported as "accepted for publication" last year/.

Honors and Awards relating to this project:

None

Serial No. NIAID-108
1. Tropical Virology
2. Mycology Section, MARU
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Studies of Histoplasmosis on the Isthmus of Panama

Principal Investigator: Capt. R. Taylor, MSC
(R & D Command, U.S. Army)

Other Investigators: Dr. F. Abildgaard, Coco Solo Hospital
Dr. C. G. Dobrovolny, MARU

Cooperating Units: Walter Reed Army Institute of Research
Gorgas Memorial Laboratory, R.de P.
Hospital del Nino, R.de P.
Gorgas and Coco Solo Hospitals, C. Z.
Ministry of Health, R.de P.
Canal Zone Health Bureau
Army, Navy and Air Force Surgeons
U.S. Army Malaria Control and Survey Branch
U.S. Army Preventive Medicine Officer
Veterinary Health Offices, C. Z.

Man Years (calendar year 1960)
Total: 3-1/2
Professional: 3/4
Other: 2-3/4

Project Description:

Objectives:

1. To determine the extent of histoplasmosis on the Isthmus of Panama and the significance of this disease in the military personnel stationed in an area of histoplasmosis endemicity.

2. To explore the epidemiology and ecology of Histoplasma capsulatum on the Isthmus of Panama and Central America.

3. To provide diagnostic aids and adequate clinical follow-up on all recognized cases of histoplasmosis in this area.

4. To evaluate experimental therapy of histoplasmosis (in cooperation with Gorgas Hospital).

Part B included Yes

Methods Employed:

In cooperation with military and civilian health authorities obtain data on hypersensitivity to histoplasmin in individuals arriving for duty on the Isthmus, as well as long time residents of the Canal Zone and the Republic of Panama. Provide diagnostic assistance to Isthmian medical facilities in locating and following all clinical cases of histoplasmosis.

Collect soil samples from areas of suspected endemicity as suggested by patient interviews to demonstrate the presence of H. capsulatum and the foci of infection. Determine the extent of naturally occurring histoplasmosis in the wild animal population as possible reservoirs of H. capsulatum and define the local areas of endemicity.

Major Findings:

In the first eleven months of 1960, forty-three cases of acute histoplasmosis in the Canal Zone were serologically confirmed. In addition, one case of disseminated histoplasmosis in an 11 month old infant was culturally proven and the child successfully treated at Coco Solo Hospital. The first known case of cavitary histoplasmosis in this area occurred in a North American employee in the Canal Zone, who was evacuated to the Clinical Center NIH for therapy.

Histoplasmin skin testing was completed in the Canal Zone School system yielding data on 9,200 children between 6 and 19 years of age. This survey provides data on the entire school-age population permitting comparison of hypersensitivity rates according to location and length of residence, race, age and sex. As expected, the percentage of reactors increases progressively from the younger to older children. A similar increase occurs in the same age group, as length of residence increases. The rate of histoplasmin sensitivity in life-residents varies from 13 to 58% among 6 year olds and 68 to 92% among 19 year olds, depending on location of residence. The Central area of the Isthmus showed the highest sensitivity rates followed by the Pacific and Atlantic areas.

A completed survey of 631 pre-school children on the Atlantic side of the Isthmus provides data on children from 6 months to six years of age. The results of this study indicate a steady increase in the rate of hypersensitivity to histoplasmin from approximately 3 years to 6 years of age when the results dovetail with the results obtained in the school system on the Atlantic side. A similar study is currently under way at Hospital del Nino to further augment the data on the younger children. This survey has been in progress for 11 months, and results on approximately 800 children are available.

Ecological and epidemiological studies resulted in the isolation of H. capsulatum from 8 soil samples: one was from the residence of a clinical case of histoplasmosis; one from a U.S. Army training area; another from the sparsely populated remote province of Darien, and four

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from below bat roosts in a cave frequented by Canal Zone residents. The remaining isolation was from a repeatedly positive tree buttress and confirms the ability of H. capsulatum to propagate in this site through both rainy and dry seasons. This brings the total number of isolations from soil up to sixteen, representing six widely separated locations on the Isthmus of Panama.

The trapping of wild animals continued this year and H. capsulatum was recovered from the livers and spleens of 5 spiney rats and 9 opossums trapped on the Atlantic side. The area of trapping is extensively utilized by the U.S. Army as a training site and is the same area where H. capsulatum was isolated from soil.

Significance to Bio-medical Research and the Program of the Institute:

As would be expected the research program has resulted in a greater local awareness of histoplasmosis in all of its clinical forms, as evidenced by recognition of three disseminated cases (two fatal and one successfuly treated) within a period of eighteen months. Previously, only one fatal case had been described since Darling's original cases in 1906.

It is hoped that the current studies on ecology and epidemiology will elucidate the clinical cases of this disease, frequently misdiagnosed as tuberculosis, carcinoma and FUO, among military and civilian personnel and their dependents in this strategically important area.

The study offers an opportunity to study the organism in a tropical climate where the ecology may be considerably different from the United States.

Proposed Course of the Project:

Clinical, ecological and epidemiological investigations will be continued utilizing methods and techniques now employed, as well as those being developed in this laboratory and elsewhere, until either the major objectives are achieved or the studies become obviously unproductive.

Serial No. NIAID-108
1. Tropical Virology
2. Mycology Section, MARU
3. Panama, Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Abildgaard, C. F. and Taylor R. L.
Generalized Histoplasmosis in a Panamanian Infant: Case Report
Am. Jour. of Trop. Med. & Hyg. 9: 400-01, (July) 1960

Taylor, R. L. and Dobrovolny, C. G.
The Distribution of Histoplasmin Sensitivity in Guatemala
Am. Jour. of Trop. Med. & Hyg. 9: 518-22 (Sept.) 1960

Honors and Awards relating to this project:

None

Serial No. NIAID-109
1. Tropical Virology
2. Mycology Section, MARU
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Studies of Superficial and Deep Mycoses in Panama and Central America

Principal Investigator: Capt. L. Taylor, MSC, R&D Command, U.S. Army

Other Investigators: Capt. A. W. McFadden, MC, Gorgas Hospital

Cooperating Units: Gorgas Hospital, C.Z.
U. S. Military Dispensaries, C.Z.
Veterinary Division, Health Bureau, C.Z.
Raymond Clinic, R.de P.
Santo Tomas Hospital, R.de P.

Man Years (calendar year 1960)

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives:

To establish: 1) the nature of common superficial mycoses in the tropics and the efficacy of newer therapeutic regimens; 2) the extent and types of deep mycoses and 3) to correlate the abundant airborne mold spores to respiratory allergy.

Methods Employed:

Standard cultural diagnostic assistance has been made available to local physicians and medical facilities where patients with mycoses are commonly seen. A survey of the airborne molds of Panama was conducted by regularly exposing Petri dishes with medium at six specified locations for one year. Hairbait cultures were utilized for recovery of keratophilic fungi from soil samples which are being processed for Histoplasma capsulatum.

Part B Included: No

Major Findings:

1. Airborne fungi in Panama. A one year survey of low altitude mold spores was completed this year. Plates were exposed twice weekly at six locations on the Pacific side of the Isthmus. Over 3,000 colonies were identified: Hormodendrum predominated, followed by Asperigillus and Penicillium species. The total counts showed a seasonal variation with a peak in July and August and a low from November to February. Meteorological data were obtained and a correlation of total counts with relative humidity, temperature, rainfall and wind velocity will be attempted.

2. Griseofulvin Therapy. Therapy of chronic dermatophytosis with griseofulvin is being conducted by Dr. A. W. McFadden, dermatologist, Gorgas Hospital, with MARU furnishing cultural diagnosis and follow-up. The patients selected are primarily those with recalcitrant nail infections. The causative agent is determined prior to therapy and the patient is followed by a series of clinical laboratory tests and additional cultures to determine toxicity and efficacy of the drug. Excellent clinical results have been obtained to date; however, no new findings on the use of the drug are anticipated.

3. Therapy of Moniliasis. A "blind" comparative clinical evaluation of Amphotericin B and Mycostatin for monilial infections is being conducted by Dr. A. W. McFadden and the physicians at the military dispensaries. All cultural material is submitted to MARU for identification of the causative agent. Preliminary results indicate one of the two drugs to be more effective (the code has not been broken). This study has brought out the major importance of moniliasis in a population living in the tropics.

4. Hair-bait Culture of Soils. Microsporum gypseum has been isolated repeatedly from soil using the hair-bait culture technique. No clinical correlation has been attempted.

5. Epidemiology of Animal and Human Dermatophytosis. The occurrence of dermatophytosis in animals and their role in human infection is being studied in collaboration with the Canal Zone Veterinary Clinic. Several isolations have been made from clinical infections in animals with only one highly suspicious but unproved case of transmission to humans.

6. Deep Mycoses. The utilization of MARU's cultural diagnostic facilities by Dr. C. Calero (Raymond Clinic in Panama) resulted in confirmation of one case of cervico-facial actinomycosis. This success may arouse further interest in diagnosis of other mycoses previously unrecognized in this area.

Significance to Bio-medical Research and the Program of the Institute:

As a by-product of the long-range major histoplasmosis project there are opportunities to explore other mycological problems. While much of this information can be classified as "geographical pathology", some of it may be obviously important to public health authorities of

the area while interesting leads of general concern may be uncovered.

Proposed Course of the Project:

The course of these studies is determined by the time and personnel available as well as the relative importance of the findings to National public health authorities of Panama and the U.S. military personnel and dependents in the area.

LABORATORY OF GERMFREE [illegible]

Summary [illegible] 1

110 - Teratogenesis in Germfree Mice..... 1
111 - The Use of Germfree Animal and Tissue Cultures in the Study of Virus..... 3
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LABORATORY OF GERMFREE ANIMAL RESEARCH

Laboratory of Germfree Animal Research

National Institute of Allergy and Infectious Diseases

Summary statement of research progress, calendar year 1960

Program developments and trends:

During the year, Laboratory plans to broaden the scope of its research activities showed considerable and rapid progress. Authorization to occupy completely the south end of the third floor of Building 8, following the departure of DBS from this space, was finally clarified. The contract for the remodeling of this area was awarded and renovations got underway early in the Fall. It is anticipated that this space will be ready for occupancy (according to the schedule) around March or April of 1961. Following the transfer of LGAR personnel to the south end, alterations in the north end, converting the entire area into space for germfree animal units and maintenance, will commence. This will be effected slowly by NIH personnel, with the view of disrupting normal activities as little as possible. When this is accomplished, additional germfree animal units will be obtained and put into operation.

As a part of the broadening in research scope, it was deemed advisable to strengthen the program by the addition of pathology and virology activities. The staffing for this has proceeded well. Of the 5 new positions authorized by the Scientific Director up through the fiscal year ending July 1961, personnel for 4 are already committed. The new personnel will enter on duty in the Spring when the space is available. For the pathology group, Dr. Edwin Lerner, Senior Surgeon, will transfer from NIAMD to our Laboratory. Dr. Fred Gorstein, S. A. Surgeon, who has had training in pathology will enter on duty about July 1. Technical assistance for these people has been arranged for. The only position outstanding for which a candidate is not yet lined up is the virologist. Efforts are currently being made to locate one at the level of a GS-11 or 12.

The alteration of the air conditioning system for other institutes on the first, and especially the second, floor during the last five or six months of the year has caused some inconvenience especially with noise, vibration and occasional shutting down of a utility. However, the research program continued at a satisfactory pace in spite of some of these drawbacks. As was true of last year, the program reflected an increased variety of interests and an increase in the number of cooperative projects planned or underway with other laboratories, and even other institutes. As examples, we have recently undertaken on a limited scale with Dr. Rowe, LID, serologic and tissue studies for the possible presence of viruses in our germfree animal colony. Such data will establish baselines for future studies with virus-free animals, or, if it turns out that way, animals containing a few known viruses. We have, in collaboration with Dr. Landy of the NCI, initiated studies on the origin and specificity of natural antibodies to enteric bacteria, and similar studies in connection with Staphylococcus with the CDC. Experiments in germfree animals would appear to provide the crucial

information in these areas. Also, such studies can provide information on the nature of the mechanism whereby endotoxin alters resistance to infection with gram negative pathogens. With Doctors Kelly and O'Gara, also of the NCI, we have initiated on a small, but satisfactory, scale a study to ascertain whether the incidence of chemically-induced lung tumors is the same in germfree mice as in conventional mice of the same strain. In view of the large numbers of viruses demonstrated as occurring in experimental mice, (Huebner, Rowe), the question arises whether the chemicals induce the tumors by activating viruses, or some other living agents that are present in the animal. Such studies in an organism-free host could provide rather significant leads in the approach to understanding tumor-virus relations.

The apparent increase in the interest in germfree animals in medical research stimulated a request by Dr. Peterson to have a film made on this subject for use by medical schools, universities, research institutions,etc. The Audio-Visual Aids section of the CDC came to the Laboratory and we made a 19-minute color movie. The popularity of the subject matter is pointed up by the fact that, recently, all 28 copies of the film which CDC had in its library were booked up on loan for a month in advance.

Significant scientific advances:

With regard to the progress in some of the research projects, an interesting series of observations has been made by Mr. Phillips on the behavior of Entamoeba histolytica in the germfree host. It is to be recalled that, in earlier studies with standardized techniques, amoebic lesions were not produced in the germfree animal following inoculation. In fact, the parasite failed to live in the intestine beyond 5 days. Recent changes have been made in the manner of rearing and handling the amoebae in vitro prior to inoculation which seemed to result in more vigorous organisms. The latter have produced lesions in the absence of bacteria, although the type and severity are still not typical of those encountered with a bacterial associate. Thus, it would appear that the latter is not the only determinant of the course and the pathogenesis of the infection.

In collaborative studies with Dr. Weinstein of LPD, we have shown that the intestinal mouse parasite, Nematospiroides dubius, does not require a flora to develop from an infective larva to the adult form in the host. However, it apparently does require bacteria, or their products, to develop from the egg to infective larva. These studies are preparatory to those to be undertaken in an analysis of the nature of the nutritional effects observed in certain parasitisms. One of the most interesting observations has been our finding that the sex of the host, which has been noted by several workers to affect the outcome of the infection in conventional (contaminated) animals, has not appeared to be an influence in the germfree host. If these findings continue to hold up, a hitherto unrecognized role (either direct or indirect) of the flora in certain observed sex effects may unfold.

In studies on the growth and biology of germfree guinea pigs, Dr. Horton has obtained several advanced pregnancies in animals maintained

on irradiated diets, although no fetus was carried to term. It is to be recalled that germfree guinea pigs have not been bred with any success. The importance of the intestinal flora to this species was pointed up by the finding that conventional (contaminated) guinea pigs reproduced normally on this same irradiated diet.

In a collaborative project with Dr. Springer of the University of Pennsylvania, Dr. Horton has also shown that the use of large dosages of a cathartic, or the application of tourniquet shock, increased the number of red cells of germfree chickens coated with human B-like antigens following mono-infection with E. coli 086. These studies are providing information on the manner in which red cells of one type may acquire antigenic characteristics of other cell types, especially B.

Our germfree mouse colony has been undergoing an intensive serologic study from several points of view. One of the most interesting has been an assay for the presence of certain so-called "natural antibodies" against a variety of bacteria. Such antibodies or antibody-like reactivities for organisms like Staphylococcus, E. coli and S. typhosa have been found to occur in a variety of uninoculated conventional animals and are presumed to originate from encounters with the viable organisms or related antigens. Animals which have lived for many generations free from contact with live bacteria are almost the sine qua non for establishing finally the validity of these ideas. Studies thus far, with the CDC and with Dr. Landy of NCI, have shown the germfree animal to be singularly free from antibody-like reactivity toward Staphylococcus and E. coli, but reactivity toward S. typhosa was obtained in several instances. We are, of course, unable to find evidence of the presence of the latter in the germfree colony. Thus, this finding strengthens sporadic reports that non-bacterial substances (perhaps in this case dietary components) can cause "cross" reactions with this organism.

We have now had our germfree animal colony and a conventional colony derived from the same stock for approximately two years. Some of our ex-breeders which we try to keep, in spite of the scarcity of germfree unit animal space, are of the order of 1-2 years of age. We have made it a practice that whenever a germfree or conventional animal not on an experiment dies, especially if it is 6 months or more of age, it is checked thoroughly for gross evidence of malformations or tumors. As of now, we have information on a total of more than 50 animals. It is of interest that we have found so-called spontaneous lung tumors in the germfree as well as the conventional mice. Also, while the numbers of animals are obviously relatively small, the incidence has been markedly higher, thus far, among the conventional animals, i.e., those exposed to external contamination, than among the germfree. This has been particularly true among animals 6-12 months of age. These observations have been discussed with Doctors Kelly and O'Gara of the NCI who find them of considerable interest. Such data will continue to be collected and will serve as corollary information along with experiments in which such tumors are induced chemically in both germfree and contaminated animals. If this difference in incidence continues, it could point up further the importance of microbial agents per se, or

stresses produced by such agents, in the induction of these tumors. While it is, of course, too soon to say much in this regard, the possibilities suggested by even this limited information are very intriguing.

Serial No. NIAID-110
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Pathogenesis of amoebiasis

Principal Investigator: Bruce P. Phillips

Other Investigators: None

Cooperating Units: NIAID-127-B

Man Years (calendar year 1960):
Total: 1 3/4
Professional: 3/4
Other: 1

Project Description:

Objectives:

To study the etiology, pathogenesis, and pathology of intestinal amoebiasis with a view toward ascertaining the role of the associated intestinal flora.

Methods Employed:

Guinea pigs are inoculated intracecally with Entamoeba histolytica trophozoites cultivated in vitro without bacteria. Conventional, germfree and ex-germfree animals with various known single or multiple bacterial infestations are used, and the disease processes produced, if any, are compared grossly and histologically. Modifications of techniques for cultivating and harvesting the amoebae are also employed in an effort to obtain maximum virulence.

Major Findings:

Amoebic lesions did not occur in germfree animals which received amoebic inocula cultivated and prepared by previously standardized procedures, even though such inocula regularly produced amoebic ulceration in conventional hosts. Amoebic ulceration varying in extent and severity did occur following introduction of similar amoebic inocula into ex-germfree animals harboring each of the following bacteria as a monocontaminant: Escherichia coli, Aerobacter aerogenes, Streptococcus faecalis, Bacillus subtilis,

Lactobacillus acidophilus, Staphylococcus aureus, Micrococcus sp. (from conventional guinea pig). More recently, amoebic lesions have been produced in germfree animals following intracecal inoculation of E. histolytica cultivated and harvested by newer procedures developed as a part of these investigations. However, these lesions were not typical of those observed in animals harboring bacteria.

Significance to Bio-Medical Research and the Program of the Institute:

The wide range of host-parasite relationships which characterize enteric amoebic infection have long been a matter of considerable scientific interest. The demonstration of bacterial influence on development of the disease, although as yet incomplete, may provide at least partial explanation for the diverse manifestations of amoebic infection which range from asymptomatic infestation to acute, sometimes fatal ulcerative enteritis. Recent success in producing enteric lesions in germfree animals following inoculation of very large members of vigorous amoebae has not altered the concept that bacterial participation is essential for the development of symptomatic intestinal amoebiasis. It does provide, however, an opportunity to study intestinal tissue changes resulting from the activity of E. histolytica, alone, disassociated from all bacterial influence.

Proposed Course of the Project:

With reference to the recent finding that amoebic lesions can be produced in germfree hosts, there are two areas in particular that require clarification. First, since the lesions appear to emanate possibly from the site of the puncture wound resulting from injecting inoculum through the cecal wall, it may be that such trauma is a prerequisite. Secondly, since E. histolytica has not been cultivated axenically, our amoebic inocula contain invertebrate forms of Trypanosoma cruzi, with which amoebae are grown in vitro. The possibility exists, therefore, that the flagellates may participate in the development of amoebic lesions in the germfree animals. Efforts will be directed toward ascertaining whether either, or both, of these points is a factor.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this Project:

Phillips, Bruce P., and Wolfe, P. A.: Pneumonic disease in germfree animals. J. Inf. Dis. (In press)

Honors and Awards relating to this project: None

Serial No. NIAID-111
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: The use of germfree animals and tissues for the study of viruses

Principal Investigator: Walter L. Newton

Other Investigators: Charles Rosenberger, Laboratory of Tropical Virology
Wallace P. Rowe, Laboratory of Infectious Diseases

Cooperating Units: NIAID-

Man Years (calendar year 1960):
Total: 1
Professional: 1/4
Other: 3/4

Project Description:

Objectives:

To explore the possibility that germfree animals and tissues might serve as particularly favorable experimental tools for certain aspects of virus study. To ascertain whether viruses are present in the germfree mouse colony.

Methods Employed:

Comparative studies are carried out on the relative susceptibilities of germfree and conventional mice to infection with certain viruses. Cultures prepared from germfree animal tissues are also used. Sera are examined for evidence of antibodies to viruses. Standard virologic techniques are employed.

Major Findings:

1. Preparations containing Dengue type I virus (Mochizuki strain) continued to cause much more CPE in kidney tissue cultures prepared from germfree mice than in cultures prepared from conventional mouse kidney. However, in recent animal tests to

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ascertain whether an increase in viral growth was associated with the difference in CPE, no evidence of such growth was obtained. Furthermore, essentially no CPE was obtained following inoculation of the same material into either cell type. Efforts are being directed toward resolving this apparent inconsistency.

2. Sera from several germfree mice 1 year or more of age have been examined for evidence of the presence of certain mouse viruses. Numbers are too few for negatives to be conclusive, but thus far no positives for polyoma and mouse adenovirus have been obtained. Evidence for the presence of Reo 3 is questionable. However, there is good evidence that K virus may be present.

Significance to Bio-Medical Research and the Program of the Institute:

The study of viruses in animals without the possibility of influence of other concomitant infections, or with known infections, should provide special insight into virus-host relations. Also, with the increase in the number of viruses shown to occur in the conventional mouse, animals with a defined viral status and for which exposure to infection can be controlled become increasingly more valuable. This is particularly true in view of the current interest in tumor-virus studies.

Proposed Course of the Project:

Efforts will be directed toward clarifying the variation in the behavior of dengue virus in the germfree animal tissue culture. Also, further serological and tissue analysis of the viral state of the germfree mouse colony will continue.

Part B Included: No

Serial No. NIAID-112
1. Germfree Animal Research
2. Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Biology of germfree animals

Principal Investigator: Richard E. Horton

Other Investigators: Walter L. Newton
William B. DeWitt, Laboratory of Parasitic Diseases
N. A. Jaworski and John L. S. Hickey, Division of Research Services

Cooperating Units: DRS 6.4

Man Years (calendar year 1960):

Total:	2½
Professional:	1½
Other:	1

Project Description:

To obtain descriptive biological data on germfree animals for the purposes of determining in what manner they may differ from the conventional animal, and establishing baseline values for other studies employing these animals.

Methods Employed:

Comparative studies are made on germfree, specifically-contaminated, and conventional animals in such areas as hematology, blood chemistry, serology, gross and microscopic anatomy, growth curves, longevity, etc. Standard laboratory techniques are employed.

Major Findings:

In efforts to improve germfree guinea pigs by maintaining them on diets sterilized by irradiation instead of steam, some success was obtained with the use of irradiated semisynthetic diet. However, neither conventional nor germfree animals on irradiated diet grew as well as conventionals on non-irradiated diet. It was observed

that the growth rate of germfree animals reared on a semisynthetic diet sterilized by 2 million rad was greater than that of animals reared on the same diet sterilized by 3 million rad. Irradiation has an obvious detrimental effect on the nutrition adequacy of the diet.

Five pregnancies have been observed in adult germfree guinea pigs maintained on the irradiated ration. None of the females was able to carry the fetuses to term; they usually aborted near the 5th or 6th week of gestation. In most instances, procidentia of the uterus was a sequela that eventually led to the death of the animal. The administration of additional amounts of Vitamins K and E to the last two pregnant animals did not appear to alter the course of their pregnancies. This failure to reproduce has not been observed in the colony of conventional guinea pigs maintained on the same sterilized diet for four generations. Histological examination of several of the older germfree guinea pigs (approximately a year old) reared on the irradiated diet has revealed fatty degeneration of the liver.

Further study of the serum proteins in germfree and conventional mice has provided data similar to those reported earlier with germfree guinea pigs: Gamma globulin levels in the germfree were the same as those in mice from a conventional colony maintained on the same sterilized diet, but both groups showed values lower than those of mice from the NIH conventional colony which is maintained on a non-sterilized diet.

Data are being accumulated on the incidence of tumors (especially lung) in our germfree and conventional mice (the same genetic stock) dying or sacrificed after 6 months of age. Lung tumors have been found in the germfree mice, but there is some evidence that the incidence is lower than in their counterparts exposed to the outside environment. The possibility of this interesting difference will continue to be explored:

Significance to Bio-Medical Research and the Program of the Institute:

Studies of this nature provide baseline data for current and future experiments that utilize the germfree animal. Also, they provide an opportunity to study the role that the "normal" flora may play in establishing hematological and serological values and general growth, longevity, and fecundity.

Proposed Course of the Project:

Studies will continue along essentially the same lines. Where differences between the germfree and the conventional "contaminated" animals are encountered, efforts will be made to establish whether

fundamental principles are involved, and whether a particular difference is worth further exploitation. Also, attempts will be made (through the use of hormones, vitamins, etc.) to determine reasons for the germfree guinea pig's failure to reproduce satisfactorily.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this Project:

Horton, R. E. and Hickey, J. L. S.: Irradiated Diets for Rearing Germfree Guinea Pigs. Proc. of Animal Care Panel. (In press)

Newton, Walter L., Pennington, Robert M. and Lieberman, Jacob E.: Comparative Hemolytic Complement Activities of Germfree and Conventional Guinea Pig Serum. Proc. Soc. for Exper. Biol. and Med., 1960, Vol. 104, Pp. 486-488.

Baer, Paul N., and Newton, Walter L.: Studies on Periodontal Disease in the Mouse. III. The Germ-Free Mouse and Its Conventional Control. Oral Surg., Oral Med. and Oral Path., Vol. 13, No. 9, Pp 1134-1144, Sep. 1960.

Honors and Awards relating to this project:

Dr. Newton was invited to present a paper on the work on gamma globulin in germfree guinea pigs at a special session of the Fifth International Congress on Nutrition.

Serial No. NIAID-113
1. Germfree Animal Research
2. Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Origin of Anti-Human Blood Group B Agglutinins in White Leghorn Chicks

Principal Investigator: Richard E. Horton

Other Investigators: G. F. Springer, University of Pennsylvania

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1
Professional: 1/4
Other: 3/4

Project Description:

Objectives:

To determine if the blood group B active polysaccharide of E. coli O_{86} B:7 is absorbed onto the erythrocytes of monocontaminated chicks harboring the organism in the intestinal tract.

Methods Employed:

Groups of germfree and conventional White Leghorn chicks are inoculated orally with E. coli 086 B:7 at selected ages. Techniques which increase permeability of bacterial endotoxin across the intestinal wall are employed: the induction of chemical enteritis by prolonged feeding of drastic cathartics; and the induction of tourniquet shock. Blood samples are taken shortly after the period of physical stress by cardiac puncture. Standard serological testing procedures are used to determine the presence of group B active polysaccharide on erythrocytes and the titer of anti B agglutinins in blood samples.

Major Findings:

In vitro studies have shown that erythrocytes of White Leghorn chicks are easily and irreversibly coated with blood group B active bacterial products. We have found that erythrocytes of the germfree

chicks used in our studies do not contain blood group B-like antigen. When healthy germfree chicks are infected with E. coli O_{86}, only a minority of the animals acquire B-like antigens on the red cells. When a series of large doses of a cathartic (cascara sagrada) is fed to the E. coli O_{86} infected chicks, the proportion of animals having antigen coated red cells is increased. Tests made on blood samples taken several hours after E. coli O_{86} infected chicks have been subjected to tourniquet shock show that almost all chicks possess varying amounts of antigen-coated erythrocytes. Two to three days after application of tourniquet shock, the anti-B titer of these animals was found to rise considerably, but there was no demonstrable decrease in coated erythrocytes.

Significance to Bio-Medical Research and the Program of the Institute:

Studies of this nature may help explain the mechanism by which human patients with severe intestinal disorders (e.g. cancer of the colon, incarcerated hernia, etc.) acquire a transitory B antigen on their erythrocytes and the altered polyagglutinability of those red cells which accompany this phenomenon.

Proposed Course of the Project:

In vivo studies will be conducted to determine extent of coating of the erythrocytes with B antigen when germfree chicks and chicks monocontaminated with E. coli O_{86} are given purified B-substance parenterally. When these results have been obtained, further work on this project will be terminated.

Part B Included: No

Serial No. NIAID-114
1. Germfree Animal Research
2. Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Studies on bacterial interactions and host bacteria relations in the germfree animal

Principal Investigator: Norman S. Ikari

Other Investigators: Walter L. Newton
Maurice Landy, National Cancer Institute

Cooperating Units: CDC, Atlanta, Georgia

Man Years (calendar year 1960):

Total:	1 1/4
Professional:	1/2
Other:	3/4

Project Description:

Objectives:

To determine whether certain bacterial phenomena observed in vitro, e.g. genetic recombination between two enteric strains of bacteria, occur within the intestine of the living mammalian host. To evaluate host responses to particular bacterial species without interference from, and in association with, other known organisms.

Methods Employed:

In vitro studies have shown that the ability to produce colicine, an antibiotic-like substance lethal to some enteric bacteria, can be genetically transferred from one bacterial strain to another. In our studies, E. coli K 12 Row (streptomycin-resistant, colicine-sensitive) is established in the germfree mouse intestinal tract by inoculation of the water bottles. Later, Paracolon CA62 (streptomycin-sensitive, colicine-positive (col+)) is fed by mouth tube, and fecal pellets are collected at intervals thereafter. Saline dilutions of these pellets are assayed for col+ colonies by plating onto streptomycin agar plates.

Sera of germfree and variously contaminated mice are compared for levels of antibody activity towards specific organisms using Ouchterlony and CF techniques.

Major Findings:

Col+, streptomycin-resistant hybrids resembling the E. coli K 12 parent were obtained at every sampling for periods ranging from 24 hours to one month after the Paracolon feeding. Further examination of the col+ colonies confirmed the stability of this transfer and revealed the possibility of an additional change(s) in these hybrids not previously shown in in vitro studies. Sharp dichotomy was noted between germfree and conventional animals with respect to staphylococcal antibody. No antigen-antibody lines were noted in the germfree serum diffused against Cowan I soluble antigens, whereas one or more lines were obtained with conventional mouse serum.

Significance to Bio-Medical Research and the Program of the Institute:

Studies on whether genetic recombination and other bacterial interactions observed in the test tube occur in natural ecological surroundings can provide information on ways in which a host may affect these phenomena. Also, since backgrounds of "normal" antibodies (e.g., to organisms like staphylococcus) that are often observed in conventional animals can complicate attempts at serologic typing and fluorescent antibody study, the potential value of the germfree animal for such studies is well worth exploring.

Proposed Course of the Project:

Other known in vitro genetic recombination systems will be attempted in the germfree animal. The finding of large numbers of very mucoid variants completely different from either parental type or the col+ hybrids has suggested possible studies in a different direction.

Thorough analysis of the germfree mouse for the presence of antibody or antibody-like reactivity to a variety of organisms, especially gram negatives, will continue.

Part B Included: No

Serial No. NIAID-115
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Behavior of parasitic protozoa in germfree hosts

Principal Investigator: Walter L. Newton

Other Investigators: Bruce P. Phillips
Lucy V. Reardon, Laboratory of Parasitic Diseases

Cooperating Units: NIAID-127-C

Man Years (calendar year 1960):
Total: 1¼
Professional: ½
Other: 3/4

Project Description:

Objectives:

To determine whether the presence of bacteria affect the course of, and response of the host to, protozoan infections other than those caused by E. histolytica.

Methods Employed:

Germfree, monocontaminated, and conventional guinea pigs are inoculated with axenically-reared or micro-isolated species of Trichomonas, Trypanosoma, or Giardia. The inoculated animals are examined for the presence and type of lesions, time of death, etc.

Major Findings:

It has been shown previously that T. vaginalis, when injected subcutaneously, soon disappears in conventional guinea pigs, but multiplies and produces a severe lesion in germfree animals. However, when the germfree animal is orally contaminated with even a single species of bacteria, its response is like the conventional animal. In recent studies, the infection has been followed in the germfree animals until subsidence -- often requiring several weeks. When such animals are later re-exposed to the parasite, the pattern has been like that in the conventional animal. The encounter with the infection, even

though it eventually disappeared (and the animal became "germfree" again), seemed to activate a defensive response which persisted.

Techniques to inoculate germfree animals with sterile Giardia cysts have been worked out. Preliminary attempts at infection have not been successful, although the numbers of organisms inoculated have been small, thus far.

Significance to Bio-Medical Research and the Program of the Institute:

Studies of this type can lead to a better understanding of the possible effects of the "normal flora" in maintaining a host's natural defensive mechanisms. It would appear that this system (involving a host and an organism to which it is resistant in the conventional but not the germfree state) provides a good tool for such studies. Also, the role of the intestinal flora in the pathogenesis of a variety of protozoal diseases has yet to be established.

Proposed Course of the Project:

Additional experiments involving the "conditioning" of germfree animals with non-living stimuli such as dead bacteria, egg albumen, etc., prior to challenging with T. vaginalis are planned. A comparative study of the tissue responses in the conditioned and unconditioned germfree animal is planned. Attempts at establishing other parasitic protozoa in germfree animals will continue.

Part B Included: No

Serial No. NIAID-116
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Studies on helminthic infections in germfree hosts

Principal Investigator: Walter L. Newton

Other Investigators: Paul P. Weinstein, Laboratory of Parasitic Diseases

Cooperating Units: NIAID-121-G

Man Years (calendar year 1960):
Total: 1½
Professional: ½
Other: 1

Project Description:

Objectives:

To ascertain what effect the bacteria and other organisms normally present in the conventional animal have upon the course of helminth infections, and upon the host's response to these parasites.

Methods Employed:

Germfree animals are fed or inoculated with sterilized eggs, or axenically-reared infective larvae, of various parasitic helminths. The development of these parasites is followed by established procedures, and lungs, intestine, and other appropriate tissues are examined histologically for the study of host response. The findings are compared with those obtained in conventional controls.

Major Findings:

Mature, fertile adult worms were obtained in germfree as well as conventional mice, inoculated with infective larvae of Nematospiroides dubius. This indicated that the lack of bacteria appeared to have little or no effect on the development of the parasite from the infective larva to the adult stage in the host.

However, development of the parasite from the egg to infective larva in feces from germfree mice was extremely poor. Fatty degenera-

tion not unlike that associated with B-vitamin deficiency occurred. If living bacteria from a conventional animal were added to the germfree feces, development of the larvae progressed normally. Apparently, feces without bacteria fail to provide certain essentials for development of the larvae.

Of special interest was the apparent indication that the sex of the host, which is a factor in parasitism in the conventional animal, was unimportant among the germfree animals. In the conventional animals, males had 2-3 times the worm burden at necropsy that the females had. Among the germfree animals, however, average worm counts were about the same in both sexes.

Significance to Bio-Medical Research and the Program of the Institute:

The fact that bacteria, per se, are apparently not required for full development of some parasites opens the way for other studies in the areas of nutrition and parasitism, chemotherapy, in vitro cultivation, and physiologic studies. Also, opportunity is provided for the study of eggs and larvae in fresh (non-sterilized) feces without bacteria, and perhaps to ascertain factors contributed by the latter toward their proper development. If the sex phenomenon holds up in future tests, interesting and potentially important relationships may be uncovered.

Proposed Course of the Project:

Of course, the validity and significance of the difference in the host sex effect between germfree and conventional mice will be studied. Efforts to complete the entire life cycle of the parasite in a sterile environment will be continued. Attempts may be made to ascertain the role of bacteria in certain parasite-diet relationships noted in conventional mice.

Part B Included: No

LABORATORY OF GERMFREE ANIMAL RESEARCH

Laboratory of Germfree Animal Research

National Institute of Allergy and Infectious Diseases

Summary statement of research progress, calendar year 1960

Program developments and trends:

During the year, Laboratory plans to broaden the scope of its research activities showed considerable and rapid progress. Authorization to occupy completely the south end of the third floor of Building 8, following the departure of DBS from this space, was finally clarified. The contract for the remodeling of this area was awarded and renovations got underway early in the Fall. It is anticipated that this space will be ready for occupancy (according to the schedule) around March or April of 1961. Following the transfer of LGAR personnel to the south end, alterations in the north end, converting the entire area into space for germfree animal units and maintenance, will commence. This will be effected slowly by NIH personnel, with the view of disrupting normal activities as little as possible. When this is accomplished, additional germfree animal units will be obtained and put into operation.

As a part of the broadening in research scope, it was deemed advisable to strengthen the program by the addition of pathology and virology activities. The staffing for this has proceeded well. Of the 5 new positions authorized by the Scientific Director up through the fiscal year ending July 1961, personnel for 4 are already committed. The new personnel will enter on duty in the Spring when the space is available. For the pathology group, Dr. Edwin Lerner, Senior Surgeon, will transfer from NIAMD to our Laboratory. Dr. Fred Gorstein, S. A. Surgeon, who has had training in pathology will enter on duty about July 1. Technical assistance for these people has been arranged for. The only position outstanding for which a candidate is not yet lined up is the virologist. Efforts are currently being made to locate one at the level of a GS-11 or 12.

The alteration of the air conditioning system for other institutes on the first, and especially the second, floor during the last five or six months of the year has caused some inconvenience especially with noise, vibration and occasional shutting down of a utility. However, the research program continued at a satisfactory pace in spite of some of these drawbacks. As was true of last year, the program reflected an increased variety of interests and an increase in the number of cooperative projects planned or underway with other laboratories, and even other institutes. As examples, we have recently undertaken on a limited scale with Dr. Rowe, LID, serologic and tissue studies for the possible presence of viruses in our germfree animal colony. Such data will establish baselines for future studies with virus-free animals, or, if it turns out that way, animals containing a few known viruses. We have, in collaboration with Dr. Landy of the NCI, initiated studies on the origin and specificity of natural antibodies to enteric bacteria, and similar studies in connection with Staphylococcus with the CDC. Experiments in germfree animals would appear to provide the crucial

information in these areas. Also, such studies can provide information on the nature of the mechanism whereby endotoxin alters resistance to infection with gram negative pathogens. With Doctors Kelly and O'Gara, also of the NCI, we have initiated on a small, but satisfactory, scale a study to ascertain whether the incidence of chemically-induced lung tumors is the same in germfree mice as in conventional mice of the same strain. In view of the large numbers of viruses demonstrated as occurring in experimental mice, (Huebner, Rowe), the question arises whether the chemicals induce the tumors by activating viruses, or some other living agents that are present in the animal. Such studies in an organism-free host could provide rather significant leads in the approach to understanding tumor-virus relations.

The apparent increase in the interest in germfree animals in medical research stimulated a request by Dr. Peterson to have a film made on this subject for use by medical schools, universities, research institutions, etc. The Audio-Visual Aids section of the CDC came to the Laboratory and we made a 19-minute color movie. The popularity of the subject matter is pointed up by the fact that, recently, all 28 copies of the film which CDC had in its library were booked up on loan for a month in advance.

Significant scientific advances:

With regard to the progress in some of the research projects, an interesting series of observations has been made by Mr. Phillips on the behavior of Entamoeba histolytica in the germfree host. It is to be recalled that, in earlier studies with standardized techniques, amoebic lesions were not produced in the germfree animal following inoculation. In fact, the parasite failed to live in the intestine beyond 5 days. Recent changes have been made in the manner of rearing and handling the amoebae in vitro prior to inoculation which seemed to result in more vigorous organisms. The latter have produced lesions in the absence of bacteria, although the type and severity are still not typical of those encountered with a bacterial associate. Thus, it would appear that the latter is not the only determinant of the course and the pathogenesis of the infection.

In collaborative studies with Dr. Weinstein of LPD, we have shown that the intestinal mouse parasite, Nematospiroides dubius, does not require a flora to develop from an infective larva to the adult form in the host. However, it apparently does require bacteria, or their products, to develop from the egg to infective larva. These studies are preparatory to those to be undertaken in an analysis of the nature of the nutritional effects observed in certain parasitisms. One of the most interesting observations has been our finding that the sex of the host, which has been noted by several workers to affect the outcome of the infection in conventional (contaminated) animals, has not appeared to be an influence in the germfree host. If these findings continue to hold up, a hitherto unrecognized role (either direct or indirect) of the flora in certain observed sex effects may unfold.

In studies on the growth and biology of germfree guinea pigs, Dr. Horton has obtained several advanced pregnancies in animals maintained

on irradiated diets, although no fetus was carried to term. It is to be recalled that germfree guinea pigs have not been bred with any success. The importance of the intestinal flora to this species was pointed up by the finding that conventional (contaminated) guinea pigs reproduced normally on this same irradiated diet.

In a collaborative project with Dr. Springer of the University of Pennsylvania, Dr. Horton has also shown that the use of large dosages of a cathartic, or the application of tourniquet shock, increased the number of red cells of germfree chickens coated with human B-like antigens following mono-infection with E. coli 086. These studies are providing information on the manner in which red cells of one type may acquire antigenic characteristics of other cell types, especially B.

Our germfree mouse colony has been undergoing an intensive serologic study from several points of view. One of the most interesting has been an assay for the presence of certain so-called "natural antibodies" against a variety of bacteria. Such antibodies or antibody-like reactivities for organisms like Staphylococcus, E. coli and S. typhosa have been found to occur in a variety of uninoculated conventional animals and are presumed to originate from encounters with the viable organisms or related antigens. Animals which have lived for many generations free from contact with live bacteria are almost the sine qua non for establishing finally the validity of these ideas. Studies thus far, with the CDC and with Dr. Landy of NCI, have shown the germfree animal to be singularly free from antibody-like reactivity toward Staphylococcus and E. coli, but reactivity toward S. typhosa was obtained in several instances. We are, of course, unable to find evidence of the presence of the latter in the germfree colony. Thus, this finding strengthens sporadic reports that non-bacterial substances (perhaps in this case dietary components) can cause "cross" reactions with this organism.

We have now had our germfree animal colony and a conventional colony derived from the same stock for approximately two years. Some of our ex-breeders which we try to keep, in spite of the scarcity of germfree unit animal space, are of the order of 1-2 years of age. We have made it a practice that whenever a germfree or conventional animal not on an experiment dies, especially if it is 6 months or more of age, it is checked thoroughly for gross evidence of malformations or tumors. As of now, we have information on a total of more than 50 animals. It is of interest that we have found so-called spontaneous lung tumors in the germfree as well as the conventional mice. Also, while the numbers of animals are obviously relatively small, the incidence has been markedly higher, thus far, among the conventional animals, i.e., those exposed to external contamination, than among the germfree. This has been particularly true among animals 6-12 months of age. These observations have been discussed with Doctors Kelly and O'Gara of the NCI who find them of considerable interest. Such data will continue to be collected and will serve as corollary information along with experiments in which such tumors are induced chemically in both germfree and contaminated animals. If this difference in incidence continues, it could point up further the importance of microbial agents per se, or

stresses produced by such agents, in the induction of these tumors. While it is, of course, too soon to say much in this regard, the possibilities suggested by even this limited information are very intriguing.

Serial No. NIAID-110
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Pathogenesis of amoebiasis

Principal Investigator: Bruce P. Phillips

Other Investigators: None

Cooperating Units: NIAID-127-B

Man Years (calendar year 1960):
Total: 1 3/4
Professional: 3/4
Other: 1

Project Description:

Objectives:

To study the etiology, pathogenesis, and pathology of intestinal amoebiasis with a view toward ascertaining the role of the associated intestinal flora.

Methods Employed:

Guinea pigs are inoculated intracecally with Entamoeba histolytica trophozoites cultivated in vitro without bacteria. Conventional, germfree and ex-germfree animals with various known single or multiple bacterial infestations are used, and the disease processes produced, if any, are compared grossly and histologically. Modifications of techniques for cultivating and harvesting the amoebae are also employed in an effort to obtain maximum virulence.

Major Findings:

Amoebic lesions did not occur in germfree animals which received amoebic inocula cultivated and prepared by previously standardized procedures, even though such inocula regularly produced amoebic ulceration in conventional hosts. Amoebic ulceration varying in extent and severity did occur following introduction of similar amoebic inocula into ex-germfree animals harboring each of the following bacteria as a monocontaminant: Escherichia coli, Aerobacter aerogenes, Streptococcus faecalis, Bacillus subtilis,

Lactobacillus acidophilus, Staphylococcus aureus, Micrococcus sp. (from conventional guinea pig). More recently, amoebic lesions have been produced in germfree animals following intracecal inoculation of E. histolytica cultivated and harvested by newer procedures developed as a part of these investigations. However, these lesions were not typical of those observed in animals harboring bacteria.

Significance to Bio-Medical Research and the Program of the Institute:

The wide range of host-parasite relationships which characterize enteric amoebic infection have long been a matter of considerable scientific interest. The demonstration of bacterial influence on development of the disease, although as yet incomplete, may provide at least partial explanation for the diverse manifestations of amoebic infection which range from asymptomatic infestation to acute, sometimes fatal ulcerative enteritis. Recent success in producing enteric lesions in germfree animals following inoculation of very large members of vigorous amoebae has not altered the concept that bacterial participation is essential for the development of symptomatic intestinal amoebiasis. It does provide, however, an opportunity to study intestinal tissue changes resulting from the activity of E. histolytica, alone, disassociated from all bacterial influence.

Proposed Course of the Project:

With reference to the recent finding that amoebic lesions can be produced in germfree hosts, there are two areas in particular that require clarification. First, since the lesions appear to emanate possibly from the site of the puncture wound resulting from injecting inoculum through the cecal wall, it may be that such trauma is a prerequisite. Secondly, since E. histolytica has not been cultivated axenically, our amoebic inocula contain invertebrate forms of Trypanosoma cruzi, with which amoebae are grown in vitro. The possibility exists, therefore, that the flagellates may participate in the development of amoebic lesions in the germfree animals. Efforts will be directed toward ascertaining whether either, or both, of these points is a factor.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this Project:

Phillips, Bruce P., and Wolfe, P. A.: Pneumonic disease in germfree animals. J. Inf. Dis. (In press)

Honors and Awards relating to this project: None

Serial No. NIAID-111
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: The use of germfree animals and tissues for the study of viruses

Principal Investigator: Walter L. Newton

Other Investigators: Charles Rosenberger, Laboratory of Tropical Virology
Wallace P. Rowe, Laboratory of Infectious Diseases

Cooperating Units: NIAID-

Man Years (calendar year 1960):

Total:	1
Professional:	1/4
Other:	3/4

Project Description:

Objectives:

To explore the possibility that germfree animals and tissues might serve as particularly favorable experimental tools for certain aspects of virus study. To ascertain whether viruses are present in the germfree mouse colony.

Methods Employed:

Comparative studies are carried out on the relative susceptibilities of germfree and conventional mice to infection with certain viruses. Cultures prepared from germfree animal tissues are also used. Sera are examined for evidence of antibodies to viruses. Standard virologic techniques are employed.

Major Findings:

1. Preparations containing Dengue type I virus (Mochizuki strain) continued to cause much more CPE in kidney tissue cultures prepared from germfree mice than in cultures prepared from conventional mouse kidney. However, in recent animal tests to

ascertain whether an increase in viral growth was associated with the difference in CPE, no evidence of such growth was obtained. Furthermore, essentially no CPE was obtained following inoculation of the same material into either cell type. Efforts are being directed toward resolving this apparent inconsistency.

2. Sera from several germfree mice 1 year or more of age have been examined for evidence of the presence of certain mouse viruses. Numbers are too few for negatives to be conclusive, but thus far no positives for polyoma and mouse adenovirus have been obtained. Evidence for the presence of Reo 3 is questionable. However, there is good evidence that K virus may be present.

Significance to Bio-Medical Research and the Program of the Institute:

The study of viruses in animals without the possibility of influence of other concomitant infections, or with known infections, should provide special insight into virus-host relations. Also, with the increase in the number of viruses shown to occur in the conventional mouse, animals with a defined viral status and for which exposure to infection can be controlled become increasingly more valuable. This is particularly true in view of the current interest in tumor-virus studies.

Proposed Course of the Project:

Efforts will be directed toward clarifying the variation in the behavior of dengue virus in the germfree animal tissue culture. Also, further serological and tissue analysis of the viral state of the germfree mouse colony will continue.

Part B Included: No

Serial No. NIAID-112
1. Germfree Animal Research
2. Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Biology of germfree animals

Principal Investigator: Richard E. Horton

Other Investigators: Walter L. Newton
William B. DeWitt, Laboratory of Parasitic Diseases
N. A. Jaworski and John L. S. Hickey, Division of Research Services

Cooperating Units: DRS 6.4

Man Years (calendar year 1960):
Total: 2½
Professional: 1½
Other: 1

Project Description:

To obtain descriptive biological data on germfree animals for the purposes of determining in what manner they may differ from the conventional animal, and establishing baseline values for other studies employing these animals.

Methods Employed:

Comparative studies are made on germfree, specifically-contaminated, and conventional animals in such areas as hematology, blood chemistry, serology, gross and microscopic anatomy, growth curves, longevity, etc. Standard laboratory techniques are employed.

Major Findings:

In efforts to improve germfree guinea pigs by maintaining them on diets sterilized by irradiation instead of steam, some success was obtained with the use of irradiated semisynthetic diet. However, neither conventional nor germfree animals on irradiated diet grew as well as conventionals on non-irradiated diet. It was observed

that the growth rate of germfree animals reared on a semisynthetic diet sterilized by 2 million rad was greater than that of animals reared on the same diet sterilized by 3 million rad. Irradiation has an obvious detrimental effect on the nutrition adequacy of the diet.

Five pregnancies have been observed in adult germfree guinea pigs maintained on the irradiated ration. None of the females was able to carry the fetuses to term; they usually aborted near the 5th or 6th week of gestation. In most instances, procidentia of the uterus was a sequela that eventually led to the death of the animal. The administration of additional amounts of Vitamins K and E to the last two pregnant animals did not appear to alter the course of their pregnancies. This failure to reproduce has not been observed in the colony of conventional guinea pigs maintained on the same sterilized diet for four generations. Histological examination of several of the older germfree guinea pigs (approximately a year old) reared on the irradiated diet has revealed fatty degeneration of the liver.

Further study of the serum proteins in germfree and conventional mice has provided data similar to those reported earlier with germfree guinea pigs: Gamma globulin levels in the germfree were the same as those in mice from a conventional colony maintained on the same sterilized diet, but both groups showed values lower than those of mice from the NIH conventional colony which is maintained on a non-sterilized diet.

Data are being accumulated on the incidence of tumors (especially lung) in our germfree and conventional mice (the same genetic stock) dying or sacrificed after 6 months of age. Lung tumors have been found in the germfree mice, but there is some evidence that the incidence is lower than in their counterparts exposed to the outside environment. The possibility of this interesting difference will continue to be explored:

Significance to Bio-Medical Research and the Program of the Institute:

Studies of this nature provide baseline data for current and future experiments that utilize the germfree animal. Also, they provide an opportunity to study the role that the "normal" flora may play in establishing hematological and serological values and general growth, longevity, and fecundity.

Proposed Course of the Project:

Studies will continue along essentially the same lines. Where differences between the germfree and the conventional "contaminated" animals are encountered, efforts will be made to establish whether

fundamental principles are involved, and whether a particular difference is worth further exploitation. Also, attempts will be made (through the use of hormones, vitamins, etc.) to determine reasons for the germfree guinea pig's failure to reproduce satisfactorily.

Part B Included: Yes

PHS-NIH
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Part B. Honors, Awards, and Publications

Publications other than abstracts from this Project:

Horton, R. E. and Hickey, J. L. S.: Irradiated Diets for Rearing Germfree Guinea Pigs. Proc. of Animal Care Panel. (In press)

Newton, Walter L., Pennington, Robert M. and Lieberman, Jacob E.: Comparative Hemolytic Complement Activities of Germfree and Conventional Guinea Pig Serum. Proc. Soc. for Exper. Biol. and Med., 1960, Vol. 104, Pp. 486-488.

Baer, Paul N., and Newton, Walter L.: Studies on Periodontal Disease in the Mouse. III. The Germ-Free Mouse and Its Conventional Control. Oral Surg., Oral Med. and Oral Path., Vol. 13, No. 9, Pp 1134-1144, Sep. 1960.

Honors and Awards relating to this project:

Dr. Newton was invited to present a paper on the work on gamma globulin in germfree guinea pigs at a special session of the Fifth International Congress on Nutrition.

Serial No. NIAID-113
1. Germfree Animal Research
2. Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Origin of Anti-Human Blood Group B Agglutinins in White Leghorn Chicks

Principal Investigator: Richard E. Horton

Other Investigators: G. F. Springer, University of Pennsylvania

Cooperating Units: None

Man Years (calendar year 1960):

Total:	1
Professional:	1/4
Other:	3/4

Project Description:

Objectives:

To determine if the blood group B active polysaccharide of E. coli O_{86} B:7 is absorbed onto the erythrocytes of monocontaminated chicks harboring the organism in the intestinal tract.

Methods Employed:

Groups of germfree and conventional White Leghorn chicks are inoculated orally with E. coli 086 B:7 at selected ages. Techniques which increase permeability of bacterial endotoxin across the intestinal wall are employed: the induction of chemical enteritis by prolonged feeding of drastic cathartics; and the induction of tourniquet shock. Blood samples are taken shortly after the period of physical stress by cardiac puncture. Standard serological testing procedures are used to determine the presence of group B active polysaccharide on erythrocytes and the titer of anti B agglutinins in blood samples.

Major Findings:

In vitro studies have shown that erythrocytes of White Leghorn chicks are easily and irreversibly coated with blood group B active bacterial products. We have found that erythrocytes of the germfree

chicks used in our studies do not contain blood group B-like antigen. When healthy germfree chicks are infected with E. coli O_{86}, only a minority of the animals acquire B-like antigens on the red cells. When a series of large doses of a cathartic (cascara sagrada) is fed to the E. coli O_{86} infected chicks, the proportion of animals having antigen coated red cells is increased. Tests made on blood samples taken several hours after E. coli O_{86} infected chicks have been subjected to tourniquet shock show that almost all chicks possess varying amounts of antigen-coated erythrocytes. Two to three days after application of tourniquet shock, the anti-B titer of these animals was found to rise considerably, but there was no demonstrable decrease in coated erythrocytes.

Significance to Bio-Medical Research and the Program of the Institute:

Studies of this nature may help explain the mechanism by which human patients with severe intestinal disorders (e.g. cancer of the colon, incarcerated hernia, etc.) acquire a transitory B antigen on their erythrocytes and the altered polyagglutinability of those red cells which accompany this phenomenon.

Proposed Course of the Project:

In vivo studies will be conducted to determine extent of coating of the erythrocytes with B antigen when germfree chicks and chicks monocontaminated with E. coli O_{86} are given purified B-substance parenterally. When these results have been obtained, further work on this project will be terminated.

Part B Included: No

Serial No. NIAID-114
1. Germfree Animal Research
2. Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Studies on bacterial interactions and host bacteria relations in the germfree animal

Principal Investigator: Norman S. Ikari

Other Investigators: Walter L. Newton
Maurice Landy, National Cancer Institute

Cooperating Units: CDC, Atlanta, Georgia

Man Years (calendar year 1960):

Total:	1 1/4
Professional:	1/2
Other:	3/4

Project Description:

Objectives:

To determine whether certain bacterial phenomena observed in vitro, e.g. genetic recombination between two enteric strains of bacteria, occur within the intestine of the living mammalian host. To evaluate host responses to particular bacterial species without interference from, and in association with, other known organisms.

Methods Employed:

In vitro studies have shown that the ability to produce colicine, an antibiotic-like substance lethal to some enteric bacteria, can be genetically transferred from one bacterial strain to another. In our studies, E. coli K 12 Row (streptomycin-resistant, colicine-sensitive) is established in the germfree mouse intestinal tract by inoculation of the water bottles. Later, Paracolon CA62 (streptomycin-sensitive, colicine-positive (col+)) is fed by mouth tube, and fecal pellets are collected at intervals thereafter. Saline dilutions of these pellets are assayed for col+ colonies by plating onto streptomycin agar plates.

Sera of germfree and variously contaminated mice are compared for levels of antibody activity towards specific organisms using Ouchterlony and CF techniques.

Major Findings:

Col+, streptomycin-resistant hybrids resembling the E. coli K 12 parent were obtained at every sampling for periods ranging from 24 hours to one month after the Paracolon feeding. Further examination of the col+ colonies confirmed the stability of this transfer and revealed the possibility of an additional change(s) in these hybrids not previously shown in in vitro studies. Sharp dichotomy was noted between germfree and conventional animals with respect to staphylococcal antibody. No antigen-antibody lines were noted in the germfree serum diffused against Cowan I soluble antigens, whereas one or more lines were obtained with conventional mouse serum.

Significance to Bio-Medical Research and the Program of the Institute:

Studies on whether genetic recombination and other bacterial interactions observed in the test tube occur in natural ecological surroundings can provide information on ways in which a host may affect these phenomena. Also, since backgrounds of "normal" antibodies (e.g., to organisms like staphylococcus) that are often observed in conventional animals can complicate attempts at serologic typing and fluorescent antibody study, the potential value of the germfree animal for such studies is well worth exploring.

Proposed Course of the Project:

Other known in vitro genetic recombination systems will be attempted in the germfree animal. The finding of large numbers of very mucoid variants completely different from either parental type or the col+ hybrids has suggested possible studies in a different direction.

Thorough analysis of the germfree mouse for the presence of antibody or antibody-like reactivity to a variety of organisms, especially gram negatives, will continue.

Part B Included: No

Serial No. NIAID-115
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Behavior of parasitic protozoa in germfree hosts

Principal Investigator: Walter L. Newton

Other Investigators: Bruce P. Phillips
Lucy V. Reardon, Laboratory of Parasitic Diseases

Cooperating Units: NIAID-127-C

Man Years (calendar year 1960):
Total: 1¼
Professional: ½
Other: 3/4

Project Description:

Objectives:

To determine whether the presence of bacteria affect the course of, and response of the host to, protozoan infections other than those caused by E. histolytica.

Methods Employed:

Germfree, monocontaminated, and conventional guinea pigs are inoculated with axenically-reared or micro-isolated species of Trichomonas, Trypanosoma, or Giardia. The inoculated animals are examined for the presence and type of lesions, time of death, etc.

Major Findings:

It has been shown previously that T. vaginalis, when injected subcutaneously, soon disappears in conventional guinea pigs, but multiplies and produces a severe lesion in germfree animals. However, when the germfree animal is orally contaminated with even a single species of bacteria, its response is like the conventional animal. In recent studies, the infection has been followed in the germfree animals until subsidence -- often requiring several weeks. When such animals are later re-exposed to the parasite, the pattern has been like that in the conventional animal. The encounter with the infection, even

though it eventually disappeared (and the animal became "germfree" again), seemed to activate a defensive response which persisted.

Techniques to inoculate germfree animals with sterile Giardia cysts have been worked out. Preliminary attempts at infection have not been successful, although the numbers of organisms inoculated have been small, thus far.

Significance to Bio-Medical Research and the Program of the Institute:

Studies of this type can lead to a better understanding of the possible effects of the "normal flora" in maintaining a host's natural defensive mechanisms. It would appear that this system (involving a host and an organism to which it is resistant in the conventional but not the germfree state) provides a good tool for such studies. Also, the role of the intestinal flora in the pathogenesis of a variety of protozoal diseases has yet to be established.

Proposed Course of the Project:

Additional experiments involving the "conditioning" of germfree animals with non-living stimuli such as dead bacteria, egg albumen, etc., prior to challenging with T. vaginalis are planned. A comparative study of the tissue responses in the conditioned and unconditioned germfree animal is planned. Attempts at establishing other parasitic protozoa in germfree animals will continue.

Part B Included: No

Serial No. NIAID-116
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Studies on helminthic infections in germfree hosts

Principal Investigator: Walter L. Newton

Other Investigators: Paul P. Weinstein, Laboratory of Parasitic Diseases

Cooperating Units: NIAID-121-G

Man Years (calendar year 1960):

Total:	1½
Professional:	½
Other:	1

Project Description:

Objectives:

To ascertain what effect the bacteria and other organisms normally present in the conventional animal have upon the course of helminth infections, and upon the host's response to these parasites.

Methods Employed:

Germfree animals are fed or inoculated with sterilized eggs, or axenically-reared infective larvae, of various parasitic helminths. The development of these parasites is followed by established procedures, and lungs, intestine, and other appropriate tissues are examined histologically for the study of host response. The findings are compared with those obtained in conventional controls.

Major Findings:

Mature, fertile adult worms were obtained in germfree as well as conventional mice, inoculated with infective larvae of Nematospiroides dubius. This indicated that the lack of bacteria appeared to have little or no effect on the development of the parasite from the infective larva to the adult stage in the host.

However, development of the parasite from the egg to infective larva in feces from germfree mice was extremely poor. Fatty degenera-

tion not unlike that associated with B-vitamin deficiency occurred. If living bacteria from a conventional animal were added to the germfree feces, development of the larvae progressed normally. Apparently, feces without bacteria fail to provide certain essentials for development of the larvae.

Of special interest was the apparent indication that the sex of the host, which is a factor in parasitism in the conventional animal, was unimportant among the germfree animals. In the conventional animals, males had 2-3 times the worm burden at necropsy that the females had. Among the germfree animals, however, average worm counts were about the same in both sexes.

Significance to Bio-Medical Research and the Program of the Institute:

The fact that bacteria, per se, are apparently not required for full development of some parasites opens the way for other studies in the areas of nutrition and parasitism, chemotherapy, in vitro cultivation, and physiologic studies. Also, opportunity is provided for the study of eggs and larvae in fresh (non-sterilized) feces without bacteria, and perhaps to ascertain factors contributed by the latter toward their proper development. If the sex phenomenon holds up in future tests, interesting and potentially important relationships may be uncovered.

Proposed Course of the Project:

Of course, the validity and significance of the difference in the host sex effect between germfree and conventional mice will be studied. Efforts to complete the entire life cycle of the parasite in a sterile environment will be continued. Attempts may be made to ascertain the role of bacteria in certain parasite-diet relationships noted in conventional mice.

Part B Included: No

LABORATORY OF PARASITIC DISEASES

Laboratory of Parasitic Diseases
National Institute of Allergy and Infectious Diseases

Summary statement of research activities, calendar year 1960

Program development and trends:

Two of the regular staff members, Dr. Jacobs and Dr. Weinbach, have been on research assignment outside the country for more than half of the year. On the other hand, we have had two persons on assignment to the Laboratory from other countries, Mr. Ian Sommerville from the McMaster Laboratory, Sydney, Australia; and Dr. Iain R. Bowman recently from the University of Aberdeen, Scotland. Mr. Sommerville has been working with Dr. Weinstein on problems related to sterile culture of worms, while Dr. Bowman has been conducting biochemical studies with Dr. von Brand. In July Dr. Allen W. Cheever was assigned to the Laboratory as a Research Associate. He has been placed in the Section on Helminthic Diseases and is conducting research on schistosome pathology, and on fundamental problems related to immunity and strain differences in susceptibility to the liver tapeworm of small rodents, with Dr. Olivier.

The Laboratory continues to emphasize fundamental studies on parasites and parasitic diseases. No important changes in the program were instituted during the year. The program of the laboratory is well diversified, considering the size of the staff, and the competencies of the various staff members cover a large proportion of the field of parasitology.

Although the emphasis in on basic studies, this does not imply a narrow viewpoint and the Laboratory is well aware of the many practical problems parasitic diseases create throughout the world. The Laboratory is often called upon for help and advice concerning prevention and control of parasitic infections and so must maintain competence, and a reputation for competence, to deal not only with basic problems of parasitism but also problems of prevention and control of parasitic diseases. Therefore, the Laboratory continues to carry on a variety of activities which help it maintain its international reputation and increase its capacity to cope with problems of parasitism. Such activity also returns benefits in the form of ideas for laboratory research and clues which may explain puzzling laboratory findings.

The Laboratory is prepared to carry out field studies when these are logical and reasonable extensions or applications of laboratory work. The project in Puerto Rico on the relation of nutrition to schistosomiasis is a case in point. Also, during the year steps were taken to extend laboratory findings and interests into foreign laboratories and into foreign field situations through the use of Public Law 480 funds in various countries. A series of project proposals has been made for work in Israel, Poland, Yugoslavia, India, and Brazil. Negotiations for two PL 480 projects in Brazil are actively under way and it is expected that both will be started in 1961.

The PL 480 projects should return substantial dividends, not only in new data which could not be obtained in the Bethesda Laboratory, but also invaluable experience and sophistication for the staff members involved. The projects are designed so as to cause minimal interference with essential laboratory activities. If the Laboratory is to consider more than a very small number of PL 480 projects it would have to add personnel to staff them and would probably need additional funds to cover incidental expenses.

The Laboratory has continued to maintain liaison with international agencies interested in health problems. Two of the staff (Olivier and Berry) have been chosen to serve on the WHO Expert Panel on Parasitic Diseases. These same staff members were loaned for short periods to WHO as consultants on schistosomiasis research and control activities. Four staff members (Berry, von Brand, Olivier, Weinstein) made important contributions to international symposia or conferences dealing with research on human disease.

The development of cooperative clinical studies with the Laboratory of Clinical Investigation has been disappointing. Relatively little significant cooperative clinical work was done during the year. This did not result from lack of desire on the part of either the Laboratory of Clinical Investigations or the Laboratory of Parasitic Diseases since as excellent rapport has been maintained, but rather because of problems inherent in procurement of useful patients and also, to some extent, to failure to form a productive "team". It is hoped that progress along this line can be made in 1961.

Scientific advances - made in 1960:

The Laboratory has produced a number of noteworthy advances in knowledge during the year. Many of these can be classified as being additions to "basic" knowledge but some have "practical" implications. Selection of items for emphasis is sometimes presumptuous and always risky since the importance of an individual item is hard to judge and often the "small" contribution may, in the long run, turn out to be "large". Nevertheless, attention is called to the following:

The studies on toxoplasmosis in New Zealand sheep (127-A) have shown that the prevalence is high. Considerable new information has been obtained concerning the distribution of the organisms in the tissues and their persistence there. After inoculation the distribution of the parasite in tissues is erratic and the parasites rapidly clear from tissues other than the muscle and placenta. Since residual infection occurs in muscle, mutton may serve as a source of human infection. Since congenital infection with Toxoplasma is an important medical problem, it is of special interest that the sheep studies have indicated that inoculation of sheep 60 days before pregnancy did not result in congenital infection or abortion but inoculation at 30 days pregnancy caused abortion or foetal death with absorption. Infection at 90 days pregnancy was less likely to be dangerous to the foetus.

The status of resistance or immunity to Toxoplasma continues to be puzzling, since living organisms fail to completely protect animals against

challenge, especially when the challenge is great, and because low grade parasitemia may persist for months in mice and rabbits in the presence of high serum antibody levels. The observation that cysts of Toxoplasma probably form in tissue cultures provides a new opportunity to study the manner of cyst formation and the factors that lead to cyst formation (127-A).

The work on the preservation of living Entamoeba histolytica and other protozoa (127-B) has practical significance since success would permit retention of strains without continuous sub-culturing. This is a relatively new field and techniques are still evolving. The work so far has shown that this approach is feasible since four species have been frozen and stored for periods ranging from one to four months depending on the species involved. Entamoeba histolytica has been kept at -197° C for 24 hours, suggesting that almost indefinite storage at this temperature may eventually be achieved.

Laboratory culture of Entamoeba histolytica continues to receive attention since it is so important to learn more concerning its nutritional requirements and its pathogenicity in the absence of other organisms. It is noteworthy that satistactory axenic culture of this species has been achieved for the first time (127-B). The protozoa are cultured in a complex diphasic medium containing no cells but including chick embryo extract. This is a long and very important step forward.

The substitution of a species of Crithidia for Trypanosoma cruzi in cultures of E. histolytica provides a more economical and rapid way of producing large cultures of the amoeba. Demonstration of the value of the Coulter Counter for the enumeration of protozoa in suspension adds a valuable tool for quantitative work and suggests this method may be applicable for counting other organisms of similar size such as tissue culture cells (127-B).

The use of germ free animals in worm-parasite studies continues to reveal the value of this tool and adds to our knowledge of the peculiar nature of the germ free state. The technique seems to be particularly useful for studying conditions that influence natural resistance and nutritional relationships of parasite and host. For example, it was found (121-G) that the roundworm, Nematospiroides dubius, develops as well in germ free as in conventional mice but while in conventional mice the worm recovery is much higher from the male animals, the recovery from germ free mice is the same for both host sexes. The cause of the difference is unknown. Also, it has been shown that the feces of germ free mice do not support development of N. dubius larvae and that bacteria in the feces provide important factors for larval development (121-G). There was further evidence that the alteration in levels of serum protein components in germ free animals is due to dietary factors (121-G).

Studies on the sterile culture of worms continues to produce fundamental information on the nutritional requirements of the parasites and brings closer the day when we can use the axenic animals for immunologic and therapeutic studies. Survival studies using relatively advanced larvae of Nippostrongylus muris has produced important results (121-E). The intent has been to try, by addition of elements to the medium, to induce the larvae to reach the adult stage. Starting with a salt mixture, dextrose was added until

the optimal level was reached. Then casein was added and survival time rose to 11 days, but there was not development of the larvae. Addition of a yeast extract to this mixture not only increased survival but permitted growth to the adult stage. Thus, a much more simple medium than used before has been evolved and the achievement of a defined medium for culture of N. muris adults is much closer. A similar approach is being used in attempts to culture microfilariae of Dirofilaria immitis (121-E).

Although the study of the relation of nutrition to schistosomiasis in Puerto Rico is still incomplete, it appears that enrichment of the diet does not affect the number of eggs passed in the feces. However, it is interesting to note that the enriched diet did cause a loss of hookworms and whipworms from the intestine (121-L). This has a bearing on the problem of the existence of hookworm infection without hookworm disease. In laboratory studies conducted in Bethesda the enhanced efficacy of stibophen in mice receiving a semi-synthetic diet was shown to be due to the absence from this diet of as yet unknown inorganic salts (121-K). Higher blood levels of the drug were maintained longer when the semi-synthetic diet was used and this may explain the greater efficacy. Demonstration of the influence of simple salts on the efficacy of stibophen suggests that other drugs may be similarly affected by diet. If the work with the stibophen-salt problem progresses satisfactorily it is hoped that a test of the effect of human diet on the action of the same drug may be tried in Puerto Rico before the study there is concluded.

Interaction of two pathogenic organisms in the same host has had relatively little attention in spite of some very provocative work done in years past. A study of simultaneous infection with encephalomyocarditis virus and Trichinella spiralis in rats has produced striking and significant results (121-A). While the virus alone does not injure adult white rats when given intraperitoneally, in the presence of Trichinella spiralis infection many of the rats are crippled and die. This potentiation of the virus pathogenicity is not due to non-specific stress but seems to be related to the presence of the worms on the muscles. The virus can be recovered from the muscle of T. spiralis-infected rats but not from muscle of rats without T. spiralis. The reason for the influence of the worm infection on the activity of the virus is unknown. The phenomenon offers an opportunity to study some of the fundamental factors in the pathogenesis of both the virus and the worm parasite. It also provokes the question as to what effect this worm infection may have on other virus infections.

Continued study of the hepato-splenic syndrome in mice infected with Schistosoma mansoni has added new evidence to show that, in mice at least, the schistosome eggs are the prime cause of liver damage and therefore the chief cause of fibrosis, portal hypertension, and collateral circulation which are so often the cause of morbidity and mortality in human schistosome infection (121-I). Diet, dead worms, and toxins produced by the worms seem to be less important in contributing to liver damage related to schistosome infection.

The study of liver damage in relation to ammonia toxicity in mice has revealed that low oxygen in breathed air greatly enhances ammonia toxicity (121-M). The mechanism of this effect is not clear. Though hepatic coma is usually considered to be related to ammonia toxicity none of the substances which exacerbate hepatic coma in man increases ammonia toxicity in mice. In fact, 6 of 10 decrease it. Ammonia toxicity in mice was greatly reduced by hypothermia and this suggests that the same measure may be useful in treating hepatic coma in man. Finally, mouse liver damage was induced in eight different ways but none caused any change in the animal's response to intravenous ammonia. Thus, though high blood ammonia levels seem to be related to liver damage, the causal relationships are by no means clear.

Fundamental physiological studies have focused on the calcareous corpuscles of tapeworms and on the phospholipids of tapeworms (123-A). The calcareous corpuscles are amorphous but, on heating, dolomite, brucite or apatite may be formed. Electron microscope pictures of corpuscles heated with KOH reveal the presence of well-formed crystals. The glycerol containing phospholipids of Taenia taeniaeformis are about half lecithid and half cephalin. Sphingomyelin is present and more than one cephalin is known to occur in the larvae of this tapeworm. Hexose-containing phospholipids occur in both larvae and adults.

Study of the mechanism of energy metabolism of sub-cellular elements has dealt, among other things, with the mechanism by which mitochondria which are depleted of high-energy phosphate intermediates are stimulated to oxidize substrates when ATP is added. This is a complex, though fundamental, bioenergetic system for which a better understanding is needed. Addition of ATP not only restored succinate oxidation but also caused reduction of intra-mitochondrial DPN. The succinate oxidation involves an energy-requiring reaction and this energy is apparently added at one site in the respiratory chain and used at another for reducing pyridine nucleotide (127-D).

Serial No. NIAID-120
1. Parasitic Diseases
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Administration and research planning and coordination.

Principal Investigator: Leon Jacobs

Other Investigators: Louis J. Olivier

Cooperating Units: None

Man Years (calendar year 1960):

Total:	2¼
Professional:	¼
Other:	2

Project Description:

This project furnishes supervisory and administrative services to all research projects in the Laboratory, as folows:

Over-all evaluation of research plans and initiation of field projects; integration of laboratory research activities with clinical studies of the Clinical Center; editing scientific and technical reports and manuscripts; preparation of reports, budget estimates, and exhibits; supervision over personnel, travel, correspondence, and maintenance; requisitioning and supervision of equipment and supplies; maintenance of reference library, etc.; and consultatory services to individuals, academic and other organizations, and liaison activities with other branches of the Service and the Federal Government.

Research projects are reviewed and, in consultation with Section heads, changes in research program are considered and initiated. The emphasis is placed on fundamental aspects of medical parasitology; extensions of projects into the fields of clinical or practical preventive medicine are attempted only when the laboratory work has progressed to a point where a sound basis exists.

Serial No. NIAID-121-A
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Host parasite relations in worm infections in laboratory animals.

Principal Investigator: Louis J. Olivier

Other Investigators: Allen W. Cheever

Cooperating Units: Laboratory of Viral Products, DBS

Man Years (calendar year 1960):

Total:	2½
Professional	1¼
Other:	1¼

Project Description:

Objectives:

To study the characteristics of infection in laboratory animals; to learn how resistance to infection may be enhanced and reduced; to learn how the host defense mechanisms act to resist infections; to study variations in resistance of host strains; to study the mechanism of natural resistance to parasites; to study how two simultaneous infections may affect each other.

Methods Employed:

Infect animals in such a way as to produce predictable infections; treat with hormones and antigens and so forth; challenge with infective worms or other agents; observe the host and parasite; and determine the degree of resistance to infection, the host reaction, and the effect of one agent on another.

Major Findings:

Encephalomyocarditis virus is pathogenic to very young rats, but is not ordinarily pathogenic to older ones. However, this virus is highly pathogenic in older rats if the latter harbor new infections with Trichinella spiralis. The rats are crippled by the infection and many die. Moreover, the virus is recoverable from trichinous muscle in greater quantity than from non-trichinous muscle. The enhanced patho-

genicity is not due to non-specific stress since other stresses, such as chilling, fighting, and so forth, do not give this result. This potentiation of virus pathogenicity by T. spiralis is very striking, but not well understood as yet. Schistosome infection does not potentiate the virus.

Significance to the Program of the Institute:

Problems of resistance and immunity are basic to understanding of host parasite relationships and pathogenicity of parasites. Natural resistance is an elusive phenomenon, but worm infections provide a useful and attractive system for its study since infective doses, antigenic mass, and lesion size can be controlled and measured quite accurately. Some parasites do not multiply in the host and are not attacked by phagocytes. These facts simplify the study of the effects of hormones and other agents on infections.

The revelation of the potentiation of EMC by T. spiralis may provide a wonderful opportunity to study the pathways of host damage by both the virus and the worms.

Proposed Course of the Project:

To test whether mice develop acquired immunity to the liver parasite of rats; to study paths of transfer of immunity of rats; to study further the affect of hormones on this resistance.

To study natural resistance to Cysticercus fasciolaris in mice and other rodents.

To follow the EMC-T. spiralis relationship and to study other possible virus worm associations. To determine the role of the reticuloendothelium system of the liver in resistance through the use of reticuloendothelial system stimulating and depressing agents. To determine whether immunologic tolerance to Cysticercus fasciolaris can be induced in rodents.

Part B included No

Serial No. NIAID-121-B
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Role of helminths in the causation of cancer

Principal Investigators: Elmer G. Berry and Louis J. Olivier

Other Investigators: None

Cooperating Units: Pathologic Anatomy Branch, National Cancer Institute, NCI—525
Laboratory of Viral Products, DBS

Man Years (calendar year 1960)

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

To test whether worm parasites can induce malignant growths in their hosts. To test whether Schistosoma haematobium infections in hamsters cause tumors of the digestive or urinary tract. To test whether the malignant tumors produced in rat liver by larval tapeworms (Taenia taeniaeformis) are virus-induced. To test whether cats, the host of the adult tapeworm, have a virus which can be related to these tumors.

Methods Employed:

Schistosoma haematobium. Hamsters are infected with S. haematobium. The animals are allowed to live as long as possible to determine whether the schistosome infection induces malignant growths.

Taenia taeniaeformis. Rats are infected and the liver cysts are ground and injected into rats and other laboratory animals. Material from cats and rats is studied by virological and immunological methods.

Major Findings:

Rat virus has been isolated from two rats having the liver sarcomas.

Significance to the Program of the Institute:

Cancer of the bladder appears to be much higher in Egypt and Mozambique than it is in other areas. Although this has been attributed to the particular strain of S. haematobium which occurs in these countries the actual cause for these differences is not known. It is hoped that this study might help to solve the problem.

The problem of virus etiology of cancer is at the center of concern in the cancer field.

Proposed Course of the Project:

Termination of the S. haematobium study when the last infected animals die.

Continued study of the rat tumor project and possible addition of similar projects.

Part B included No

Serial No. NIAID-121-C
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Destruction of molluscs by chemical means.

Principal Investigator: Louis J. Olivier

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

To discover more effective means for killing snail vectors of schistosomiasis. To study the mode of action of chemicals used as molluscicides. To develop more effective means for study of snail poisons in the laboratory.

Methods Employed:

Snail vectors, or their eggs, are exposed to chemicals and the destructive efficacy of the chemicals is recorded. The optimal conditions for use of chemicals against snails are sought. By quantitative methods the optimal duration of exposure, concentration, temperature, etc. are determined.

Major Findings:

Using standardized methods the sodium pentachlorophenate LD50 was determined for eggs and adults of Australorbis glabratus. Eggs are more susceptible to the chemical than adults by a chemical concentration factor of about 5. Strain differences in susceptibility were not great. Very small differences were found between young and old post-enbryonic snails.

Following failure of NaPCP to kill snails as predicted in some field situations it was shown that ultra-violet light distroys the compound rapidly and that this could explain some of the field failures. Quantitative laboratory data showed the half-life of NaPCP solutions in sunlight may be less then an hour whereas the duration of an effective field concentration probably has to be 8 or more hours.

Significance to the Program of the Institute:

For the present, at least, the best means to control schistosomiasis is interruption of transmission from man to man by destruction of the snail intermediate hosts. The molluscicides now available are not ideal. The project attempts to find better and more economical ways to use the available molluscicides. The evidence from the work done so far suggests the possibility that more efficient use may be made of molluscicides if they are directed against the snail eggs than against the adults. The project puts the Institute in a better position to take part in measures directed against the schistosomes which are rapidly coming to prominence as dangerous parasites of man.

Proposed Course of the Project:

To continue with efforts to standardize the laboratory method for study of molluscicides with the aim of perfecting a method for highly analytical quantitative work with snail poisons and also with the aim of providing laboratories interested in chemical screening with a better tool. To investigate the action of chemicals already studied upon other species of vector snails. To try, under the same conditions, other molluscicides of high promise so that there may be accurate and quantitative data by which molluscicides can be compared. To devise a method for screening for compounds which may be active but insoluble.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Olivier, L. and Haskins W. T. The effects of low concentrations of sodium pentachlorophenate on the fecundity and egg viability of Australorbis glabratus. Amer. Jour. Trop. Med. Hyg. 9; 199-205 (1960)

Olivier, L. Factors affecting the survival of aestivating pulmonate vectors of schistosomiasis. Anniversary Volume for Dr. Caballero y Caballero. pp. 215-225 (1960)

Hiatt, C. W., Haskins, W. T., and Olivier, L. The action of sunlight on sodium pentachlorophenate. Amer. Jour. Trop. Med. Hyg. 9; 527-531. (1960)

Awards: None

Honors: Dr. Louis J. Olivier served in Geneva as Consultant to the WHO Section on Endemic Diseases from 18 September to 10 October 1960. During this time he took part in a meeting of the WHO Expert Comittee on Bilhorziasis which met from 26 September to 10 October 1960.

Dr. Louis J. Olivier was invited to present one of the papers in the Fourth Conference of the Industrial Council for Tropical Health which met on July 20 - 22 in the Harvard School of Public Health.

Under the sponsorship of WHO Dr. Louis J. Olivier visited three laboratories studying chemicals useful as animal toxins in water in order to gather information and increase exchange of information and ideas among those interested in the subject.

Dr. Louis J. Olivier has been chosen to serve on the WHO Expert Advisory Panel on Parasitic Diseases.

Serial No. NIAID-121-D
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Investigation of intermediate hosts and vectors of human diseases caused by worms.

Principal Investigator: Elmer G. Berry

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total:	1 3/4
Professional:	3/4
Other:	1

Project Description:

Objectives:

To investigate the distribution, life history, and ecology of snail intermediate hosts and to develop a rational and useful system of classification and means of identification.

Methods Employed:

Colonies of snails which serve as intermediate hosts of schistosomes are maintained in the laboratory where they are available for intensive study. Laboratory-reared specimens are exposed to miracidia to evaluate the susceptibility and to determine whether strain differences are present.

Major Findings:

Additional specimens belonging to the genera Bulinus and Biomphalaria were collected from the Belgian Congo, Mozambique, Kenya, and the Transvaal. Colonies of these species are now established in the laboratory. Continued progress has been made toward clarification of taxonomic problems.

Significance to the Program of the Institute:

This knowledge is essential in understanding the epidemiology of bilharziasis and its control.

Proposed Course of the Project:

Continuance of the anatomical studies of the snails which serve as intermediate hosts and to compile this information into a manual.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project: None

Awards: None

Honors: Dr. Elmer G. Berry served as a Consultant and Leader of Discussion on Snail Control by Chemical Means at the African Symposium on Bilharziasis which met in Lourenco Marques, Mozambique, 30 March to 8 April 1960.

Dr. Elmer G. Berry served as Consultant on bilharziasis research at the Expert Committee on Bilharziasis (Molluscicides) which met in Geneva, Switzerland 26 September to 1 October 1960.

Dr. Elmer G. Berry was appointed to serve on the World Health Organization Expert Advisory Panel on Parasitic Diseases for a period of five years, beginning June 1, 1960.

Serial No. NIAID-121-E
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Development of methods for the cultivation of parasitic helminths in vitro, and the determination of the nutritional requirements of such organisms in vitro.

Principal Investigator: Paul P. Weinstein

Other Investigators: I. R. Sommerville (visiting scientist)
T. K. Sawyer

Cooperating Units: None

Man Years (calendar year 1960):

Total:	4
Professional:	2 1/4
Other:	1 3/4

Project Description:

Objectives:

To develop media and physical conditions suitable for the in vitro cultivation of helminths throughout their life cycle, and to obtain information on their specific nutritional requirements.

Methods Employed:

Worm eggs, infective larvae, or partially matured worms are isolated from charcoal cultures, rodent hosts, or mosquitoes, depending on the helminths involved. These are inoculated into culture media to be tested for growth-promoting properties, and are observed for development and differentiation.

Major Findings:

1. Cultivation of trichostrongylids. Survival of fourth stage larvae of Nippostrongylus muris was studied in salt solutions to which nutrients were added. In a modified Krebs-Ringer salt solution, survival was limited to 3 or 4 days, but the addition of dextrose increased the survival time to 6-8 days. The optimal concentration of dextrose was 0.014 M. Change in phosphate concentration was without effect. When

casein was added to the solution of salts and dextrose, survival time increased to 11 days, but no developmental changes were observed. Replacement of casein with either enzymic or acid hydrolysates of casein gave inferior results, and acid hydrolysates appeared to be toxic. The addition of a water-soluble extract of yeast to a solution containing casein, dextrose and salts not only enhanced survival but yielded adult worms. Better survival, but no development was obtained when a mixture of L-amino acids in the proportions in which they occur in casein was used in place of casein.

Although N. muris does not mate when grown in a complex medium composed of chick embryo extract, vitamin mixture and serum, it was found that the third, fourth, and early fifth stages, if taken from such cultures and put directly into the small intestine of rats will mate and produce viable eggs.

The closely related nematode, Nematospiroides dubius, was placed in the same medium which was not conducive to the mating of N. muris. Under these conditions, N. dubius was observed to mate. This makes more likely the possibility that a complete generation of a parasitic nematode may be obtained in vitro.

2. Cultivation of Dirofilaria immitis microfilariae. Survival in balanced salt solutions alone is very short. Addition of dextrose increased survival time about fourfold. There is a direct relation between concentrations of magnesium and dextrose and survival time. With sodium to potassium ratios near that of mammalian blood (29:1) or close to that of some insect fluids (5:1 or less), survival is good, whereas at intermediate ratios (20:1, 10:1) it is relatively poor.

3. Nematode coelomocytes and vitamin B_{12}. This vitamin added to a defined medium resulted in the development of a deep rose-pink pigment in the coelomocytes of the dog hookworm Ancylostoma caninum, similar to what was previously reported for N. muris. It is suspected that this pigment represents a specific concentration of B_{12}.

Significance to the Program of the Institute:

The growth in vitro of parasitic helminths will provide an important tool with which to study the physiology and metabolism of these organisms, and should facilitate the isolation and preparation of metabolic antigens involved in the development of functional immunity.

Proposed Course of the Project:

Work will continue on the specific nutritional requirements of N. muris and N. dubius in vitro.

The study of the interaction of B_{12} and coelomocytes will be extended to other nematodes.

Dirofilaria uniformis infections in rabbits have now been established in the laboratory, and coupled with D. immitis from dogs, studies will continue on the growth requirments of filariids in vitro.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Weinstein, Paul P.: Excretory mechanisms and excretory products of nematodes: An appraisal. In "Host Influence on parasite physiology." Rutgers University Press: 65-92. (1960).

Weinstein, Paul P.: The current status of the axenic cultivation of helminths. (In press).

Honors: Dr. Weinstein was invited to participate in the Annual Conference on Protein Metabolism entitled, "Host Influence on Parasite Physiology," sponsored by Rutgers University, January 1960.

Dr. Weinstein was invited to present the "Annual Address" to the Annual Meeting of the Midwestern Conference of Parasitologists held in conjunction with the International Symposium on Growth at Purdue University to commemorate the dedication of the Life Sciences Building, June 1960.

Dr. Weinstein was invited by the First Pan-American Congress on Biology and Experimental Pathology to participate in a symposium entitled, "Parasitic Biodynamics," held in Caracas, Venezuela, September 1960.

Awards: None

Serial No. NIAID-121-G
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The development of helminths in germfree animals.

Principal Investigator: Paul P. Weinstein

Other Investigators: I. R. Sommerville, (visiting scientist), LPD
Thomas K. Sawyer, LPD

Cooperating Units: Laboratory of Germfree Animal Research, NIAID Serial No. NIAID-116

Man years (calendar year 1960):
Total: ½
Professional: ¼
Other: ¼

Project Description:

Objectives:

To establish helminth infections in germfree animals so that problems involving host-parasite relations, nutrition, immune response, and pathology can be studied in the absence of other complicating microorganisms.

Methods Employed:

Larvae are reared to the infective stage under axenic conditions, and eggs and larvae are also isolated from natural sources and rendered axenic; these are used to infect germfree animals.

Major Findings:

Axenically reared Nematospiroides dubius larvae will consistently mature in germfree mice. Total worm recoveries from germfree animals have been equivalent to those from conventional ones. While in conventional animals there is a considerable higher worm recovery from male than from female mice, in germfree mice worm recovery was essentially the same from both sexes. Apparently, diet does not affect this difference.

N. dubius larvae which hatched from eggs passed in axenic feces did not develop normally in such feces maintained axenically. When feces from

conventional animals containing bacteria was added to such cultures, rapid, normal larval development to the infective stage occurred, indicating that bacteria in feces provide important components for larval development.

Significance to the Program of the Institute.

These experiments demonstrate the feasibility of using the axenic helminth parasite and host in studying factors which concern the host parasite relationship. This technique has value particularly where intestinal parasites are concerned in studying conditions which influence natural resistance, host specificity, and nutritional relationships of parasite and host.

Proposed Course of the Project:

Further work will be done to compare the susceptibility to infection of male and female conventional and germfree mice with a helminth parasite. It is also planned to attempt to establish the infection on a continuing basis in the germfree environment through successive generations of host and parasite, and to follow the course of the infection under such conditions. Nutritional factors relating to infection are to be studied. It is also planned to use worms developing in axenic hosts as inocula for cultures for *in vitro* study under axenic conditions.

Part B included No

Serial No. NIAID-121-I
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Pathological physiology of worm infections

Principal Investigators: Kenneth S Warren and Allen W. Cheever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1¼
Professional: 3/4
Other: ½

Project Description:

Objectives:

To study the dynamic relationships existing between worms and their hosts. Specifically to study the syndrome in mice which resembles human hepato-splenic schistosomiasis mansoni and learn its causes; to use this syndrome as a tool for the study of liver diseases; to study the pathogenesis of schistosome infection, using mice as tools.

Methods Employed:

Use unisexual and bisexual infections to determine the cause of schistosome liver disease; evaluation by use of portal pressure determinations, study of tissues, surgical procedures, nutritional changes, liver function tests, and physiological studies. Study vascular damage in liver by injection of colloidal carbon or carmine. Study importance of eggs, dead worms, etc. as factors in schistosome liver damage.

Major Findings:

In mice hepato-splenic schistosomiasis occurs in the presence of good nutrition and is not exacerbated by poor nutrition; unisexual infections do not cause typical hepato-splenic schistosomiasis, but in male infections there is a moderate, transient splenomegaly and an increase in portal pressure. The production of eggs by the worms appears

to be necessary before hepato-splenic schistosomiasis develops. There is no lung shift of worms following the use of high doses of Fuadin. There is some evidence that the liver recovers rapidly from damage due to dead worms.

Significance to the Program of the Institute:

Much controversy exists concerning the relative roles played by ova, toxins, dead worms, and immunity in the production of schistosoma liver disease. The dispute is of practical importance since those who believe dead worms to be the chief pathogenic agent do not treat infected patients. In general, pathogenesis of worm parasites needs more attention. Its study should return fundamental information of broad usefulness.

Proposed Course of the Project:

To continue to study the relations of schistosomes to their host by studying the role of male schistosomes in the cause of liver damage; by injecting schistosome eggs into the portal system in order to observe the damage they cause alone; by attempting to produce immunologic tolerance; by studying the role of immunity in host damage; by testing further the role of dead worms in causing liver damage; and other similar approaches to the problem.

It is also intended to study the pathogenesis of tapeworm infections in rat liver.

Part B included No

Serial No. NIAID-121-J
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Serum protein studies on germfree animals infected with various parasites.

Principal Investigator: William B. De Witt

Other Investigators: None

Cooperating Units: Laboratory of Germfree Animal Research, NIAID-112.

Man Years(calendar year 1960):

Total:	1/4
Professional:	0
Other:	1/4

Project Description:

Objectives:

To determine the effects of a germfree environment on the relative distribution of various protein components of germfree guinea pigs, mice, and other small laboratory animals. This work is being done to obtain base line values in preparation for contemplated serologic studies on the pathogenesis of parasitic infections in germfree hosts.

Methods Employed:

Serum from animals maintained in germfree tanks on a sterilized ration is compared with that obtained from animals housed in conventional cages and given the same ration. A third group of animals, housed in conventional cages and fed a commercial pellet diet is also studied. Paper electrophoretic studies are carried out according to standard techniques.

Major Findings:

This project has been relatively inactive during the past year. However, further confirmatory evidence was obtained, indicating that previously observed alterations in levels of serum protein components in the germfree mouse was due mainly to dietary factors rather than to the germfree state.

Significance to the Program of the Institute:

Before the effects of parasitic infections on the relative distribution of serum protein components of the germfree animal can be studied, it is first necessary to establish baseline values in the axenically reared animal. It is hoped that serum protein studies on animals experiencing for the first time the invasion and development of parasites will shed light on the role played in the defense of the host by circulating antibodies associated with the various globulin components.

Proposed Course of the Project:

The present study is to be extended to determine the effect of age and various nutritional factors on the relative distribution of the serum protein components. Later the response to infection with a variety of parasites will be measured.

Part B included No

Serial No. NIAID-121-K
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Effects of nutrition on chemotherapy of parasitic diseases

Principal Investigator: William B. DeWitt

Other Investigators: None

Cooperating Units: Laboratory of Parasite Chemotherapy, NIAID-131-D.

Man Years (calendar year 1960):

Total	1 3/4
Professional:	1/2
Other	1 1/4

Project Description:

Objectives:

To determine whether the nutrition of the host can influence the efficacy of parasite chemotherapy.

Methods Employed:

Animals maintained on defined diets either enriched or deficient in certain components are exposed to a single species of parasite and the developing infection is treated either in the prepatent or patent periods. The influence of the diet on treatment is determined by (1) the reduction of the number of parasites, (2) condition of the parasites, (3) growth and survival of the host, and (4) pathologic conditions developing in the host.

Major Findings:

The efficacy of stibophen (Fuadin) therapy on mature Schistosoma mansoni infections in mice was increased up to 16 times by feeding a balanced semi-synthetic diet. The toxicity of the drug was not similarly increased. The enhancement of curative action by the purified semi-synthetic diet was found to be due to the absence of as yet unidentified inorganic salt(s) that interfere with drug activity. It was found in mice fed on the purified semi-synthetic diet that higher blood levels of the drug were maintained for a longer period than when the same amount

of Fuadin was injected into mice fed on the commercial pellet diet, suggesting that the increased cure-rate was due to the higher blood drug level.

Significance to the Program of the Institute:

Since parasitic infections occur most frequently in backward areas where malnutrition is also prevalent, it is necessary to determine the relation between the nutritional status of the people and the results obtainable by chemotherapy. Failure of chemotherapy is a common experience in such areas.

Proposed Course of the Project:

The present study is to be extended to determine the specific nutritional factors involved in the enhancement of Fuadin efficacy in schistosomiasis. Other drugs used for the treatment of this and other parasitic diseases will be investigated under similar experimental conditions.

Part B included Yes

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Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Luttermoser, George W., and DeWitt, William B.: Studies on interrelations of nutrition and treatment of schistosomiasis I. Enhancement of stibophen (Fuadin) activity against Schistosoma mansoni in mice by feeding purified semi-synthetic diets. (In press)

Honors: None

Awards: None

Serial No. NIAID-121-L
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Effects of improving the nutrition of malnourished people infected with Schistosoma mansoni.

Principal Investigator: William B. De Witt

Other Investigators: None.

Cooperating Units: School of Medicine, San Juan, P. R.
Rio Piedras Hospital, Rio Piedras, P.R.

Man Years (calendar year 1960):

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

To evaluate the effects of providing malnourished people infected with Schistosoma mansoni with an enriched diet containing an abundance of animal protein.

Methods Employed:

Malnourished people suffering from schistosomiasis are given high-protein, high-caloric, vitamin-enriched food over an extended period so that the effects of the improved diet on their well-being and on the parasitic infections may be determined. Such factors as the following are observed before and at various intervals after the patients are given the enriched diet: (1) Nutritional status as determined by physical examinations and biochemical tests; (2) egg-production and viability of eggs of the parasite; (3) antibody levels; (4) skin sensitivity to schistosome antigens; (5) intestinal uptake of essential nutrients; (6) liver function, as determined by BSP and thymol turbidity measurements; and (7) liver biopsy examinations. After the effects of the improved diet on the health of the patients have been determined, the patients will be given a standard course of treatment so that the effects of the improved nutrition on the efficiency of the drug can be determined. Suitable control patients are likewise to be studied.

Major Findings:

The first part of this project has been completed and preliminary analysis of the data indicates: (1) Malnourished people suffering from schistosomiasis respond favorably to an enriched diet. Weight gain and improvement in general well-being were particularly marked. (2) The effects of the enriched diet on passage of schistosome eggs did not appear to be significant. However, with some patients a decrease was noted in the number of schistosome eggs passed. (3) Several patients with concomitant hookworm and whipworm infections apparently underwent self-cure while on the enriched diet. Similar cures were not observed among the control subjects. (4) Biochemical studies made on patients before they received the improved diet revealed a complete absence of urinary vitamin C in more than half of those examined. Most of those studied also had low serum vitamin A levels.

Significance to the Program of the Institute:

This project is a direct extension of some of our basic laboratory studies which indicate that the normal growth and development of schistosomes is prevented when the experimental host is given a deficient diet. Many of the worms remain sexually immature and are unable to produce eggs, which are important factors in the pathogenic mechanism of schistosomiasis. No information is available concerning the interrelation of diet and schistosomiasis in humans. If, as has been shown in animal experiments, the efficacy of treatment of schistosomiasis in humans can be greatly enhanced by providing an enriched diet, it would be a major accomplishment. As to date, no satisfactory treatment of the disease has been discovered.

Proposed Course of the Project:

The second phase of the project is concerned with determining the effects of dietary improvement on the efficacy of drugs against schistosomiasis. The curative action of the drug (Fuadin) will be evaluated when different regimens are employed and the occurrence and severity of side reactions will be noted.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project: None

Honors: Dr. William B. De Witt was made editor of Tropical Medicine and Hygiene News.

Awards: None

Serial No. NIAID-121-M
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Ammonia metabolism and toxicity in relation to liver disease.

Principal Investigator: Kenneth S Warren

Other Investigators: None

Cooperating Units: Laboratory of Clinical Investigations, NIAID

Man Years (calendar year 1960):
Total: 1½
Professional: 1
Other: ½

Project Description:

Objectives:

To study the biochemical pathway of ammonia toxicity and the possible relation between ammonia toxicity and hepatic coma. More specifically, to determine whether drugs which exacerbate hepatic coma or are used in its treatment effect ammonia toxicity; to test whether the susceptibility of animals to ammonia toxicity is altered by different types of liver disease.

Methods Employed:

Ammonia toxicity to mice is established by determining the intravenous LD_{50}. The modification of the LD_{50} by drugs, anti-metabolites and liver disease is then determined. Blood and brain ammonia concentrations, glutamine concentrations, and blood pH are also studied.

Major Findings:

1. Low oxygen in breathed air enhances ammonia toxicity, but hypoglycemia and cyanide have no such effect. Uncoupling agents have a slight effect and substances which effect the lower part of the Embden-Myerhoff cycle and the Krebs cycle up to succinate increase ammonia toxicity. Ammonia toxicity bears no relation to brain acetyl-choline

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Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Warren, K. S, Iber, F. L., Dolle, W., and Sherlock, S: The effect of alterations in blood pH on the distribution of ammonia from blood to cerebrospinal fluid in patients in hepatic coma. J. Lab. Clin. Med. 56: 687

Warren, K. S, and Schenker, S.: Hypoxia and amonia toxicity. Am. J. Physiol. (Dec. 1960).

Honors: None

Awards: None

Serial No. NIAID-121-N
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Parasitological investigations at the Marine Biological Laboratory, Woods Hole, Mass.

Principal Investigator: Paul P. Weinstein

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: ¼
Professional: ¼
Other: None

Project Description:

Objectives:

A. To study the ectocommensal associates of the horseshoe crab, Limulus polyphemus, and the factors which influence the host-ectocommensal relationship.

B. To study the little-understood life cycles of trypanorhynchid and tetraphyllidean tapeworms.

Methods employed:

A. Various stages of Limulus were collected and the parasites were studied in vivo and in vitro.

B. Usual biological methods were used to study life cycles of the tapeworms.

Major Findings:

A. A new species of oncholaimid nematode was discovered as an ectocommensal on the ventral surface of Limulus. Its entire life cycle has been found to take place in this habitat, and various stages from the egg to the adult have been studied. Information has been obtained on seasonal changes in growth of the worm, prevalence rate on different instars of Limulus, and the relationship of molting of Limulus to continued "infestation.

The turbellarian ectocommensals, Bdelloura candida, B. propinqua and Syncoelidium pellucidum were studied "in vitro", and information was obtained on feeding response to various substances. Cocoon deposition, which ordinarily only occurs on the gills of Limulus, was obtained in vitro, and these cocoons were successfully embryonated and hatched.

Studies made on Limulus from the trilobite stage to the adult gave some information on the sequence of "infestation" of this host with its nematode and turbellarian ectocommensals, and has clarified somewhat the "epidemiological" picture.

B. Studies done with the trypanorhynch, Lacistorhynchus tenuis indicate that certain copepods will support rapid development of procerci after exposure to coracidia. No procerci were observed following exposure of copepods to the eggs of two species of Calliobothrium (tetraphyllidean), nor were procerci of either genus found in the exposed benthic fauna.

Significance to the Program of the Institute:

A. The ectocommensals of Limulus have a strict host specificity; they are found nowhere else in nature. Delineating the factors underlying such a relationship should contribute toward an understanding of ectocommensalism, which is one of the interesting categories of animal association, and is in an evolutionary sense a forerunner to parasitism.

B. The tapeworms studies have given information on the first intermediate hosts of a relatively primitive group of tapeworms, whose life cycles are essentially unknown. The trypanorhynchid cycle may prove to be similar to that of Diphyllobothrium latum, the fish tapeworm of man.

Proposed Course of the Project:

This project has been terminated to permit time to develop plans for the contemplated filariasis program under PL 480.

Part B included No

Serial No. NIAID-123-A
1. Parasitic Diseases
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Fundamental physiological and biochemical studies on parasites, intermediate hosts, and parasitized animals.

Principal investigator: Theodor von Brand

Other investigators: Eleanor J. Tobie, Patricia A. McMahon, Iain B. Bowman (Guest worker).

Cooperating Units: Laboratory of Histology and Pathology, NIDR.

Man Years (calendar year 1960)

Total:	4 1/2
Professional	3
Other	1 1/2

Project Description:

Objectives:

The objective is to gain knowledge on the chemical composition and the metabolism of parasites, intermediate hosts, and parasitized animals.

Methods Employed:

Trypanosoma cruzi, T. gambiense and T. rhodesiense are cultivated in wholly fluid media and trehalose utilization is studied by quantitative methods. Insight into a major metabolic pathway of Trypanosoma cruzi is sought by estimating, with the help of radioactive CO_2, the carbon dioxide fixation into succinic acid. The aerobic and anaerobic metabolism of Taenia taeniaeformis are studied by determining quantitatively the elimination of various metabolic end-products and the utilization of carbohydrate. The calcareous corpuscles of the same tapeworm are isolated by various means, treated in a mufflefurnace, and then turned over to the collaborating laboratory for diffraction and electronmicroscopic studies. The phospholipids of larval and adult Taenia taeniaeformis are fractionated and studied by electrophoretic, chromatographic, and chemical methods, stress being laid on the quantitative relationships between various components.

Major Findings:

The trypanosome species studied are not capable of utilizing trehalose and the presence of this disaccharide does not render cultures of African trypanosomes infective. The production of succinic acid by the culture form of T. cruzi is dependent on the presence of carbon dioxide in its surroundings. Taenia taeniaeformis produces anaerobically much more succinic acid than it does aerobically. Stored lipids are not utilized by this worm during short periods of aerobic or anaerobic starvation. Upon heating the calcareous corpuscles of larval or adult Taenia taeniaeformis to 300° C diffraction lines appear that are very close, if not identical, to those of dolomite. Upon heating to higher temperatures, the dolomite-like substance is decomposed to calcium oxide and magnesium oxide. Upon treating of corpuscles with KOH or NaOH, no dolomite is formed, but brucite and apatite are produced. Highly magnified electron-microscopé photographs of KOH-isolated corpuscles reveal the presence of well formed crystals. The glycerol-containing phospholipids of both larval and adult Taenia taeniaeformis are approximately half lecithin and half cephalin. Electrophoretic studies confirmed the presence of sphingomyelin and indicated two cephalin fractions in larval T. taeniaeformis as well as a lecithin differing from that of the adult tapeworm. Hexose-containing phospholipids have been found in both the larval and the adult worms.

Significance to the Program of the Institute:

The studies on metabolism and chemical composition of parasites, intermediate hosts, and parasitized animals are essential for an understanding of the pathogenesis of parasitic diseases. They also lay a foundation for a rational approach to chemotherapy.

Proposed Course of the Project:

The studies on the metabolism of trypanosomes will be continued with emphasis on tracer studies. The studies on the overall metabolism of Taenia taeniaeformis will be terminated within the next few months. A study of the influence of oxygen tension and carbon dioxide tension on the metabolism of tapeworms will be initiated. The studies on the calcareous corpuscles will be continued, but may be terminated some time during the coming year. The studies on phospholipids will be continued with emphasis on electrophoretic studies.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

von Brand, Theodor: Recent advances in carbohydrate biochemistry of helminths. Helminthological Abstracts 29: 97-111 (1960).

von Brand, Theodor: Der Stoffwechsel der Trypanosomen. Ergebnisse der Biologie 12: 30-46 (1960)

von Brand, Theodor: Influence of oxygen on life processes. In: Sasser, J.N., and Jenkins, W.R. (Editors): Nematology. The University of North Carolina Press. pp 242-248. (1960).

von Brand, Theodor: Influence of size, motility, and age on metabolic rate. In: Sasser, J.N., and Jenkins, W.R. (Editors): Nematology. The University of North Carolina Press. pp. 233-241. (1960).

von Brand, Theodor: Influence of pH, ions, and osmotic pressure on life processes. In: Sasser, J.N. and Jenkins, W.R. (Editors): Nematology. The University of North Carolina Press. pp. 249-256. (1960).

von Brand, Theodor: Influence of temperature on life processes. In: Sasser, J.N. and Jenkins, W.R. (Editors): Nematology. The University of North Carolina Press. pp. 257-266. (1960).

von Brand, Theodor: Introductory remarks. In: Stauber, L.A. (Editor): Host influence on parasite physiology. Rutgers University Press. pp. 1-3. (1960).

von Brand, Theodor, Mercado, Teresa I., Nylen, M.U., and Scott, D.B.: Observations on function, composition, and structure of cestode calcareous corpuscles. Exper. Parasitol. 9: 205-214. (1960).

Thompson, M.J., Mosettig, E., and von Brand, T.: Unsaponifiable lipids of Taenia taeniaeformis and Moniezia sp. Exper. Parasitol. 9: 127-130. (1960).

Bowman, Iain B.R., von Brand, Theodor, and Tobie, Eleanor J.: The cultivation of trypanosomes in the presence of trehalose with observations on trehalase in blood serum. Exper. Parasitol. (In press).

von Brand, Theodor: The metabolism of trypanosomes with special reference to Trypanosoma cruzi. Proc. 1. Internat. Congr. Chagas Dis. (In press).

von Brand, Theodor: Influencia del tamano, motilidad, ayuno y edad sobre la actividad metabolica. Biologia 28: 117-128. (1959). (Issued April 1960 and therefore not reported as published in 1959).

Awards: None

Honors: Theodor von Brand presented the introductory remarks on the occasion of the symposium "Host influence on parasite physiology," Rutgers University's 16th Annual Conference on Protein Metabolism, New Brunswick, January 1960.

Theodor von Brand served as coordinator of the panel "Impact of modern instrumentation on medicine in the tropics." Fourth Conference on Research Needs in Tropical Medicine. New Orleans, La. April 1960.

Theodor von Brand served as chairman of the"Symposium sobre biodinamia parasitaria". I. Congreso Panamericano de Biologia y Patologia Experimental, Caracas, Venezuela, September 1960.

Theodor von Brand gave a series of seven lectures on parasite physiology before the Instituto de Medicina Tropical, Universidad Central de Venezuela, September 1960.

Theodor von Brand was nominated "Huesped de Honor" by the Medical Faculty of the Universidad Central de Venezuela, September 1960.

Serial No. NIAID-123-B
1. Parasitic Diseases
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Pathological physiology and histochemistry of parasitic diseases.

Principal Investigator: Teresa I. Mercado

Other Investigators: Theodor von Brand

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2¼
Professional: 1¼
Other: 1

Project Description:

Objectives:

To gain a better understanding, by means of studies at the cellular level, of the pathological physiology of parasitic diseases as related to interaction between parasite and host.

Methods Employed:

Normal rats and rats infected with Plasmodium berghei are fed large amounts of hytakerol (dihydrotachysterol) for 4, 5, or 6 days. They are then killed and various organs are fixed. Sections are stained for calcium, primarily by the von Kossa method, and for mucopolysaccharides by the alcian blue procedure. The slides are then studied in respect to the possible presence of quantitative and qualitative calcification differences between normal and infected animals.

Major Findings:

In most organs studied, such as kidney, heart, lung, and other essentially the same type of calcification occurred in normal and parasitized animals; the question of possible occurrence of quantitative differences (either in respect to the number of animals showing calcifications, or in respect to the degree of calcification) requires further analysis of the prepared slides. A significant qualitative difference between malarious and control rats was observed in stomach calcification. The majority of infected animals showed pronounced

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Mercado, Teresa I., and von Brand, Theodor: Histochemical studies of liver glycogen and lipid in some parasitic infections. J. Infect. Dis. 106: 95-105. (1960).

Honors: None.

Awards: None.

calcium deposits between the glands of the stomach mucosa, resulting in a mosaic-like pattern. This was found only in a few non-infected animals; when the latter showed calcification, the deposits were seen more frequently in the muscular coat.

Significance of the Program to the Institute:

Histochemical studies concerned with the pathological physiology of parasitic diseases allow the study of some facets of the complicated interaction between parasite and host that cannot be investigated by purely chemical means. As such, they may be used to throw light on such fundamental questions as the elusive question concerning the possible production of specific parasite substances damaging the host, the intimate distribution of enzyme patterns, and others; all of them important for the advancement of modern chemotherapeutic and immunological concepts.

Proposed Course of the Project:

The calcification studies will be terminated during the coming year. It is proposed to study by modern histochemical procedures certain enzymes, such as succinoxidase or cytochrome oxidase, in the liver of normal and parasitized rats.

Part B included Yes

Serial No. NIAID-123-C
1. Parasitic Diseases
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biochemical mechanisms of energy metabolism in normal and parasitized animals.

Principal Investigator: E. C. Weinbach

Other Investigators: None

Cooperating Units: Laboratory of Parasite Chemotherapy; Wenner-Grens Institut, Stockholm, Sweden.

Man Years (calendar year 1960)

Total:	1¼
Professional:	1
Other:	1/4

Project Description:

Objectives:

The objective is to conduct fundamental studies on the mechanism of energy metabolism in normal and parasitized animals.

Methods Employed:

Mammalian tissues are fractionated by mechanical disruption of the cells and the subcellular elements are isolated by differential centrifugation. Mitochondria are fragmented further by various chemical and mechanical means to obtain membranes bearing the electron transport system. These cellular and subcellular fractions are employed as biological catalysts to study electron transport, oxidative phosphorylation, and related exergonic reactions. Intramitochondrial substrates and cofactors of metabolism are determined by suitable enzymatic, chemical, radiochemical, and spectrophotometric techniques. Manometric procedures and oxygen electrode recordings are used to measure oxidation rates. Radioisotope techniques are used to investigate subtle metabolic changes. Similar studies are conducted with tissues obtained from parasitized animals.

Major Findings:

The stability of isolated liver mitochondria is related to the endogenous oxidative phosphorylation. Oxygen electrode recording of

the respiratory control index has revealed that liver mitochondria are more stable when they exhibit a high endogenous metabolism.

Phosphoenolpyruvate is formed from endogenous substrates in isolated liver mitochondria.

Work at the Wenner-Grens Institut:

Studies are conducted on the oxidation of flavosubstrates by the mitochondrial respiratory chain and on the regulation by flavosubstrates of the redox state of the intramitochondrial pyridine nucleotides. Specifically, the present study concerns the mechanism by which the addition of ATP to mitochondria which are depleted of their endogenous high energy-phosphate intermediates stimulates the oxidation of a flavosubstrate such as succinate and, simultaneously, reduce pyridine nucleotides. In effect, this is a "reversal" of oxidative phosphorylation and it appears to be the most powerful tool to date for studying the mechanism of this complex bioenergetic process.

Major Findings:

Mitochondria, depleted of endogenous high-energy phosphate, exhibit a marked diminution in the capacity to oxidize succinate. Addition of ATP restored the succinate oxidation and, simultaneously, initiated reduction of intramitochondrial DPN. Since the ATP effect was obtained in the presence of uncoupling agents, it must be exerted at a site which is not accessible to mitochondrial ATPase. This suggests compartmentalization in the mitochondria and preliminary studies with P^{32} supports this concept. The important fact is that the oxidation of succinate is linked to an energy-requiring reaction, and that this energy is invested at one site of the respiratory chain and then utilized at another site for reducing pyridine nucleotide.

Significance to the Program of the Institute:

This investigation, because of its fundamental nature in elucidating one of the vital processes in all living cells, has obvious significance to biochemistry in general, and provides a firm basis for our understanding of the subtle biochemical changes in energy metabolism associated with parasitism. In addition, it provides a solid foundation for future studies on the complex bioenergetic mechanisms of parasitic forms.

Proposed Course of the Project:

Efforts aimed at obtaining additional information on the basic metabolism of mitochondria, and an increased understanding of the fundamental mechanism of oxidative phosphorylation will be continued.

Specifically at the Wenner-Grens Institut:

We intend to measure the requirement for high-energy phosphate of different systems capable of succinate oxidation. Since the ATP effect is not found with non-phosphorylating submitochondrial preparations, we plan to study the aerobic oxidation of succinate in submitochondrial fragments which have different degrees of phosphorylating capacity. An intense effort, employing radioisotopes, will be made to examine the compartmentalization concept of mitochondrial organization.

Part B included Yes

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Weinbach, E.C.: Biochemical changes in mitochondria associated with age. In: Strehler, B.L. (Editor): The Biology of Aging. A Symposium. American Institute of Biological Sciences, Washington, D.C. Publication No. 6: pp. 328-331. (1960).

Weinbach, E.C.: Oxidative phosphorylation with endogenous mitochondrial substrates. Acta Chem. Scand. (In press).

Schellenberg, K.A., and Weinbach, E.C.: The endogenous formation of phosphoenolpyruvate by rate liver mitochondria. Biochem. et Biophys. Acta. (In press).

Honors: None

Awards: None

Serial No. NIAID-123-D
1. Parasitic Diseases
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biology of trypanosomes.

Principal Investigator: Eleanor J. Tobie

Other Investigators: None

Cooperating Units: Applied Immunology Section, Laboratory of Immunology, NIAID. Serial No. NIAID-148.

Man Years (calendar year 1960):

Total:	1 3/4
Professional:	3/4
Other:	1

Project Description:

Objectives:

To clarify the life cycle of Trypanosoma rangeli in relation to its vertebrate and invertebrate hosts; to study ways of maintaining and enhancing infectivity of strains.

Methods Employed:

Young nursling rats and Rhodnius prolixus are used as hosts. A colony of R. prolixus is maintained. One strain of T. rangeli is maintained in R. prolixus and several are maintained in vitro. Rats and R. prolixus are infected, both by artificial and natural means. Infections in rats are determined by microscopic examination of blood or by in vitro cultivation of blood. Infections in R. prolixus are proved by microscopic examination of fecal material and hemolymph, feeding on fresh rats, and by dissection, with microscopic examination of body fluids and organs. Fresh frozen sections are made of infected R. prolixus, stained and examined microscopically. Culture material is grown and harvested for immunizing of rabbits.

Major Findings:

Various strains of T. rangeli react differently in the white rat as well as in R. prolixus. Evidence suggests that R. prolixus may not be

the natural invertebrate host for all strains of T. rangeli or that trypanosomes designated as T. rangeli actually represent more than one species. Passage through R. prolixus, following inoculation into the hemocoele enhanced the ability of one strain to invade the salivary glands, making it possible to maintain the strain by cyclical transmission. The infectivity of in vitro strains can be extended by repeated isolation in vitro and passage through the rat. R. prolixus does not develope resistance to T. rangeli when the infection does not pass beyond the digestive tube. Artificial transfer of parasites from fecal material to the hemocoele resulted in completion of the cycle.

Significance of the program to the Institute:

Intensive biological studies of this species may provide new and valuable information general to trypanosomes. This is a valuable laboratory tool.

Proposed Course of the Project:

Studies will be directed to determining the life cycle of the parasite within the insect host by localizing the organism during its stages of development in the various organs by means of the fluorescent antibody technique.

Part B included Yes

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Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

von Brand, Theodor, and Tobie, Eleanor J: The mechanism of elimination of certain strains or species of trypanosomes when mixed in experimental infections. J. Parasitol. 46 (2): 129-136. (1960).

Tobie, Eleanor J.: Experimental transmission and biological comparison of strains of Trypanosoma rangeli. Exp. Parasitol. (In press).

Honors: None

Awards: None

Serial No. NIAID-127-A
1. Parasitic Diseases
2. Protozoal Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies on toxoplasmosis

Principal Investigator: Leon Jacobs

Other Investigators: Milford N. Lunde, Marjorie L. Melton, Anastasia S. Stanley

Cooperating Units: Ophthalmology Branch, NINDB

Man Years (calendar year 1960):

Total:	5½
Professional:	3½
Other:	2

Project Description:

Objectives:

The accumulation of data on the occurrence of toxoplasmosis as an acute and chronic infection of man and animals; the study of the biology of the proliferative parasite and the cyst, with the aim of evaluating their importance in the epidemiology of toxoplasmosis and in the production of chronic disease; the determination of the usefulness of immunological tests in diagnosis, and the identification of antigens and antibodies; the description of factors related to the virulence of Toxoplasma, and of those involved in susceptibility and immunity of the host, and in the occurrence of congenital disease; the elucidation of the mechanism of cyst formation, relative to factors in the host; the critical study of toxoplasmicidal agents against proliferative parasites and cysts; the study of the role of hypersensitivity in ocular toxoplasmosis.

Methods Employed:

Standard procedures of animal inoculation plus special techniques for injection into the anterior or posterior chambers of the eye. A microisolation apparatus is used for handling cysts. Histochemical procedures are being initiated to complement electron-microscopic work.

Tissue cultures are used for morphological, histochemical, and electron-microscopic studies and for attempts at growing Toxoplasma cysts in vitro. The dye and hemagglutination tests are used for serological studies, and the antigens are investigated by biochemical and electrophoretic techniques.

Major Findings:

Sheep Studies in New Zealand: The prevalence of Toxoplasma in New Zealand sheep is very high. The distribution of parasites in the tissues of sheep is erratic. Parasitemia was found only in the first week of infection with two strains of Toxoplasma given by various routes. Toxoplasma in the sheep is rapidly cleared from tissues except for muscle and placenta. Residual infection in sheep is more likely to persist in muscle than in brain. Mutton can therefore definitely serve as a source of human infection.

The following statements concerning the circumstances of congenital transmission appear justified from data obtained, although not yet complete: Infection 60 days prior to onset of pregnancy does not result in congenital infection or abortion. Abortion or foetal death and resorption occurs following infection of the ewe at 30 days pregnancy. Infection at 90 days pregnancy, by various routes, sometimes results in abortion or death of the lamb soon after birth. In other cases, the lamb survives, although the foetal cotyledons are infected. Natural immunity or active immunity produced by vaccination with live parasites prior to mating does not protect ewes from Toxoplasma abortion when the challenge inoculum is high. When congenital transmission does occur, only one of twin lambs may be affected. The foetal cotyledons are more consistently infected than are the tissues of the lambs.

Studies at N.I.H: The failure of immunization with live organisms completely to protect animal from a challenge infection has been demonstrated in further tests in guinea pigs.

Further studies have been conducted on parasitemia in chronically infected mice and additional work has been done on rabbits. Both species may exhibit low grade parasitemia for months. Since these animals have high antibody levels, it is difficult to explain the persistent activity of the parasite.

Various domestic animals have a heat-labile, non-specific, anti-Toxoplasma activity in their serum. It has been found also in human sera. The use of citrate in the collection of accessory factor sera for the dye test diminishes or eliminates this non-specific activity.

A serological survey of cattle sera indicates that in these animals there is considerable difference in hemagglutination and dye test titers.

Ultra violet absorption at 260 and 280 millimicrons shows a relatively large amount of nuclear protein material in the hemagglutination antigen. Agar gel diffusion with the hemagglutination antigen and its rabbit antiserum indicates at least two separate antigen-antibody systems are present.

The complement-fixation test for toxoplasmosis has been set up as an additional diagnostic procedure.

Use of pyrimethamine in mice with chronic infections has reduced the number of cysts. This has important implications, possibly, relative to control of toxoplasmic chorioretinitis. The same effect has been observed with sulfadiazine.

Cysts of Toxoplasma have been found at all levels of the intestinal tract and in the lung of chronically infected mice three to four months after infection. There is evidence that cysts of Toxoplasma form in tissue cultures. Suspensions of cultures have infected mice after pepsin-HCl digestion, a treatment which kills proliferative forms.

Significance to the Program of the Institute:

Knowledge of the transmission of Toxoplasma in nature is requisite as a basis for recommendations for prevention of the infection. Continued work is necessary to explain mechanisms of transmission to herbivores and vegetarian human beings.

The analysis of antigens derived from Toxoplasma has importance in relation to an understanding of the course of the disease and the action of various antibodies, as well as diagnosis. Continued work on the hemagglutination test may establish it as the best practicable procedure for diagnosis of human toxoplasmosis.

Extensive and intensive basic study of Toxoplasma infection will furnish explanations for clinical observations and contribute to a general understanding of chronic infections. This is of great importance in relation to ocular disease and may also reveal instances of chronicity in systemic infections.

Proposed Course of the Project:

Attention will be paid to: the epidemiology of toxoplasmosis in herbivorous animals; the circumstances of cyst-formation and rupture in chronic infections; the relation of cysts and dormant parasites to chronic disease; the cultivation of cysts in tissue culture; the mechanism of proliferation of Toxoplasma in cells in tissue cultures; the relation of antibodies to particular antigenic components and to persistence of

chronic infections; the effect of drugs on cyst and on activity of the parasite during chronicity; the relation of local tissue immunity to the spread of challenge infections; and the significance of non-specific anti-Toxoplasma activity of serum.

Part B included Yes

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Jacobs, Leon, Remington, Jack S., and Melton, Marjorie L. The resistance of the encysted form of Toxoplasma gondii. J. Parasitol. 46: 11-21. (1960)

Jacobs, Leon, Remington, Jack S., and Melton, Marjorie L. A survey of meat samples from swine, cattle, and sheep for the presence of encysted toxoplasma. J. Parasitol. 46: 23-28 (1960)

Remington, Jack S., Jacobs, Leon, and Kaufman, Herbert. Toxoplasmosis in the adult. New England Journal Medicine 262: 180-186, 237-241. (1960)

Frenkel, J. K., Weber, R. W., and Lunde, M. N. Acute toxoplasmosis. Effective treatment with pyrimethamine, sulfadiazine, leucovorin calcium, and yeast. J.A.M.A. 173: 1471-1476. (1960)

Jacobs, Leon. Ocular toxoplasmosis: Laboratory contributions to diagnosis and chemotherapy. Reprinted from Human Toxoplasmosis Copenhagen: Munksgaard. (1960)

Remington, Jack S., Jacobs, Leon, and Melton, Marjorie L. Chronic toxoplasma infection in the uterus. (in press) J. Lab and Clin. Med.

Remington, Jack S., Jacobs, Leon, and Melton, Marjorie L. Congenital transmission of toxoplasmosis from mother animals with acute and chronic infections. J. Inf. Dis. (in press)

Honors and Awards:

Dr. Leon Jacobs was awarded a Fulbright Fellowship for study of problems in the epidemiology and pathology of toxoplasmosis in New Zealand. He departed on this assignment May 1.

Dr. Leon Jacobs visited Brisbane, Sydney, and Melbourne from 10 to 24 October on a visiting lecturer under the Australian Fulbright Program. He lectured in universities, hospitals and veterinary research institutions.

Dr. Leon Jacobs was awarded a Guggenheim Fellowship which will permit him to visit a number of Asian and European parasitology laboratories on his return trip from New Zealand.

Serial No. NIAID-127-B
1. Parasitic Diseases
2. Protozoal Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies on Entamoeba histolytica and other parasitic protozoa.

Principal Investigator: Louis S. Diamond

Other Investigators: Harry D. Baernstein
Lucy V. Reardon

Cooperating Units: Biophysics Laboratory, Naval Institute for Medical Research.

Man Years (calendar year 1960):

Total	2½
Professional:	1½
Other:	1

Project Description:

Objectives:

To study metabolism of E. histolytica, methods of diagnosis, mechanisms of pathogenesis, factors in host susceptibility, methods of culture, immunology, and variation. To study the cultural requirements of axenically cultivated Entamoeba spp. of lower animals in order to extend our knowledge of the biology of these important parasites. To devise a method for the axenic cultivation of Trichomonas tenax, the oral trichomonad of man. To develop techniques for the preservation of parasitic protozoa by freezing and storage at low temperatures and by freezing and storage in the dry state.

Methods Employed:

Usual methods for axenic studies and for use of single bacterial or protozoan associates; micro-isolation methods; immunological methods, including adsorption and extraction. For preservation studies, protozoa are suspended in a variety of suitable agents and cooled slowly from ambient temperatures to -25° C, then stored at this temperature or at temperatures of -79° C and -197° C; or the protozoa are frozen rapidly in vacuo at a temperature of -30° C, then stored at -197° C; or protozoa are supercooled, frozen and dried in vacuo.

Major Findings:

Axenic cultivation of E. histolytica and T. tenax was achieved in an anaerobic medium supplemented with a cell free extract of chick embryo. Neither species grew in the absence of the extract. Furthermore, growth of E. histolytica occurred only when the medium was made up in a diphasic form, i.e., a solid agar slant with a liquid overlay.

A substitute for T. cruzi as an associate in the monoxenic cultivation of E. histolytica was found in the form of a new species of Crithidia isolated from the gut of a hemipteran. The new associate increases the yield of amoebae by a factor of 4, does not require a separate medium for maintenance of stock cultures, and is not known to be a parasite of man.

Several batches of antigen have been prepared from monoxenic E. histolytica - T. cruzi cultures.

Studies on the cultural requirements of E. invadens resulted in the following: a) development of a technique for counting the amoebae with a Coulter Electronic counter; b) the finding that the amoebae reacted adversely to any change in concentrations of trypticase, yeast extract or horse serum; c) that yeast extract could not be replaced by Eagle's vitamin B mixture.

There has been progress toward development of a method for preservation of E. histolytica and other protozoan species by freeze-drying, but so far it has not been possible to store the freeze-dryed material successfully and still preserve viability.

Attempts to preserve the protozoans by freezing without drying had been more successful. The slow freezing and subsequent storage at -79° C of T. vaginalis, T. hominis, T. gallinae, and E. histolytica suspended in dimethyl sulfoxide has resulted in preservation, to date, of these protozoa for periods of 4 months, 3 months, 30 and 29 days respectively. E. histolytica cooled to a temperature of -197° C by a similar technique has been stored successfully at this temperature for a period of 24 hours. This last finding is most encouraging, since theory predicts that a cell capable of withstanding exposure to -197° C, even for such a short time, should be capable of almost indefinite storage at such temperatures.

Significance to the Program of the Institute:

The development of a technique for growing E. histolytica under axenic conditions should open the way to the solution of many unsolved problems in amoebiasis. It should be possible, for example, to determine whether or not E. histolytica, by itself, is capable of initiating the

lesions of amoebiasis; to prove or disprove the existence of lytic substances of amoebic origin; and to determine growth requirements in vitro. Solution of the technical problems associated with axenic cultivation of the amoebae and T. tenax should find application in solving similar problems met with in the cultivation not only of other parasites, but mammalian cells as well.

Techniques developed for the preservation of parasitic protozoa by freezing and freeze-drying should be applicable to preservation of other types of cells.

Development of a technique for enumerating protozoa in culture with the aid of the Coulter Electronic counter has extended the use of this instrument, which was originally designed to count blood cells, and gives indication that this instrument could be utilized profitably in counting mammalian cells from tissue culture.

Proposed Course of Project:

Efforts will be made to define and improve media employed in axenic cultivation of E. histolytica and T. tenax; to develop methods for axenic cultivation of other parasitic protozoa of man; to develop more sensitive antigens for serological diagnosis; to study factors associated with invasiveness and pathogenicity of strains of E. histolytica in experimental hosrs; to study cytochemical changes during division of E. histolytica; to refine techniques for preservation of protozoa by freezing and freeze-drying.

Part B included No

5S

Serial No. NIAID-127-C
1. Parasitic Diseases
2. Protozoal Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Trichomoniasis

Principal Investigator: Lucy V. Reardon

Other Investigators: Louis S. Diamond

Cooperating Units: Laboratory of Clinical Investigations, NIAID
Laboratory of Germfree Animal Research, NIAID
Laboratory of Pathology and Histochemistry, NIAMD
Clinical Investigations Branch, NIDR

Man Years (calendar year 1960):

Total:	1-3/4
Professional:	3/4
Other:	1

Project Description:

Objectives:

Continued study of the pathogenesis of Trichomonas vaginalis; transfer of genetic characters in strains of T. vaginalis; antibody formation and hypersensitivity in relation to resistance and pathogenesis; the action of purported trichomonacides (as well as amoebacides) as determined by in vitro tests; the possible relation of oral protozoa to peridontal disease.

Methods Employed:

Pathogenesis of trichomonads is tested in mice by intraperitoneal injection of concentrated organisms. Microbe-free cultures, maintained without use of antibotics, furnish the inocula.

Pathogenesis is also studied in germfree guinea pigs. Microbe-free material is injected subcutaneously into germfree pigs, monocontaminated pigs, and conventional pigs. The effect of the sera from the above guinea pigs on washed concentrates of trichomonads is observed microscopically.

60

For studies on genetic transfer, organisms of the non-virulent R strain are associated with homognates of the virulent C-1 strain processed to contain DNA of the strain and then injected into mice for evidence of alteration of characters. Genetic transfer is also attempted through efforts to produce an aureomycin resistant strain of the organism. The haemagglutination test is used for evidence of antibody production. Human sera and vaginal exudates are likewise tested. Evaluation of purported trichomonacides (as well as amoebacides), is made by in vitro tests. Diseased and normal tissues from patients with peridontal disease are examined for oral protozoa, both by direct examination of the material and by cultures.

Major Findings:

Virulent and avirulent T. vaginalis were retested in mice after having been in continuous in vitro cultivation 4 and 5 years respectively. No change in virulence was observed.

Experimental studies of germfree guinea pigs, monocontaminated pigs (as well as conventional pigs) showed as in previous experiments, large, palpable swellings or abscesses occurring at the site of injection in germfree pigs, less marked swelling in the monocontaminated pigs, and none in conventional pigs. Germfree pigs, rechallenged with trichomonads after subsidence of swellings, failed, in one experiment, to develop new swellings. Sera from germfree or monocontaminated pigs appeared to have a deleterious effect on the trichomonads. Sera from the conventional pigs had no effect on the trichomonads other than to cause agglutination.

Additional experiments on transformation of virulence by association of the non-virulent strain with DNA of the virulent, showed no evidence of such transformation.

The preparation of T. vaginalis antigens was continued. Production in guinea pigs of high titers of haemagglutinins against T. vaginalis demonstrated the usefulness of whole organisms as a source of antigen. Haemagglutinins against an enzyme preparation of sonicated T. vaginalis were also produced in guinea pigs. These enzyme haemagglutinins tested in assay system for malic dehydorgenase showed decided inhibitory action against this enzyme.

Humatin, (Paromomycin sulfate), failed to cause complete inhitition of activity of T. vaginalis in concentrations of the drug up to 800 ug/ml of medium. E. histolytica in association with Trypanosoma cruzi was not inhibited in concentrations of the drug up to 64 ug/ml. However, in association with a Crithidia (an insect flagellate) as its supporting organism, E. histolytica appeared inhibited in concentrations of 32 and 64 ug/ml but not in lower concentrations. The Crithidia was likewise not inhibited in the lower concentrations of the drug.

Significance to the Program of the Institute.

Such studies should give new basic information on the trichomonads and lead to a better understanding of their pathogenecity, variation, transmission, life history, etc.

Proposed Course of Project:

Attempts will be made to induce haemagglutinis against individual enzymes of T. vaginalis and to develop a hemagglutination test for trichomoniasis. Transformation and strain studies will be continued.

Part B included No

Serial No. NIAID-127-D
1. Parasitic Diseases
2. Protozoal Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biochemistry of parasitic protozoa.

Principal Investigator: Harry D. Baernstein

Other Investigators: L. V. Reardon
L.S. Diamond

Cooperating Units: None

Man Years (calendar year 1960):

Total:	3/4
Professional:	3/4
Other:	None

Project Description:

Objectives:

To study the biochemistry of the parasitic protozoa: especially the intracellular enzymes and biochemical problems related to axenic and monoxenic cultivation.

Methods Employed:

Cultivation of various species and evaluation of growth rates in relation to composition of the medium by use of the Coulter Counter.

The isolation and purification of intracellular enzymes by conventional precipitation methods and by electrophoresis, and spectrophotometric methods to evaluate activities related to the concentration of protein present. Relations of the organisms to their hosts are studied by analysis for antienzymes and other antibodies developed in serum against purified antigens.

Major Findings:

Studies on the enzymatic activation of acetate in T. vaginalis showed the presence of such activation as determined by hydroxamic acid production. However, the type of hydroxamic acid produced must await purification of the enzyme and identification. Preliminary experiments on acetylation of sulfanilamide were negative.

Significance to the Program of the Institute:

The elucidation of biochemical properties of these parasitic organisms should help us understand their pathogenicity and their susceptibility to drugs. Specific enzyme-inactivating antibodies can be useful in the study of enzyme function and differentiation and identification of enzymes. The techniques developed for producing these anti-enzymes should be applicable in the study of enzymes of a variety of cells.

Proposed Course of Project:

Analysis of the complex components of media employed in cultivation of parasitic protozoa. Analysis of the enzyme systems present in the organisms. Better preparations with fewer interfering enzymes should result by application of electrophoresis. The problem of multiple enzymes having the same substrate specificity (isoenzymes) will be studied. The special biochemical problems associated with anaerobiasis such as electron transport will be explored.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Baernstein, H. D.: Malic dehydrogenase of Trichomonas vaginalis. J. Parasitol. (in press).

Honors: None

Awards: None

LABORATORY OF PARASITIC CHEMOTHERAPY

LABORATORY OF PARASITE CHEMOTHERAPY

Laboratory of Parasite Chemotherapy, NIAID

Summary Statement of Research Activities, Calendar Year 1960

This Country's commitment of 38 million dollars in Fiscal Year 1961 toward a program of world-wide malaria eradication and the long-term interest in malaria by most of the senior staff resulted in a research effort, during the past year, largely directed toward problems in that field. Special emphasis was given to the study of simian malaria in man and in monkeys because malaria in simians might be a real deterrent to the eradication program. Clinical facilities at the Atlanta Penitentiary were enlarged and the staff increased. A laboratory was established at Kuala Lumpur, Malaya in cooperation with the Malaya Institute of Medical Research and the United States Medical Research Unit. Studies on several aspects of the simian-human-malaria problem have been in progress there since mid-August.

As a result of the above development, it was decided to move the Section on Cytology, now located at Memphis, to Chamblee, Georgia, early in 1961. Alterations necessary to accommodate this move are nearing completion. This arrangement will bring the simian hosts closer to the human volunteers at the penitentiary, and the insectary maintained by the Section will be geared to accommodate the work at Chamblee and at the prison.

Dr. Jeffery of the Section on Epidemiology is spending a year in graduate study at Yale University and therefore the Section has been without his services since July. The program of the Laboratory was reviewed by the Board of Scientific Councilors who visited the Laboratory at Columbia, November 9-10.

The work of the Section on Chemotherapy has been curtailed due to two resignations, Drs. Gaudette and Schellenberg, during the past year. During this period, Dr. Jacobs joined the staff of that Section and has initiated a program of research aimed at an assessment of the place of nutrition in chemotherapy.

MALARIA - HUMAN Plasmodium falciparum (McLendon strain): Chloroquine (300 mg, base) and primaquine (45 mg, base) given together beginning 3 days after mosquito bites and weekly thereafter for a total of 8 doses, resulted in suppressive cure in 5/5 subjects. Controls were positive 11-15 days after infection. After 2 days of parasitemia, each control was given the above drug combination which was repeated weekly for a total of 3 doses. Parasites were removed promptly and cure was obtained based on no evidence of infection during 227 days of observation.

Primaquine, at daily doses of 0.75 mg, had some sporontocidal effect upon Plasmodium falciparum gametocytes but none against those of P. vivax (one case). Therapeutic doses (1.4 gm in 3 days) of amodiaquine had no sporontocidal effect against gametocytes of P. falciparum (one case). The effect referred to is against the development of the malaria parasites in the mosquito.

A strain of Plasmodium falciparum from Colombia, South America, was found to be resistant to chloroquine. This finding is of utmost importance in terms of malaria eradication.

Plasmodium vivax (Chesson strain): A drug combination of primaquine (45 mg) and pyrimethamine (50 mg) given weekly beginning 7 days after mosquito bite and continuing for a total of 4 doses, gave suppressive-cure in 4/5 subjects; the other subject developed a patent infection 240 days after infection. Pyrimethamine (50 mg) given alone, as above, produced suppressive-cure in 1/4 subjects; the other 3 came down on days 82, 83 and 84. Five controls all came down 12 to 13 days after infection.

The Russian 8-aminoquinoline, quinocide, was compared with primaquine and found to be distinctly inferior as a curative drug against early and late primary attacks of Chesson vivax malaria particularly from the standpoint of the occurrence of second and third relapses.

Another 8-aminoquinoline, Win 5037, was studied in 5 subjects. Toxic effects and failure to cure made further investigation unwarrented.

Plasmodium malariae: The results of a 14-year study of the biology of Plasmodium malariae were drawn together for publication. The highest infectivity for mosquitoes occurred during the 8th to 10th weeks of the primary attack. Although the infection rate of mosquitoes was ordinarily low, the relatively long period during which mosquitoes could be infected may explain the persistence of P. malariae in nature. The ability of the symptom-free malarious patient to infect mosquitoes at a rate similar to that of the symptomatic patient makes eradication difficult.

MALARIA - SIMIAN Plasmodium cynomolgi bastianellii: In early May, two accidental sporozoite-induced infections with Plasmodium cynomolgi bastianellii occurred at our Memphis Laboratory. This happening was of signal importance because it showed that simian malaria, contrary to the generally held opinion, was infectious to man. In that light, full scale study of human infections was undertaken at our Atlanta Penitentiary installation.

Two infections were induced in inmate volunteers by inoculation of infected blood obtained from one of the accidental sporozoite-induced infections in man. Twenty inmate volunteers were infected by bites of Anopheles quadrimaculatus or Anopheles freeborni which had fed on infected monkeys. The prepatent period ranged from 14 to 29 days and the parasite density ranged from 5 to 500/cmm. The most constant symptom was headache and the most significant signs were fever, splenomegaly and hepatomegaly. Infections were allowed to run their course, generally without treatment.

Anopheles freeborni were infected from two patients but attempts to infect volunteers by their bites have yielded equivocal results. The finding that P. c. bastianellii will grow consistently and produce clinical illness in man suggested the possibility that malaria is a zoonotic disease, that is, a disease which man can acquire from animals with which he is associated. Whether

or not such transfer occurs in nature is not yet determined, but should it occur, it would be of greatest significance to the world-wide malaria eradication program.

Plasmodium cynomolgi cynomolgi: Eleven inmate volunteers were bitten by Anopheles freeborni infected with P. c. cynomolgi on 8 September, and to date (14 December) three have exhibited evidence of infection (i.e., fever). Parasitemia has been demonstrated in only one, on the 58th day after mosquito bites. These results show that this strain infects man far less readily than P. c. bastianellii.

FIELD STUDIES IN MALAYA

Three staff members, Drs. Eyles, Dobrovolny, and Mr. Clinton S. Smith, were detailed to Malaya during the year where they engaged in the study of simian and human malaria in cooperation with the Malayan Institute for Medical Research and the U. S. Army Medical Research Unit at Kuala Lumpur.

The epidemiology of monkey malarias is being studied and the feeding habits of some of the Anopheles determined. By injection of uninfected monkeys with sporozoites from natural infections, it was determined that Anopheles hackeri is a natural vector of Plasmodium knowlesi. This is a most important discovery, especially since the vector of this parasite has been sought for repeatedly during the last 25 years.

Studies of malaria in aborigenes associated with monkeys have been made. Blood passed from aborigenes to monkeys have thus far produced no patent infection in the monkeys.

EE STAGES AND DRUG ACTION

Studies were continued on the direct effect of drugs on the exoerythrocytic stages of primate malaria. When sulfonamides were used with pyrimethamine to exploit the possible synergism of the two drugs, monkeys developed parasitemia 30 to 40 days after inoculation with sporozoites even though all parasites observed in liver biopsies were damaged. The curative efficacy of quinocide, the Russian drug, was compared with primaquine. Even when administered at twice the dosage used with primaquine, quinocide was less effective. Chloroquine had no observable effect upon the liver forms of Plasmodium cynomolgi. Young parasites appeared in the blood in large numbers on the 8th, 16th and 24th day indicating the existence of secondary exoerythrocytic generations.

INSECT TISSUE CULTURE

Blood cells from caterpillers and cells of the ovariole sheath of several species of moth pupae have been cultivated in several different media. The virus of St. Louis encephalitis has been maintained in cultures of hemocytes from larvae of the catalpa sphinx for 10 days. Oocysts of Plasmodium gallinaceum attached to the midgut of Aedes aegypti have shown growth in vitro and sporozoites have been produced.

BIOCHEMICAL STUDIES It was shown that mosquitoes infected with malaria have higher levels of ribonucleic acid than uninfected mosquitoes. Chromatographically, the acid-hydrolysate of ribonucleic acid from a pyrimethamine-resistant strain of Plasmodium falciparum differs from the acid-hydrolysate of ribonucleic acid from a pyrimethamine-susceptible strain. Bephenium hydroxynaphthoate inhibited glutamic acid transaminase of Nippostrongylus muris. Bephenium chloride and quinacrine reduced the rate of glucose absorption by the tapeworm Hymenolepis diminuta but low concentrations of dithiazanine iodide stimulated glucose absorption by this cestode.

INTESTIONAL PARASITES Epidemiological studies on the inmates of a mental institution show a high persistence of Trichuris and hookworm for six years, with an apparent decrease in Strongyloides. To test dithiazanine and tetrachlorethylene, alone and in combination, heavily parasitized mental patients were given the drugs for about one year. A large number of worms were removed but the cure rate was low and transmission was not stopped. Bephenium hydroxynaphthoate and bephenium chloride were used with good results against hookworm, Ascaris and Trichuris.

SCHISTOSOMIASIS The activity of griseofulvin observed in mice infected with Schistosoma mansoni was not well developed in hamsters or monkeys. A series of tetracycline analogues which show an affinity for microfilaria did not combine with schistosomes and were without activity. One of these analogues was significantly more active against microfilariae of Dirofilaria immitis than tetracycline.

In many tests, the efficacy of stibophen (Fuadin) therapy on mature Schistosoma mansoni infections in mice was increased up to 16 times by feeding a balanced semi-synthetic diet. The toxicity of the drug was not similarly increased. The enhancement of curative action by the purified semi-synthetic diet was thought to be due to the absence of, as yet unidentified, inorganic salt(s) that interfere with drug activity. It was found in mice fed on the purified semi-synthetic diet that higher blood levels of the drug were maintained for a longer period than when the same amount of Fuadin was injected into mice fed on the commercial pellet diet, suggesting that the increased cure-rate was due to higher blood drug level. Similar drug advantage was observed in mice given tartar emetic while on the purified diet.

DRUG COMBINATIONS Various combinations of primaquine, chloroquine, amodiaquine, and pyrimethamine proved to be no more effective than single drugs against blood induced Plasmodium berghei in mice.

NUTRITION AND MALARIA An intensive study of the influence of nutritional deficiencies on the activity of antimalarials has been initiated.

ADRENAL HORMONE AND MALARIA

The influence of cortisone on antimalarial activity was investigated in Plasmodium berghei and Plasmodium gallinaceum. The drugs used were primaquine, chloroquine, and pyrimethamine. The only combination in which cortisone exerted an adverse effect on antimalarial action was primaquine and Plasmodium gallinaceum. In Plasmodium berghei, cortisone has a slight synergistic effect when given concurrently with antimalarial drugs.

Serial No. NIAID-130
1. Parasite Chemotherapy
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Administration, research planning and coordination

Principal Investigator: G. Robert Coatney

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1 3/4
Professional: 3/4
Other: 1

Project Description:

This project furnishes certain technical, supervisory, and administrative services to all research projects in the Laboratory, as follows:

(a) Over-all planning of research and the coordination of research activities in the various Sections of the Laboratory.

(b) Integration of Laboratory research activities with clinical studies of the Clinical Center.

(c) Supervision over personnel, maintenance, travel, and correspondence.

(d) Requisitioning and supervision of equipment and supplies.

(e) Preparation of reports, budget estimates, and exhibits and other public relations materials.

(f) Maintenance of reference library, reprint files, card catalogues, and specimen collections.

(g) Consultatory services to individuals, commercial organizations, nongovernmental organizations, Government agencies including liaison activities with other branches of the Service, foreign governments, and international agencies.

(h) Reviewing, editing, and revising scientific and technical reports and manuscripts.

Progress on research projects is reported quarterly and, when necessary, are discontinued or revised in order to meet current objectives. During the past year some changes have been made in the research program of the laboratory, impetus given to others, and certain activities directed into new channels. The discovery that simian malaria was infective to man brought about changes in three directions: (1) Plans were laid for moving the Memphis activities to Chamblee, Georgia in order to have a flourishing insectary and the simian hosts close to the inmate volunteers at the Atlanta prison, (2) A laboratory was established at Kuala Lumpur, Malaya, to carry out investigations in the field relative to all aspects of the simian-human-malaria chain, and (3) Studies in human volunteers at the Atlanta prison were tailored to an all-out study of simian infections in man. Continued emphasis was placed on the fundamental aspects of other problems in tropical parasitology and chemotherapy. These included the culture of insect tissues, intensive and extensive study of the development and chemotherapy of fixed tissue stages of certain mammalian malarias, the tissue culture of mammalian malarias, the mechanism of drug action on parasites and the inhibiting effect of antimetabolites, the effect of nutrition on chemotherapy, drug resistance by parasites, chemotherapy of intestinal parasites, and the carriage of viruses by parasites.

The cooperative study with Dr. John Tobie, LI (Ser. No. NIAID-148), on the application of fluorescent antibody techniques to the staining of human plasmodia for mass screening with a mechanical scanner was continued.

Part B included - Yes

PHS-NIH
Individual Project Report
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Gaudette, Leo E. and Coatney, G. R. Stability of primaquine diphosphate under various conditions. Am. J. Trop. Med. & Hyg. 9:532-535, 1960.

Coatney, G. R., Elbel, R. E., and Kocharatana, P. Some blood parasites found in birds and mammals from Loei Province, Thailand. J. Parasitol. 46: 701-702, 1960.

Coatney, G. R. and Greenberg, J. The effect of a diet deficient in Factor 3 on the course of Plasmodium berghei infection in mice. Proc. VI Internatl. Congress Trop. Med. & Mal. (in press).

Coatney, G. R. and Hinman, E. H. Malaria. Cyclopedia of Medicine, Surgery and Specialties (in press).

Honors and Awards relating to this project:

Visiting Professor, Department of Preventive Medicine and Public Health, School of Medicine, Howard University.

Visiting Lecturer on Tropical Public Health, Harvard School of Public Health.

Visiting Lecturer on chemotherapy, Malaria Eradication Training Center, Kingston, Jamaica.

Consultant to Chief, Section on Malaria Eradication, Pan American Sanitary Bureau.

Vice President, American Society of Tropical Medicine and Hygiene.

President-Elect, American Society of Tropical Medicine and Hygiene.

Vice Chairman for the meeting of the Expert Committee on Malaria held in Geneva, Switzerland, 25-30 July 1960.

Chairman, Technical Meeting on Malaria, Geneva, Switzerland, 14-19 November 1960.

Member of the Expert Advisory Panel on Malaria of the World Health Organization.

Consultant to the Director, Division of Malaria Eradication, World Health Organization, Geneva, Switzerland, on the place of drugs in malaria eradication in Africa, and in that capacity, visited Liberia, French Equitorial Africa, Belgian Congo, Zanzibar, Tanganyika, Southern Rhodesia, and Geneva, during March and April, 1960.

Serial No. NIAID-130-A
1. Parasite Chemotherapy
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in human malaria

Principal Investigator: G. Robert Coatney

Other Investigators: Morton E. Getz, Henry K. Beye, Harvey Elder, Don E. Eyles, and Martin D. Young

Cooperating Units: Department of Justice, Bureau of Prisons, and Institute for Medical Research, Christ Hospital, Cincinnati, Ohio

Man Years (calendar year 1960):

Total:	3 3/4
Professional:	1 1/4
Other:	2 1/2

Project Description:

Objectives:

1. To evaluate new antimalarial drugs and/or drug combinations and methods of administration applicable to mass chemotherapy as applied to malaria eradication.

2. To study the clinical manifestations and parasitology of simian malaria infections in man with special emphasis on Plasmodium cynomolgi bastianellii.

Methods Employed: Normal inmate volunteers at the Federal Penitentiary, Atlanta, Georgia, are screened before they are admitted to the project. Volunteers are under the care of the resident investigators whether handled as outpatients or as inpatients. They take drugs as prescribed, submit to necessary laboratory procedures and to infection by bites of infected mosquitoes. Participation may be for 6 weeks to 2 months or up to one year depending on the nature of the project.

Major Findings:

McLendon strain of falciparum malaria.

Chloroquine (300 mg, base) and primaquine (45 mg) given together in a

single oral dose beginning day 0-3, and weekly thereafter for a total of 8 doses, resulted in suppressive cure of 5/5 subjects. Controls were positive 11-15 days after infection. After 2 days of parasitemia, each control was given the above drug combination as a single dose and then weekly for a total of 3 doses. Parasites were removed promptly and cure was obtained based on no evidence of infection following 227 days of observation.

Chesson strain of vivax malaria.

Primaquine, given 30 mg weekly (single dose) beginning day 0-1 and continuing on the same day each week and, to another group, at 3 mg daily starting day of infection, failed to prevent or suppress infection. Treated and control patients all exhibited patent infections 9 to 12 days after infection.

A drug combination of primaquine (45 mg) and pyrimethamine (50 mg) given in a single dose weekly, beginning on day 0+7, and weekly thereafter for a total of 4 doses, gave suppressive-cure to 4/5 subjects; the other subject developed a patent infection 240 days after infection. Pyrimethamine (50 mg) given alone, as above, produced suppressive-cure in 1/4 subjects; the other 3 came down on day 82, 83, and 84, respectively, after infection. The controls (5) all came down 12 to 13 days after infection.

An 8-aminoquinoline allied to primaquine and known as CN1115 and Win 10,448 in the United States and as quinocide by the Russians was purported to produce radical cure and result in less toxicity than primaquine. The effective dosage of each drug was supposed to be the same, 15 mg daily x 14.

Primaquine compared with quinocide against sporozoite-induced Chesson strain P. vivax; each drug at 15 mg daily x 14 after 600 mg base chloroquine

Type of attack	1st relapse (Days)	2nd relapse (Days)	3rd relapse (Days)	Days of observation*
		PRIMAQUINE		
E. P.	1/5 (48)	----	---	281-446
D. P.	1/5 (35)	1/5 (250)	---	401-593
		QUINOCIDE		
E. P.	3/4 (7-356)	3/4 (21-218)	2/4 (46-68)	405-446
D. P.	2/4 (32-140)	1/4 (161)	---	443-446

E. P. = early primary attack
D. P. = delayed primary
( ) = days to relapse since last Rx
* = as of 1 December 1960

The results show that primaquine is distinctly superior to the other compound.

Reports on a new 8-aminoquinoline, 6 methoxy-8(5 propylaminoamylamino) quinoline phosphate, Win 5037, indicated that this drug was as therapeutically effective as chloroquine and as effective as primaquine in producing cure. At dosages of 20 mg b.i.d. for 2 days, and then 10 mg daily x 12, and at 30 mg b.i.d. x 2, and then 20 mg daily x 12, the parasites were removed and the fever subsided almost as rapidly as following 600 mg of chloroquine, but the entire regimen did not produce cure. Those on regimen 1 (3 pts) relapsed after 35 to 45 days and were re-treated on two occasions; at neither time was the blood cleared of parasites. Those on regimen 2 (2 pts.) each relapsed on day 103 and were cured with chloroquine and primaquine. Because of toxic effects, failure to cure, and drug resistance after the initial trial, further investigation was not considered warranted.

Simian malaria, Plasmodium cynomolgi bastianellii.

In early May, two infections were induced in inmate volunteers by inoculation of infected blood obtained from a sporozoite-induced accidental infection. Following these infections, individuals (whites and Negroes) have been infected by bites of infected Anopheles quadrimaculatus or Anopheles freeborni fed on infected monkeys. In cases where typical parasites could be demonstrated, the prepatent period ranged from 14 to 29 days. The parasite density ranged from 5 to 500/cmm. In some patients, parasitemia was demonstrated before the onset of symptoms. The most frequent symptom was headache, followed in decreasing frequency by anorexia, abdominal pain, joint pain, nausea, myalgia, back pain, vomiting, chest pain, chills, and cramping. Headache was generally described as an aching, bilateral, frontal pain, fairly persistent and often extending to the back of the head and down the back and the lateral aspects of the neck. There was often muscle tenderness in the head and neck. Abdominal pain was often in the right and left lower upper quadrants. It occasionally was increased on deep inspiration. The most significant findings were splenomegaly and hepatomegaly usually associated with tenderness. In those patients exhibiting a tertian fever, the pattern was initiated between the 19th and 23rd day; there was no correlation between parasitemia and febrile response. Observations to date would show no febrile response after the 75th day of infection (based on 24 patients).

Anopheles freeborni were infected after feeding on two different patients with gametocytes as evidenced by finding normal looking oocyts on the gut and 'normal' sporozoites in the salivary glands, but attempts to infect volunteers

by mosquito bite have so far yielded equivocal results. One patient became patent after 58 days but the source of the circulating parasites is open to question.

Plasmodium cynomolgi cynomolgi.

Eleven inmate volunteers were bitten by large numbers, up to 40, of infected mosquitoes (A.f.) on 8 September, and to date three patients have exhibited evidence of infection (i.e., fever); parasitemia has been demonstrated in only one and that on the 58th day after infection. Blood was drawn from each man on day 8, and subsequently when there was clinical evidence of infection, and inoculated into clean monkeys at Dr. Schmidt's laboratory, Cincinnati. So far no infections have developed in monkeys except in those inoculated with blood from the one patient shown to have had parasites on smear. These results show that P. c. cynomologi will produce infection in man but that it is far less infective than P.c. bastianellii.

Significance to Bio-medical Research and the Program of the Institute: This country, along with other countries of the world, is committed to a program of world-wide eradication of malaria and to that end the Congress appropriated thirty-eight million dollars for fiscal year 1961. It was considered by malariological consultants that non-human reservoirs would not be a problem in this effort, and this may still be true. However, the ease with which P. c. bastianellii of monkeys can be transmitted to man raises an important question about the place of simian hosts in human disease. Studies in volunteers have a two-fold purpose in terms of malaria eradication: (1) clinical and parasitological studies of simian malarias in man, and (2) evaluation of drugs and drug combinations. It is expected that the knowledge obtained can be applied in this field.

Proposed Course of the Project: This project is being enlarged in order to include studies on simian malarias in man along with the assessment of methods of mass chemotherapy and the study of parasite resistance in terms of malaria eradication.

Part B included - Yes

PHS-NIH
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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Eyles, Don E., Coatney, G. R., and Getz, M. E. Vivax-type malaria parasite of Macaques transmissible to man. Science 131:1812-1813, 1960.

Serial No. NIAID-131
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Drug resistance in experimental malaria

Principal Investigator: William F. Cantrell

Other Investigators: None

Man Years (calendar year 1960):
Total: 1 1/2
Professional: 1/2
Other: 2/3

Project Description:

Objectives: To obtain strains of malaria resistant to a variety of antimalarial drugs and to study the genetics of drug resistance by producing hybridization of these strains in the mosquito phase of the life cycle.

Methods Employed: Large populations of Plasmodium gallinaceum are exposed to the pyrimethamine, chloroquine or primaquine by treating infected chickens. After one to three days, 0.5 ml of blood is transferred to a new chicken and treatment is repeated.

Major Findings: Repeated exposure of large populations of malaria parasites resulted in resistance only in the case of pyrimethamine. It appears that even in very large populations parasites resistant to chloroquine or primaquine do not occur. An attempt was made to select a chloroquine resistant strain from the pyrimethamine resistant strain without success.

Significance to Bio-medical Research and the Program of the Institute: The development of resistance to chloroquine or primaquine is a greatly feared possibility in the world-wide malaria eradication program. Fundamental knowledge about the process by which malaria parasites become resistant may lead to means for preventing drug resistance or combating drug resistant strains.

Proposed Course of the Project: Other drugs will be tried in Plasmodium gallinaceum in order to obtain genetic markers. Experiments will be made with Plasmodium berghei in which species chloroquine-resistant strains are known. Here the mosquito phase of the work will be more difficult and will require special study.

The possibility of using X-ray to speed up the development of resistant strains will be explored.

The pyrimethamine resistance characteristic will be employed in studies directed toward the prevention of the development of resistance by simultaneous administration of drugs and other means.

Part B included - No

Serial No. NIAID-131-A
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The effect of adrenal cortical hormone on chemotherapy of experimental malaria

Principal Investigator: William F. Cantrell

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1 1/2
Professional: 1/2
Other: 1

Project Description:

Objectives: To determine whether adrenal cortical hormones affect the action of antimalarial drugs adversely or otherwise.

Methods Employed: Chickens infected with Plasmodium gallinaceum or mice infected with Plasmodium berghei are treated simultaneously with cortisone and one of the antimalarials and the effect is compared with the effect of the antimalarial alone and cortisone alone.

Major Findings: In the case of P. gallinaceum, cortisone (or hydrocortisone) alone was without effect on the course of the infection. In the case of P. berghei, cortisone (or hydrocortisone) alone has a weak but significant antimalarial effect. In the case of P. gallinaceum treated with primaquine, cortisone has an adverse effect on antimalarial action. In the case of P. gallinaceum, treated with chloroquine or with pyrimethamine, cortisone is without effect even in high doses. In the case of P. berghei, the antimalarial action of cortisone (or hydrocortisone) is combined with the antimalarial action of chloroquine, primaquine or pyrimethamine giving a slightly increased antimalarial effect.

Significance to Bio-medical Research and the Program of the Institute: The adverse effect of cortisone on host defenses in a number of experimental infections and in a few human diseases suggested that more information with regard to malarial infections would be useful. The diverse responses found with different drugs and different host-parasite systems indicates that no direct application should be made to human malaria. On the other hand, the

finding that there is system in which cortisone has a favorable effect on therapy is of some value from the point of view of experimental chemotherapy as a science. The finding that cortisone interferes with chloroquine activity against P. gallinaceum but not with primaquine nor pyrimethamine suggests that primaquine activity depends more on a cooperative action by the host defense system.

Proposed Course of the Project: No further exploration of this phase of the work is planned, but studies of other physiological factors affecting the response of malaria to chemotherapy will be made.

Part B included - No

Serial No. NIAID-131-B
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Antimalarial drugs and nucleic acid synthesis

Principal Investigator: Karl A. Schellenberg

Other Investigator: G. Robert Coatney

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1 1/3
Professional: 2/3
Other: 2/3

Project Description:

Objectives: To determine the effects of antimalarial drugs on nucleic acid synthesis in experimental malaria.

Methods Employed: The incorporation of radioactive phosphate into ribonucleic acid and deoxyribonucleic acid was measured and the influence of several antimalarial drugs and other substances was determined in Plasmodium gallinaceum and Plasmodium berghei.

Major Findings: Quinine, chloroquine, and quinacrine inhibited the incorporation of P32 into both RNA and DNA, whereas pyrimethamine and the triazine metabolite of chloroguanide specifically inhibited incorporation of P32 into DNA. The inhibition by pyrimethamine was not reversed by folic acid, folinic acid, thymidine, deoxyuridine, uracil, thymine, glycine or adenine. Chloroguanide was active in vivo but not in vitro.

Significance to Bio-medical Research and the Program of the Institute: The highly specific effect of pyrimethamine on deoxyribonucleic acid metabolism occurring at concentrations which may be reached in actual therapy affords an explanation of drug action on a more fundamental level than is available for most drugs. This linking of drug action to synthesis of the genetic material of a parasitic organism affords an approach to the discovery of new drugs through advances in the highly active field of nucleic acid enzymology.

Proposed Course of the Project: Dr. Schellenberg has resigned in order to continue advanced study in biochemistry. Continuation of this important line of research depends on finding a suitably trained person.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Schellenberg, K. A. and Coatney, G. R. The Influence of antimalarial drugs on nucleic acid synthesis in Plasmodium gallinaceum and Plasmodium berghei. Biochem. Pharmacol. (in press).

Schellenberg, K. A. and Weinbach, E. C. The endogenous formation of phosphoenolpyruvate by rat-liver mitochondria. Biochim. et Biophys. Acta 45:593-595, 1960.

Serial No. NIAID-131C
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Experimental chemotherapy of helminthic diseases

Principal Investigator: George W. Luttermoser

Other Investigators: None

Cooperating Units: Dr. B. Prescott, LID, NIAID-61A and Dr. J. Tobie, LI, NIAID-148

Man Years (calendar year 1960):
Total: 1 1/2
Professional: 1/2
Other: 1

Project Description:

Objectives: To discover new and more effective schistosomacides and compounds active against other helminth parasites. To learn how the chemicals selectively kill those parasites.

Methods Employed: Mice are infected with cercariae of Schistosoma mansoni and treatment is begun (1) immediately (prophylaxis) or (2) after 35 days (curative). The mice are examined 50 days or more after exposure to infection or for the presence of dead worms. In critical evaluation of drug activity, the liver and portal system are perfused and the live or dead worms are counted. Live mature worms removed from treated and untreated animals are observed in maintenance culture for activity and survival. Drugs are added to some of the cultures. Monkeys are infected with the same parasite and dogs, with S. japonicum (Formosan). A test for either prophylactic or curative activity of promising compounds was conducted in the larger animals in a similar fashion to the mouse test except that fecal egg counts were also utilized as an indicator of the status of the infection.

For studies on nematode parasites, dogs naturally infected with Dirofilaria immitis and mice infected with pinworms were utilized. Observations were made on the parasiticidal effects of the drugs in vivo and in vitro. Fluorescence of the helminth was read in UV light which indicated whether the drugs were absorbed.

Major Findings: Completion of the test for prophylactic activity of the antibiotic S1629 (Griseofulvin or Fulvicin) indicated that oral administration of 50 mg/kg of the drug daily starting the fourth day before exposure to S. mansoni and continuing for 12 days brought about a reduction in the numbers of schistosomes developing in these animals. This was not found in similar experiments with hamsters. There was, however, an indication of weak prophylactic activity in two monkeys started on the drug two days before exposure and continued on a daily dose for 9 days.

Since filariids and other helminths absorb tetracycline and will fluoresce (Tobie and Beye, 1960), a study was made of the effects of tetracycline and 12 analogues of tetracycline on Dirofilaria immitis and on mouse pinworms. A comparison was made of the longevity of the larvae and adults of these nematodes in maintenance media with and without drugs added at final concentrations of from 1 µg to 1 mg per ml. One of the analogues (1686-7) killed the microfilariae of D. immitis at in vitro concentrations as low as 10 µg/ml in a period of 4 to 9 hours, while tetracycline itself did not do so. The oral administration of 15 daily oral doses of 20 mg of this analogue per kg body weight likewise caused a marked reduction in the microfilarial blood count. Nevertheless, many of the micro- and macrofilaria which fluoresced after in vitro exposure to other of these "tetracyclines", continued to live for several hours afterward. In mice with pinworm infection, the analogue 1686-7 was not more active than tetracycline itself in clearing the animals of pinworms. However, a 2 day regimen of another of the analogues (1689) cleared the pinworm infections from most of the mice while a similar regimen of an active piperazine did not. Weak fluorescence was only observed in small areas of the upper intestine and of the genital system of the pinworm whereas strong fluorescence was observed throughout the body wall and intestine of the filariids.

The analogues of tetracycline tested have not been found to be schistosomacidal nor have they been found to cause the schistosome to fluoresce.

Significance to the program of the Institute: New information with regard to a reaction between a chemical and a parasite may be a "lead" for the development of better drugs needed for control of parasitic infections such as schistosomiasis and filariasis. Current treatments for these infections are inadequate.

Proposed Course of the Project: The principal investigator will be Visiting Professor at the American University of Beirut, Lebanon, during 1961. Studies of effects of new drugs on schistosomes will be continued by Dr. William Cantrell.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Honors and Awards relating to this project:

President of the Helminthological Society of Washington, 1960.

Appointed consultant on schistosomiasis to WHO and accepted assignment to Ministry of Health of Venezuela for the period 22 January to 18 March, 1960.

Named Visiting Research Professor in the Department of Tropical Health, American University, Beirut, Lebanon, for the year 1961.

Serial No. NIAID-131-D
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Effects of nutrition on chemotherapy of parasitic diseases.

Principal Investigator: George W. Luttermoser

Other Investigators: None

Cooperating Units: Dr. W. DeWitt, LPD, NIAID-121K

Man Years (calendar year 1960):
Total: 1 1/2
Professional: 1/2
Other: 1

Project Description:

Objectives: To determine whether the nutrition of the host can influence the chemotherapy of parasitic infections.

Methods Employed: Animals maintained on defined diets either enriched or deficient in certain components are exposed to a single species of parasite and the developing infection is treated in either the prepatent or patent period. The influence of the diet on treatment is determined by (1) the reduction of the number of parasites (2) the condition of the parasites (3) growth and survival of the host and (4) the pathologic conditions developing in the host.

Major Findings: In many tests, the efficacy of stibophen (Fuadin) therapy on mature Schistosoma mansoni infections in mice was increased up to 16 times by feeding a balanced semi-synthetic diet. The toxicity of the drug was not similarly increased. The enhancement of curative action by the purified semi-synthetic diet was thought to be due to the absence of, as yet unidentified, inorganic salt(s) that interfere with drug activity. It was found in mice fed on the purified semi-synthetic diet that higher blood levels of the drug were maintained for a longer period than when the same amount of Fuadin was injected into mice fed on the commercial pellet diet, suggesting that the increased cure-rate was due to higher blood drug level. Similar drug advantage was observed in mice given tartar emetic while on the purified diet.

Significance to the Program of the Institute: Since parasitic infections occur most frequently in backward areas where malnutrition is also prevalent, it is necessary to determine the relationship between the nutrition of the people and the results obtainable by chemotherapy. Failure of chemotherapy is a common experience in such areas. Projects for screening drugs for schistosomacidal activity might be improved if more optimum dietary conditions for experimental chemotherapy are found.

Proposed Course of the Project: During the next year the Principal Investigator will be Visiting Professor at the American University, Beriut, Syria. During his absence Dr. DeWitt (LPD) will continue the investigation to determine the specific nutritional factors involved in the enhancement of the efficacy of stibophen in schistosomiasis. Other drugs used for the treatment of this and other parasitic diseases will be investigated under similar conditions with different animals.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Luttermoser, G. W. and DeWitt, W. B. Studies on interrelations of nutrition and treatment of schistosomiasis I. Enhancement of stibophen (Faudin) activity against Schistosoma mansoni in mice by feeding purified semi-synthetic diets. Am. J. Trop. Med. & Hyg. (in press).

Serial No. NIAID-131-E
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Comparison of antimalarial drugs administered singly and in various combinations.

Principal Investigator: Richard L. Jacobs

Other Investigators: William F. Cantrell

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1/3
Professional: 1/6
Other: 1/6

Project Description:

Objectives: To determine the dosage-level of chloroquine, primaquine, amodiaquine and pyrimethamine required to give any desired degree of suppression of parasitemia in mice. To compare the effectiveness of various combinations of these drugs to single-drug treatment.

Methods Employed: NIH Swiss female mice, averaging 20 gm/each, were infected by tail-vein injection of one million RBC infected with P. berghei NYU-2 strain. Drugs were administered orally at various levels for five consecutive days, beginning on day of infection. Blood smears were made on four consecutive days, beginning on the third day after infection, for evaluation of the effect of different treatments on the course of parasitemia. Each trial involved five animals per group and all trials were repeated at least one time.

Major Findings: Chloroquine, primaquine, and amodiaquine, when reduced to base equivalents, were equally effective in suppressing parasitemia in mice by the method described. Approximately 50 percent suppression in parasitemia resulted from administering 40 µg/mouse/day. Pyrimethamine is equally effective at one-third the dosage level of the above drugs.

Various combinations of these four drugs were no more effective than single-drug treatment. When combined, results indicated consistently that the effect was additive, or less than additive.

Significance to Bio-medical Research and the Program of the Institute: The practice of combining drugs has been used extensively, often with favorable results. Reports have indicated that certain combinations of antimalarial drugs exhibit a synergistic effect in suppression of parasitemia (quinine and pamaquine in chicks). Also, clinical trials have indicated that antimalarial combinations are more effective in preventing relapse in vivax malaria. The results of this study indicate that combinations of the drugs tested offer no advantage over single-drug therapy in suppressing erythrocytic forms of P. berghei. Suppressive levels of drugs have been established for use in other studies.

Proposed Course of the Project: This project has been completed.

Part B included - No

Serial No. NIAID-131-F
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Effect of nutritional status of host on the action of antimalarial drugs.

Principal Investigator: Richard L. Jacobs

Other Investigators: William F. Cantrell

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives: To study the effect of the nutritional status of the host animal on the course of parasitemia in experimental malarial infection, both with suppressive levels of antimalarial drugs and in the absence of drug therapy. It is hoped that the results of this study will yield information regarding the mode of action of antimalarial drugs.

Methods Employed: The parasite P. berghei, NYU-2 strain is being studied in mice of altered nutritional status. Young NIH Swiss female mice are obtained averaging nine to ten grams each and are maintained on various test diets for approximately four weeks prior to infection. An alternate method consists of the administration of antimetabolites prior to, or at, time of infection. Effects are studied both in absence of and in suppressive levels of chloroquine.

Major Findings: Preliminary results indicate that alterations in the course of parasitemia in mice by dietary deficiencies are variable. The variability is due, primarily, to the degree of the deficiency. Also, the possibility of multiple-deficiencies cannot be neglected when the semi-purified diet is employed.

In tests employing folic acid deficient diets, the effect on the course has been variable; however, a striking suppression of parasitemia has

consistently been observed when aminopterin is administered.

The effect of nutritional deficiencies appears to be influenced by suppressive drug treatment. Pyridoxine-deficient animals without drug treatment showed an increased parasitemia; when a 50% suppressive dose of chloroquine was administered, the pyridoxine-deficiency appears to inhibit parasitemia.

Vitamin deficiency states that appear to favor the course of parasitemia include: Pantothenic acid, biotin, riboflavin and choline.

Deficiencies that suppress parasitemia include: ascorbic acid and niacin.

Significance to Bio-medical Research and the Program of the Institute: It is believed that the results of this study may give some insight into the mode of action of antimalarial drugs.

Proposed Course of the Project: Investigations along the lines outlined above will be continued and the use of antimetabolites will be extended. Further work will be initiated to clarify promising leads.

Part B included - No

Serial No. NIAID-132
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies on Human Malaria

Principal Investigator: Martin D. Young

Other Investigators: None

Cooperating Units:

Man Years (calendar year 1960):
Total: 3
Professional: 1/2
Other: 2 1/2

Project Description:

Objectives:

To add to the biological knowledge of malaria by observations and experiments on induced malaria. To determine the sporontocidal activity of antimalarial drugs and how developed resistance influences transmission by mosquito vectors. To study the development and inheritance of resistance to drugs by the parasites. To determine if the development of resistance to insecticides by mosquitoes alters their ability to transmit malaria. To serve as probably the only source in the nation for established strains of malaria for use on a nationwide basis for therapy of neurosyphilis, nephrosis, Parkinsonism, and for experimental malaria studies.

Methods Employed:

Malaria is induced in neurosyphilitic patients and in volunteers under normal and experimental conditions. The patent infections are challenged by drugs. When resistance occurs measurements are made of its characteristics. Mosquitoes are allowed to bite patients with induced infections before and at intervals after drug administration. The resulting infections in the mosquitoes are correlated with the presence or absence of drug resistance of the malaria infection in the human host. Insecticide-resistant and normal mosquitoes are fed simultaneously for comparison of ability to transmit malaria.

Major Findings:

Primaquine given 1.5 mg. daily had sporontocidal effect upon Plasmodium vivax and P. falciparum but little effect upon the asexual parasites. Daily doses of 0.75 mg. had some sporontocidal effect upon P. falciparum but none against one P. vivax case. Therefore, 1.5 mg. daily appears to be the lowest amount that is reliably sporontocidal against both P. falciparum and P. vivax.

Therapeutic doses (1.4 gm in 3 days) of amodiaquine had no sporontocidal effect upon one P. falciparum case.

P. falciparum from South Rhodesia was susceptible to pyrimethamine.

Preliminary tests indicate no difference in vectorial ability of insecticide-susceptible and insecticide-resistant mosquitoes.

P. vivax was maintained in a viable condition for five years at -70° C. The adding of glycerine to blood containing chicken and rat malaria aids in its preservation at low temperatures.

A 14-year study of the biology of P. malariae, during which time it had been passaged through neurosyphilitic patients mainly by blood transfusions, revealed the following: there was no apparent reduction in viability; the infection and completion of the sporogonous cycle in Anopheles aztecus; the comparative susceptibilities of the mosquitoes tried, in decreasing order, were: A. freeborni, A. punctipennis, A. aztecus, and A. quadrimaculatus; no infections were found in A. crucians and A. albimanus; the length of the sporogonous cycle in A. quadrimaculatus ranged from 19 to 23 days when incubated at 76° F.; A. freeborni when paired with A. quadrimaculatus also had an identical 23-day cycle; of 7,354 A. quadrimaculatus dissected, 3.1 per cent were infected; the oocysts per infected gut averaged 3.0; only 3.9 per cent of the guts had 10 or more oocysts; transmission of the malaria to six patients was accomplished, three by the bites of infected mosquitoes and three by the intravenous injection of the glands of these mosquitoes; there was wide variation in the infectiousness of different malarious patients to mosquitoes; mosquitoes became infected in nearly every week of the first six months with the highest infectivity occurring during the eighth to tenth weeks of the primary attack; the asymptomatic parasitemias infected mosquitoes at about the same rate as the symptomatic parasitemias.

an autochthonous chronic asymptomatic case of P. malariae of at least 18 months duration infected mosquitoes; although the infection rate of mosquitoes in ordinarily low, the relatively long period of infection during which mosquitoes can be infected may explain the persistence of P. malariae in certain areas; the ability of the symptom-free malarious patient to infect mosquitoes at a rate similar to that of the symptomatic patient makes case finding more difficult, especially in important areas of malaria eradication.

A strain of P. falciparum induced into neurosyphilitic patients was resistant to normal and above normal doses of chloroquine.

Significance to Bio-Medical Research and the Program of the Institute:

The finding of resistance of malaria parasites to chloroquine is of importance to the malaria eradication programs in many parts of the world as drugs are being used as supplemental measures in some programs. It is important to determine if there is cross-resistance between the 4-aminoquinoline drugs. Additional information is needed on the biology of the parasite especially in relationship to drugs.

The comparative ability of insecticide-susceptible and insecticide-resistant mosquitoes to transmit drug-susceptible and drug-resistant parasites needs to be determined, especially to indicate whether the change in the vector or parasite has significance in the epidemiology of malaria.

The low but persistent infectivity of P. malariae to mosquitoes helps explain its epidemiology and especially its persistence in nature.

Proposed Course of the Project:

To determine more fully the nature and extent of the chloroquine resistance in human malaria, as well as cross-resistance to other drugs such as the 4-aminoquinolines. Further investigate the ability of insecticide-susceptible and insecticide-resistant mosquitoes to transmit drug-susceptible and drug-resistant malaria parasites. Continue to obtain basic information, especially epidemiological and host-parasite relationships on induced human malarias, both domestic and foreign. Important strains of malaria will be added to those now in the local repository which apparently is the only existing one for the preservation of malaria.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B.

Honors, Awards, and Publications.

Publications other than abstracts from this project:

Young, M. D. The Effect of Small Doses of Primaquine Upon Malaria Infections. Ind. J. Mal. 13:69-74. 1960.

Young, M. D. MALARIA - A Study of Scientific Exploration and Achievement. Bull. S. C. Acad. Sci. 21:32-37. 1960.

Young, M. D. Malaria (Chapter in Manual of Tropical Medicine, third edition, by Hunter, Frye, and Swartzwelder. Saunders). 1960.

Jeffery, G. M. Infectivity to Mosquitoes of Plasmodium vivax and Plasmodium falciparum Under Various Conditions. Am. J. Trop. Med. and Hyg. 9:315-320. 1960. May.

Jeffery, G. M. Book Review: The Ecology of Human Diseases, by Jacques M. May. Am. J. Trop. Med. and Hyg. 9: 350-351. 1960.

Young, M. D. Book Review: Parasitology (Protozoology and Helminthology) in Relation to Clinical Medicine, by K. D. Chatterjee. Am. J. Trop. Med. and Hyg. 9: 351.

Young, M. D. Chemotherapeutic agents and malaria eradication. Proceedings of Sixth International Congresses on Trop. Med. and Mal. (In press). 1960.

Garnham, P. C. C., Jeffery, G. M., and Young, M. D. Preservation of strains of malaria parasites. Proceedings of Sixth International Congresses on Trop. Med. and Mal. (In Press.) 1960.

Jeffery, G. M. Inoculation of Human Malaria into a Simian Host, Macaca mulatta. J. Parasit. (In Press.)

Young, M. D., and Burgess, R. W. The Infectivity to Mosquitoes of Plasmodium Malariae. Am. J. and Hyg. (In press). 1960.

Honors and Awards Relating to this Project:

For Dr. Martin D. Young:

Visiting Lecturer, Department of Microbiology, Meharry Medical School.

Visiting Lecturer and Temporary Advisor on Chemotherapy PAHO Malaria Eradication Training Center, Kingston, Jamaica.

Member, City Board of Health, Columbia, South Carolina.

Member, Expert Advisory Panel on Malaria, World Health Organization.

Member, Editorial Board, American Journal of Tropical Medicine and Hygiene.

Serial No. NIAID-132-A
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Chemotherapy of intestinal parasites.

Principal Investigator: Martin D. Young

Other Investigators: Geoffrey M. Jeffery

Cooperating Units: South Carolina State Hospital

Man Years (calendar year 1960):

Total:	2 5/6
Professional:	5/6
Other:	2

Project Description:

Objectives:

To find adequate drugs for the prophylaxis and treatment of parasitic infections which constitute serious health problems in certain types of mental patients as well as in more normal populations; to determine the relationship of parasite load to psychological or psychiatric considerations in the patients; also to investigate factors influencing parasite spread and persistence among patients.

Methods Employed:

Various segments of the institutional population are examined to determine qualitatively, and quantitatively when possible, their parasitic infections. Promising compounds in varying dosages and regimens are tried against selected cases; post-treatment evaluation determines the effectivity of the drug against the various parasites. Occasionally careful pre- and post-treatment psychological, psychiatric and physical evaluations are done to determine the possible effect of parasite removal. The results are based upon thousands of patient-drug (individual regimens) trials.

Major Findings:

Epidemiological studies on a defined population show a high persistence of Trichuris and hookworm for six years, with an apparent decrease in Strongyloides. The yearly studies show fluctuations in protozoal infections indicating that transmission is occurring.

To test the therapeutic and prophylactic effect of dithiazanine and tetrachloroethylene alone and in combination, a group of mental patients heavily parasitized were given drugs at various intervals for about one year. A large number of worms were removed but the cure rate was low. Treatments subsequent to the initial one had reduced effectiveness. Transmission was not stopped. These drugs do not appear to be adequate prophylactic agents under conditions of high exposure such as represented in this study.

Bephenium hydroxynaphthoate continued to exhibit excellent results against hookworm and Ascaris infections found in mental patients. A retrial of bephenium chloride was begun. The results so far indicate that it may be more effective in single comparative doses than the bephenium hydroxynaphthoate. It is showing promising activity against Trichuris. Combined with an anti-nausea drug, Marezine, the side effects of nausea and vomiting appear to be reduced.

Significance to Bio-Medical Research and the Program of the Institute:

Currently available drugs in the regimens tried have not been shown to be adequate in the prevention of transmission of Trichuris and hookworm parasites in areas of high exposure risks. Better drugs, or better regimens of the present ones, are needed. Information obtained should be of value in mental hospitals and in many tropical areas.

Proposed Course of the Project:

The evaluation of promising compounds for better prophylactic and parasiticidal agents will be continued with especial interest on different regimens of bephenium chloride. Parallel investigations are underway on the mode of action of drugs on parasites. Efforts are being made to explain the failure of effective drugs to produce radical cures, the reduced effectiveness of drugs after the initial treatment, and similar problems. Further investigations on the epidemiology of parasitic infections in mental hospital populations will be continued.

Part B included: Yes.

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Serial No. NIAID-132-A

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Jeffery, G. M. A Three-Year Epidemiologic Study of Intestinal Parasites in a Selected Group of Mental Patients. Am. J. Hyg. 71:1-8. 1960.

Young, M. D., Jeffery, G. M., Morehouse, W. G., Freed, J. E., and Johnson, R. S. The Comparative Efficacy of Bephenium Hydroxynaphthoate and Tetrachloroethylene against Hookworm and other Parasites of Man. Am. J. Trop. Med. & Hyg. 9:488-495.

Serial No. NIAID-132-B
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Physiology of human parasites especially as related to nucleic acids and drug action.

Principal Investigator: Kenneth O. Phifer

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives:

To determine the place of action of drugs upon malaria and intestinal parasites of man. The study of the action of drugs on malaria includes the mechanism and locale, the development of resistance and a search for methods to avoid the development of resistance or how to overcome it. Similar studies are planned on the developmental chemotherapy of other parasites, especially the worms of the intestinal tract.

The nucleic acids of malaria parasites are being examined qualitatively and quantitatively in order to determine whether drug-resistance affects the synthesis of these substances. To ascertain whether malaria-infected mosquitoes have higher nucleic acid levels than non-infected mosquitoes. To determine what effect anthelmintics have on the in vitro metabolism and in vivo distribution of helminths.

Methods Employed:

Malaria-infected red blood cells and infected mosquitoes are homogenized and fractionated into ribonucleic acid; this

fraction is then measured colorimetrically. Qualitative analysis of the nucleic acid fraction is carried out through the use of paper chromatography and paper electrophoresis.

Helminths are incubated both in the presence and in the absence of anthelmintics and several measures of metabolism are utilized; e.g., transaminase reaction and rate of glucose uptake. Also noted is the effect of anthelmintics on egg production and distribution of the worms in the intestine of the host.

Major Findings:

It was shown that mosquitoes infected with malaria parasites tend to have higher levels of ribonucleic acid than do uninfected mosquitoes.

Chromatographically, the acid-hydrolysate of ribonucleic acid from a pyrimethamine-resistant strain of Plasmodium falciparum appears to be different than the acid-hydrolysate of ribonucleic acid from a pyrimethamine-susceptible strain of this species. Preliminary experiments point toward similar findings in the case of chloroquine resistance.

Bephenium hydroxynaphthoate inhibited glutamic acid-alanine transaminase and glutamic acid-aspartic acid transaminase in Nippostrongylus muris in vitro. This drug also appears to remove more worms from the anterior part of the intestine of infected rats than from more posterior segments of the gut.

Bephenium chloride and atabrine reduced the rate of glucose absorption by the tapeworm, Hymenolepis diminuta, from 30 to 50 per cent. At low concentrations the cyanine dye, dithiazanine iodide stimulated glucose absorption by this cestode.

Significance to Bio-Medical Research and the Program of the Institute:

Since several antimalarial agents have chemical structures similar to those of components of nucleic acids it seems advisable to determine whether these drugs affect the parasite's basic nuclear compounds. It is felt that if it were demonstrated that nucleic acids are altered by antimalarials, this clarification of mechanism of drug action would provide a more logical rationale for the synthesis of new drugs.

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Resistance of parasites to drugs is a serious world problem with respect to malaria eradication. Since resistance has been shown to be under genetic control in many instances, demonstration of changes in nucleic acid composition in response to exposure to a drug should be of value in determining mechanisms of resistance. Once mechanisms of resistance are delineated, the problems associated with combatting drug resistance might be more readily solved.

A corollary aim of this project is the attempt to develop a biochemical means of detection of infection of mosquitoes before morphological stages in the parasite's life cycle are evident. If such a means proved reliable, some insight could be gained as to the changes occurring and the experimental procedures would be shortened.

Study of the effect of anthelmintics on helminth metabolism will aid in a clearer understanding of drug action and might be valuable in the design of new drugs.

Study of the effect of anthelmintics on the distribution of helminths in the host intestine is important since in human cases of helminthiasis, a few worms often remain after therapy and the question of whether this is due to resistance to the drug or a re-location in the gut should be answered.

Proposed Course of the Project:

Studies of nucleic acid composition of malaria parasites from drug-resistant and drug-susceptible strains will be continued using both paper chromatography and absorption spectrum analysis via spectrophotometry.

Infected mosquitoes will be examined for ribonucleic acid level to determine conclusively whether increased nucleic acid levels can be reliable indices of infection.

Studies with respect to the permeation of drugs into the malaria parasite will be carried out, comparing the uptake of drug in susceptible and resistant strains.

Animals infected with helminths will be drugged with anthelmintics and the worms will be counted, position and size of worm noted, and relative metabolic activity assayed. Both cestodes and nematodes will be utilized.

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Part B included: No

Serial No. NIAID-132-C
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Virus-Parasite Association

Principal Investigator: Geoffrey M. Jeffery

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total:	1 1/3
Professional:	1/3
Other:	1

Project Description:

Objectives:

To determine experimentally if parasites might be associated with some of the virus diseases of man or animals as vectors or in some other capacity. To study such relationships under natural conditions and to investigate known virus infections and known populations to determine existing relationships, including the study of possible intestinal viruses among institutionalized mental patients. In view of current findings, further study the relationships of virus-malaria and virus-endoparasite associations under experimental conditions. Investigate the possibility that certain parasites may themselves be afflicted with harmful virus infections. Investigate the effects of parasitic infections upon virus infections and vice versa.

Methods Employed:

Small animals and their common parasites are used in attempts at transmission of viruses. Viruses, such as lymphocytic choriomeningitis (LCM), polio, St. Louis encephalitis (SLE), rabbit papilloma and fibroma are maintained in small animals by serial transmission, providing infective virus material for

experimental work. Besides the common helminth parasites of mice, Nippostrongylus muris, Nematospiroides dubius, Hymenolepis nana, and others, the malaria parasites Plasmodium berghei and P. gallinaceum are also maintained as experimental parasites. Viruses and parasites are brought together in various ways, such as by simultaneous infection in animals, during cultivation of the free-living stages, or by simultaneous exposure of the host to infective parasite stages and virus suspensions. Tissue culture or small animals are used for detection of virus infection carried by parasites from virus-infected animals, for virus detection in parasites suspected of harboring viruses in nature, and for detection of virus infection in arthropods. Incidence of intestinal viruses and their possible association with parasites in institutionalized mental patients is studied with animal inoculation, tissue culture, and other means.

Major Findings:

In mice inoculated with SLE virus intraperitoneally, the presence of P. berghei aided in the transport of the virus to the central nervous system as shown by symptoms and by death of the mice. The same occurred when LCM virus and P. berghei were combined. The virus appeared earlier and persisted longer when malaria was present. SLE virus and P. gallinaceum combined caused death earlier in some chicks and enhanced the viremia in others over that found when malaria alone was present.

Significance to Bio-Medical Research and the Program of the Institute:

Many problems associated with the transmission of viruses in human and animal populations remain unsolved. New viruses in mammals and arthropods are continually being discovered. There are suggestions in the literature and theoretical possibilities that parasites may act as reservoirs and agents of transmission for viruses of importance to man. Elucidation of association of viruses and parasites, both experimental and natural, will do much toward bringing about a more complete understanding of the epidemiology of diseases produded by such organisms, alone or in combination. The possible role of malaria in transmitting virus from host to host either by extending the viremia or by actually transporting the virus in the sporozoite is of epidemiological importance.

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Proposed Course of Project:

The general course of the project will be changed little. Repetition and expansion of the experiments combining nematode parasites and viral agents in the vertebrate host, during free-living stages, and in culture, will be carried out. An increased amount of effort will be directed toward the association of viruses and malaria parasites, and possibly other protozoans. The detection of viruses in malaria parasites, especially the sporozoites by fluorescent antibody techniques and by inoculation will be attempted. Further refinement of viral detection methods will be investigated and incorporated in the work when practicable. Most of the experimental work planned is in the realm of artificial viral-parasite associations, but the possibility of extension of the studies to naturally occurring associations will be always in mind and such studies will be pursued as opportunities arise.

Part B. included: Yes.

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Serial No. NIAID-132-C

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Part B Honors, Awards, and Publications

Honors and Awards Relating to this Project:

Member Council, South Carolina Academy of Science.

Editor, ASB Bulletin, published by the Association of Southeastern Biologists.

Recipient of Jefferson Award of the South Carolina Academy of Science.

Serial No. NIAID-132-D
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Development of human pathogens in immature insects.

Principal Investigator: William E. Collins

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total:	1 1/2
Professional:	1/2
Other:	1

Project Description:

Objectives:

To determine experimentally to what extent immature stages of arthropods will support growth of pathogenic organisms with particular emphasis on the viruses and protozoans in mosquito larvae. If experimental evidence indicates the growth or maintenance of these microorganisms in insect larvae, investigations will be made to determine the possible role larval infection plays in the transmission of these infections to man.

Methods Employed:

The larvae of mosquitoes belonging to several different genera are exposed to virus suspensions under varying conditions. The quantity of virus present in larvae, pupae, and adults subsequent to exposure is determined by small animal inoculations. Transmission of the virus by the adult mosquitoes is made using small animals and blood pools.

Major Findings:

Further studies confirmed the results of the prior year where the virus of St. Louis encephalitis was taken up by the larvae of Aedes aegypti and an increase in virus titer found in subsequent larval and pupal mosquitoes. The larvae of Culex quinquefasciatus exposed under similar conditions take up the virus to a lesser extent, and the adults have not demonstrated the virus. Transmission of the virus to baby chicks has been confirmed using mosquitoes 9 to 26 days after adult emergence. The rate of adult infection and virus transmission using St. Louis encephalitis virus and Aedes aegypti mosquitoes has been less than one per cent under these experimental conditions.

Significance to Bio-Medical Research and the Program of the Institute:

The presence of viruses and protozoans in mosquito breeding pools as a result of improper sanitation procedures or by death and decomposition of infected animals and arthropods poses the possibility that these organisms may be taken up by the mosquito larvae and subsequently transmitted by the adults. Information on such larval infection will add to a more complete understanding of the epidemiology of a number of arthropod-borne diseases.

Proposed Course of the Project:

The general course of the project will be changed little. The relative ability of mosquito larvae of different genera to take up the virus of St. Louis encephalitis and the effect of initial virus titer, incubation temperature and larval instar on the rate of infection, virus titer and adult transmission will be tested.

Part B included: No.

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Serial No. NIAID-132-E
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Insect Tissue Culture

Principal Investigator: William E. Collins

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

To establish insect tissues in culture in order to study better those factors necessary for insect growth and metamorphosis and the effect of microorganisms on insect tissues. To determine which of the viruses, protozoans or helminths will persist, develop, or multiply in the different insect tissues grown in vitro using both natural vectors and other species.

Methods Employed:

Tissues and cells from moth larvae and pupae and from mosquito larvae, pupae and adults are removed and set up in experimental culture media using several different culture methods. The presence of mitosis, tissue contractility or general cell condition are used as indications of tissue growth or maintenance. Viruses are added to such cultures and periodic samples collected and titrated in small animals. Protozoans are added and microscopic examinations made to determine persistence or development of the parasites. Guts of mosquitoes previously infected with Plasmodium sp. are removed and set up in culture. Periodic examination and measurement of the parasite development are made.

Major Findings:

Blood cells from caterpillars and cells of the ovariole sheath of several species of moth prepupae and pupae have undergone growth in culture in several different experimental media. The virus of St. Louis encephalitis has been maintained in cultures of third, fourth, and fifth instar hemocytes from the catalpa sphinx (Ceratomia catalpae) for 10 days with little drop in titer over that which occurred during the first 24 hours. The oöcysts of Plasmodium gallinaceum attached to mosquito midguts in vitro have exhibited growth and on several occasions, sporozoites have been produced.

Significance to Bio-Medical Research and the Program of the Institute:

Insects serve as alternate hosts for a number of virus and protozoan diseases of man. Insect tissue culture would offer the opportunity to study these parasites and their growth in insect cells in vitro, and thus quite possibly shed new light on: (1) parasite growth requirements (2) arthropod tissue specificity and (3) reasons why many human pathogenic viruses will multiply in the insect with no visible or apparent pathology. These studies may yield information on those factors controlling the ability of insect vectors to transmit virus and protozoan diseases whereas closely related species of insects do not.

Proposed Course of the Project:

Various tissues of mosquito larvae, pupae, and adults, and moth larvae and pupae will be established in culture and tested for their ability to support the virus of St. Louis encephalitis. Microscopic studies will be made in efforts to detect any cytopathology of the tissue as a result of virus infection. In addition, fluorescent antibody studies will be made to determine in which cells or tissues the virus is present. Guts from mosquitoes infected with Plasmodium sp. will be set up in different culture media in an attempt to determine those factors necessary for the development of sporozoites in vitro. Further studies on the effect of virus on caterpillar blood cells and ovariole tissue will be made.

Part B included: No.

Serial No. NIAID-133
1. Parasite Chemotherapy
2. Cytology
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies of the biology of exoerythrocytic phases of primate malaria.

Principal investigator: Don E. Eyles

Other investigators: Robert L. Ingram, Nell C. Owen, Frances E. Jones and C. S. Smith

Cooperating Units: None

Man Years (calendar year 1960):

Total	3
Professional:	1 1/2
Other:	1 1/2

Project Description:

Objectives: To extend the knowledge of the exoerythrocytic stages of primate malaria, determine the type of cells parasitized, and trace the development from sporozoite to mature schizont. To determine if the liver is the sole tissue parasitized. To determine the origin and nature of the late tissue stages and to determine their relationship to relapse. To define in detail quantitative relationships in the exoerythrocytic cycle. To study the exoerythrocytic cycle as it is related to the immune processes of the monkey host. To determine factors influencing the degree of exoerythrocytic infection. To provide materials and correlative information to accompany projects on growth of malaria parasites in vitro and the chemotherapy of malaria.

Methods Employed: Plasmodium cynomolgi, which is closely related to P. vivax of man, is maintained in Macaca mulata monkeys. Strains of P. gonderi (also a simian vivax-type parasite) and P. inui (related to human P. malariae) are also maintained. Anopheles mosquitoes of several species are produced and mosquitoes infected in order to produce very large numbers of infected mosquitoes. Salivary glands are dissected from these mosquitoes and injected intravenously in order to produce patent liver infection.

At predetermined intervals, liver specimens are taken by laparotomy. Histological studies are made on living and stained material. Flourescent-antibody as an aid in locating parasites is employed, and other labelling methods will be used as necessary.

Major Findings: Continued study was made on quantitative aspects of the problem such as the relationship of inoculm size to the development of liver parasites and subsequent parasitemia. Other studies were made which indicated that secondary generations of exoerythrocytic parasites in the liver do occur. Experiments on route of inoculation (ie. portal circulation versus femoral vein) indicated no difference in degree of liver infection.

Work on this project was curtailed due to the urgency of work on malaria as a zoonosis.

Significance to Bio-medical Research and the Program of the Institute: Confirmation of the liver cycle of malaria puts work on chemotherapy on a sound basis. Unelucidated aspects of the cycle may have significance in chemotherapy and basic biology of the parasites. Study of simian species is especially appropriate as the close relationship to the human species indicates that any findings may be directly applied to human infection with few reservations. The present project is closely correlated with chemotherapy and tissue culture projects, and is another phase of the integrated malaria study of the Laboratory of Parasite Chemotherapy.

Proposed Course of the Project: During the coming year special emphasis will be placed on study of the exoerythrocytic stages of newly isolated strains of simian malaria and on comparative biology of the parasites of different species of primate malaria. Work is again proposed on the influence of immunity on exoerythrocytic parasites, and the project will continue to be a source of material for tissue culture work and for chemotherapy studies.

Part B included - Yes

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Eyles, D. E. Anopheles freeborni and A. quadrimaculatus as experimental vectors of Plasmodium cynomolgi and P. inui. J. Parasit., 46:540. 1960.

Eyles, D. E. The exoerythrocytic cycle of Plasmodium cynomolgi and P. cynomolgi bastianellii in the rhesus monkey. Amer. J. Trop. Med. & Hyg., 9:543-555. 1960.

Eyles, D. E. Susceptibility of Anopheles albimanus to primate and avian malarias. Mosquito News, In press. 1960.

Eyles, D. E. and Coleman, N. The effect of metabolites on the antitoxoplasmic action of pyrimethamine and sulfadiazine. Am. J. Trop. Med. & Hyg., 9:277-283

Eyles, D. E. The treatment of toxoplasmosis. From Human Toxoplasmosis, Munksgaard, Copenhagen, pp. 127-145. 1960.

Gibson, C. L. and Jumper, J. R. The prevalence of canine toxoplasmosis in Memphis, Tennessee. J. Parasit., 46:559-565. 1960

Note: Last three papers not directly associated with project but represent previous projects not reported this year.

Serial No. NIAID-133-A
1. Parasite Chemotherapy
2. Cytology
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Chemotherapeutic studies of the direct effect of drugs on the exoerythrocytic stages of primate malaria.

Principal Investigator: Don E. Eyles

Other Investigators: Robert L. Ingram, Nell C. Owen, Clinton S. Smith

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2 1/2
Professional: 1 1/2
Other: 1

Project Description:

Objectives: To determine the direct effects upon exoerythrocytic parasites of primate malarias of established antimalarial drugs. To describe morphological and histochemical changes and relate these to mode of action. To determine dosages necessary for effect. To study effect as related to time of administration; that is, before infection and at various times during infection. To study possible synergists. To study the reaction of the primate host to killed or damaged parasites. In the case of incomplete effects, to determine how parasites survive treatment and produce relapse.

To study the direct effect of various substances of known metabolic or antimetabolic activity on primate exoerythrocytic stages. To relate findings to physiological processes of the parasites, with the ultimate objectives of obtaining leads toward development of more effective drugs. It is quite possible that incomplete effects, not detectable by indirect studies of relapse, might be seen directly, and provide information useful in future work.

Methods Employed: Plasmodium cynomolgi, the vivax-type parasite of macaques, is the principal parasite used; however, we plan to use the malariae-type, Plasmodium inui, as suitable techniques are developed. For chemotherapeutic studies, infections are established in the livers of monkeys by the intravenous inoculation of massive numbers of sporozoites. Patent infections can be produced consistently. Drugs or other substances are administered either before or after infection is produced. In the event of prior drug administration,

controls are established using identical inocula. When treatment is begun after the infection is established, pre-treatment and post-treatment biopsies are made and the parasites compared with those found in control animals. The infections, both erythrocytic and exoerythrocytic, are followed after treatment to obtain information on relapse.

Parasites affected by the drugs are studied morphologically and histochemically by the application of various staining techniques. Drug levels are obtained when necessary. A flexibility of procedure is maintained so that the methods may be modified to apply to specific problems as they arise.

Major Findings: Further study of pyrimethamine confirmed the fact that when administered on the sixth day of sporozoite induced infection all parasites seen on subsequent biopsy were severely damaged or killed, but parasitemia developed 30 to 40 days later indicating some parasites escaped the action of the drug. When sulfonamides were used with pyrimethamine to exploit the possible synergistic interaction of the two drugs, similar results were obtained--monkeys developed parasitemia 30 to 40 days after infection even though all parasites seen were damaged.

Quinocide, the Russian 8-aminoquinoline drug, was compared with Primaquine. Even when administered at twice the dosage used with Primaquine the drug was less effective. Treatment on the sixth day did not prevent the appearance of large numbers of blood forms on the ninth day, but the liver parasites after five days of treatment did appear damaged.

Untreated parasites and parasites treated with pyrimethamine were fixed in Carnoy's and stained with toluidine blue before and after exposure of sections to the action of ribonuclease. Preliminary conclusions are that the synthesis of DNA is markedly inhibited by the drug.

Chloroquine had no observable effect upon the liver forms of Plasmodium cynomolgi, normal exoerythrocytic stages being observed after treatment. Ring stage parasites appeared in the blood in large numbers on the 8th, 16th and about the 24th day after inoculation indicating the existence of secondary exoerythrocytic stages.

Significance to Bio-medical Research and the Program of the Institute: This project is part of an intergrated program of the Laboratory of Parasite Chemotherapy to study this subject in all of its aspects. The methods used allow direct evaluation of antimalarial drugs as illustrated by the tests with Quinocide, and provide a means for studying drug action histochemically. Since the exoerythrocytic stages are those responsible for relapse and must be eliminated for radical cure, the studies have significance on the use of drugs in malaria eradication.

Proposed Course of Project: The project was somewhat curtailed due to the urgent investigation of malaria as a zoonosis. During the coming year it is expected to increase the emphasis along lines proposed in the previous year's report; that is, studies on Plasmodium inui and studies on mode of action using metabolites and cytochemical methods. Studies of resistant strains are also contemplated.

Part B. included - No.

Serial No. NIAID-133-B
1. Parasite Chemotherapy
2. Section on Cytology
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project title: Studies of simian malarias in man to determine if malaria is a zoonotic disease in areas in which man and monkey are closely associated.

Principal investigators: Don E. Eyles, Charles G. Dobrovolny

Other investigators: Clinton S. Smith, local Malayan staff

Cooperating units: Institute for Medical Research
Federation of Malaya, Kuala Lumpur

U. S. Army Medical Research Unit
Kuala Lumpur, Federation of Malaya

Man Years (Calendar year 1960)

Total	4
Professional	1
Other	3

Project Description:

Objectives:

To determine the degree to which established strains of monkey malaria, particularly Plasmodium cynomolgi, will grow in man. To isolate new strains of monkey malaria for comparison with established strains and for study in man. To work out the epidemiology and transmission of monkey malarias in the field, and apply knowledge obtained to the problem of inter-relationship between human and monkey malarias. To determine if monkey malarias are acquired by man in the field and if they can maintain themselves in man. To collect and correlate all information on distribution and host relationships of primate malarias.

Methods employed:

Strains of monkey malaria are maintained in captive monkeys in the laboratory. Using insectary reared mosquitoes, these strains are

passed from monkey to monkey and from monkey to man. Clinical study of the malaria produced in man is described in a separate project at the Atlanta Federal Penitentiary. Strains of malaria are compared before and after passage through man and attempts are made to transmit the infections man to man.

A field party is operating in the Federation of Malaya cooperating with the Malayan Institute for Medical Research and the U. S. Army Medical Research Unit. A systematic study is being made of the malarias present in far eastern macaques and other monkeys. Isolation of strains is being attempted from selected monkeys from a variety of species and from several geographical areas. Studies are being made of the vectors of monkey malaria as compared with the vectors of human malaria. The prevalence of human malaria is studied in aborigines groups closely associated with monkeys.

Major findings:

It was found that laboratory personnel bitten by mosquitoes infected with Plasmodium cynomolgi subspecies bastianellii will become infected and clinically ill with malaria. This finding was confirmed immediately by experimental inoculation of prison inmates. Clinical and parasitological characteristics of the infections in man are being studied (separate project).

It was found that the malarias could be passed back to monkey easily and identified by cross-immunological experiments. It was found possible to infect mosquitoes on human infections, and one instance of man to man passage by mosquitoes has been seen, the malaria being then reintroduced into monkey successfully. Man to man blood passage was accomplished readily.

In the field notable progress has been made in delineating the prevalence of malarias of various types in monkeys. A number of strains have been isolated or are in the process of being isolated. Other infected animals are on hand for future isolation attempts. Many new findings on host distribution and on geographic distribution of monkey malarias have been made. Studies of malaria in aborigines associated with monkeys have been made in Malaya revealing many malaria infections. Blood passed from aborigines to monkeys have thus far produced no patent infections in the monkeys.

Studies of the epidemiology of monkey malarias have been initiated and the feeding habits of some of the Anopheles determined. By injection of uninfected monkeys with sporozoites from natural infections it was determined that Anopheles hackeri is a natural vector of Plasmodium knowlesi.

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Significance to Bio-medical Research and the Program of the Institute:

The finding that Plasmodium cynomolgi subspecies bastianellii will grow consistently and produce clinical illness in man suggests the possibility that malaria is a zoonotic disease; that is, a disease which man can acquire from animals with which he is associated. Whether or not such transfer of infection occurs in nature is not yet determined, but should it occur it would be of greatest significance to the worldwide malaria eradication program.

More basically, the findings of the study taken in conjunction with previous findings indicate that there is not the strict specificity of primate malarias previously supposed but rather that the specificity is a matter of degree and varies within the species. The possibility of variants with greater infectivity to man occuring naturally is a matter of concern.

The full significance of the study cannot be completely evaluated until study has been carried further, but the potential importance makes it essential that the project be prosecuted vigorously.

Proposed Course of Project:

Isolation of strains from different monkeys and from different geographical areas shall be continued, with the view of studying these in man and comparing them immunologically and morphologically. Work in Asia at present should eventually be extended to Africa and the new world tropics.

Studies shall be continued to determine if transmission from monkey to man occurs in nature, and if so whether or not man is a "deadend" host or can pass the malaria on man to man. This study requires further study of populations associated with monkeys and the study of the epidemiology of malaria in monkeys, a subject which has not been at all well elucidated.

Part B included -- Yes.

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Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Eyles, D. E., Coatney, G. R. and Getz, M. E. Vivax-type malaria parasite of macaques transmissible to man. Science, 131:1812-1813. 1960.

Honors and awards relating to this project:

None.

Serial No. NIAID-133-C
1. Parasite Chemotherapy
2. Section on Cytology
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project title: In vitro studies of malaria parasites and liver tissue.

Principal investigator: Robert L. Ingram

Other investigators: John R. Jumper

Cooperating units: None

Man years (Calendar Year 1960):

Total:	2-3/4
Professional:	1
Other:	1-3/4

Project Description:

Objectives:

To develop techniques of maintaining adult liver tissue in vitro in order to study the biology of the exoerythrocytic stages of malarias, to test antimalarial drugs and antimetabolites on the course of the infection of the tissue and other forms of the organism and to investigate, if possible, certain liver metabolic pathways and ways in which the parasite may interfere with normal metabolism of the hepatic cells. Also, to study the degradation of various therapeutic antimalarial agents by the liver, and the parasites which inhabit the hepatic cells. And to trace the cycle of the organism in the host.

Methods employed:

Routine tissue culture methods are used, but the mediums employed, accessory factors, conditions of the cultures, methods of preparation, etc., are devised to produce the desired effects.

These include considerations of the composition of the medium, accessory factors such as glycolytic intermediates, hormones which stimulate growth or otherwise affect metabolic pathways, gas requirements for the proper oxygen tension, buffering capacity, etc. The effect of various agents against sporozoites of the malaria parasite were determined by bioassay using P. gallinaceum and baby chicks. Standard bacteriological procedures were used to assess the effect of the compounds on the microflora of the mosquito.

Histological sections were made and material inoculated into experimental animal to determine the results of the studies. Staining of the material with fluorescein labelled antibodies and tagging with radioactive isotopes, using the autoradiographic techniques for detection of the parasite were also used.

P^{32} and H^3- and C^{14}- labelled thymidine were obtained from commercial sources. At first approximately 5μc of the H^3 and C^{14} compounds were injected into the body cavity of infected mosquitoes. This produced a high mortality which did not appear to be due entirely to traumatization of the insect. Thereafter, P^{32} and tritrated thymidine was added to the food of the mosquitoes. The mosquitoes were examined later for oocysts and in some experiments dissections were made for sporozoites. After 14 days the mosquitoes were offered a blood meal on the experimental animal and the glands or whole ground mosquitoes were injected into the animal. Tissue sections were monitored and film strip radioautographs were made.

Chemical fraction of the nucleic acids, nucleotides and purine and pyrimidine bases were carried out by methods of Schmidt and Thannhauser, Schneider, and others. Separation and identification of the nucleotides and bases have been attempted by ionexchange and paper chromatography and spectrophotometric analysis.

Fetal human material was obtained from a local hospital.

Major findings:

Slight improvements have been made for maintaining the liver tissue over that reported last year. However, many additional factors and conditions which needed to be tested have been studied for their effect on liver maintenance. Also, greater success has been had with outgrowth of "hepatic" cells which meet a single criterion of hepatic cells, e.g. the selective uptake of neutral red dye as reported in the literature. Sufficient quantities of material have not been available to do further studies, especially of a biochemical nature, to characterize the cells.

We have continued to grow the EE stages of the parasite whenever these are available in sufficient numbers in the livers of P. cynomolgi bastianellii infected monkeys. Also, we have demonstrated by tissue culture that the parasite is in the liver four days after sporozoite inoculation. This represents a somewhat closer elucidation of the cycle of the parasite in the animal.

Sulfapyrazine, 6-mercaptopurine, and pyrimethamine, at 10μ M per ml adversely affected the in vitro survival of organized liver tissue, especially when chick plasma was used as substrate (in a tissue culture sense, not in a biochemical sense) for the tissue fragments. Aminoptein at the same level showed little deleterious effect. Human plasma as substrate greatly counteracted the inimical effect of the drugs on the survival of the tissue.

Penicillin and streptomycin at 500-1000 units and microgram per ml respectively showed very definite parasiticidal activity. Sulfa drugs such as sulfathiozole, Gantricin, and Elkosin at 500 micrograms per ml also showed some parasiticidal activity, but less than that shown by the penicillin and streptomycin combination. The broad spectrum antibiotic, Achromycin, had less effect on the sporozoites than did the sulfa drugs. The addition of 250 micrograms of Mycostatin, a fungicidal drug, to certain of the other drugs did not increase their effect on the survival of the sporozoites.

Achromycin in combination with Dow A, a fungicidal compound, or Mycostatin eliminated or greatly inhibited the growth of most of the microorganisms associated with the mosquito. Penicillin and streptomycin and the sulfa drugs gave only partial control of the micro flora. The studies indicate that in attempts to infect organized tissue, cellular outgrowths, or cell lines, with sporozoites, penicillin and streptomycin, the commonly used antibiotics to combat contamination in tissue cultures, should not be used -- first, because they will adversely affect the sporozoites and secondly they are ineffective against the mosquito flora.

The mosquitoes which were given p^{32} showed fewer oocysts and in some cases the oocysts at 14 days was the size of a normal 7 day old oocyst. To date, no sporozoites have been demonstrated in these mosquitoes either microscopically or by the mosquitoes being able to infect an experimental animal.

Mosquitoes which were given the H^3- thymidine showed more oocysts than did those which received the p^{32} which were allowed to feed upon the same infected monkey. About 90 per cent of Anopheles freeborni

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H^3 fed mosquitoes were positive for oocysts; many had over 100 oocysts on the gut. They also showed high sporozoite infections. Experimental animals inoculated with glands from these mosquitoes became infected. However, because of faulty film and other technical difficulties radioautographs of the parasite has not, as yet, been demonstrated. However, we have promising slides in the process of being exposed.

The nucleic acid studies are in the formative stages with the greatest effort at present being directed toward separation and identification using commercially available known compounds.

Significance to Bio-medical Research and the Program of the Institute:

The liver has such a varied and interesting function that any possibility of maintaining it in vitro would be of interest, especially, to biochemists; also, to tissue culture workers. The results of the in vitro maintenance of the liver would be of interest to other workers who are making studies with malaria similar to ours. The finding should be of interest to workers who are studying infectious hepatitis and other liver infections, of which there are a large number of parasitic ones.

The importance of the drugs, tested for their effect on the survival of organized liver tissue in vitro, as antimetabolites and antimalarial are such that any information concerning their mode of action would be of general interest to biochemists, pharmacologists, and malariologists. Also, any clues to substances which might counter their actions such as competitive substrates, etc., would be of general interest to workers studying chemotherapeutic agents and to biochemists. Many bio-medical scientists are attempting to isolate viruses from mosquitoes and other Diptera by means of tissue culture. This is especially true of the encephalitides, dengue and related viruses. The findings reported here concerning the effect of antibiotics against the microflora of mosquitoes should be useful to these investigators. The results should be especially useful to those who attempt to infect tissue culture material with sporozoites or who attempt embryonic inoculations with sporozoites and hope to eliminate the possible concomitant bacterial and fungal infections.

If we are successful in tracing the cycle of the malaria parasite in the host by the radioisotope technique this will advance our knowledge of the parasite and should be a very useful finding. It will probalby encourage others to use the method in similar studies. Biochemical knowledge related to malaria organisms is extremely fragmentary and it is believed that our approach to the subject will produce fruitful results. Nucleic

acid studies are among the forefront of bio-medical studies at the present time; this apparently indicates the sugnificance with which biological scientists regard these compounds.

Proposed course of the project:

We intend to make use of the results reported here to make a number of studies concerning the biology of the EE stages of malaria if we discover ways in which to produce high infections in the liver.

Work will be extended with the antimalarial and antimetabolite drugs. We especially wish to screen a number of these drugs for their antimalarial potency by means of tissue culture.

The tracer work will be continued unless it is found that the organism will not incorporate some of the radioactive label; this seems highly unlikely. We shall also look for mutagenic effects of the isotopes on the parasite. Hopefully this method can be used to determine the mode or site of action of some of the drugs to be tested.

Part B. included -- No.

Serial No. NIAID-133-D
1. Parasite Chemotherapy
2. Section on Cytology
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project title: Immunological studies of malaria parasites.

Principal investigator: Robert L. Ingram

Other investigators: John R. Jumper

Cooperating units: None.

Man years (Calendar Year 1960):

Total	1
Professional	1
Other	0

Project Description:

Objectives:

To develop ways of distinguishing closely related malaria organisms to devise means of detecting the organisms in the tissue phase as soon as possible after infecting the experimental animal so as to delineate the cycle of the organism, and basic immunochemical studies concerning antigen-antibody reactions of protozoa.

Methods employed:

Antigenic material was prepared from both the erythrocytic and the sporozoite stages of the malaria parasites. White rabbits were given a series of injections of these preparations and after a suitable time, serums were collected. These were pooled, the gamma globulin was fractionated and conjugated with fluorescein isothiocyanate (F.I.C.). This preparation was used to stain the parasites which were then examined using fluorescent microscopy equipment.

Major findings:

Blood forms of P. gallinaceum were stained with a serum prepared by using sporozoites of this species as the antigenic material. P. fallax and P. lophurae also were stained with this labeled serum. Blood forms of P. cynomolgi bastianellii failed to stain with this preparation. However, F.I.C. labeled serum from experimentally infected convalescent monkeys was used to stain P. cynomolgi bastianellii. P. gallinaceum, P. fallax and P. lophurae failed to stain with this preparation.

Significance to Biomedical Research and the Program of the Institute:

The use of the fluorescent antibody technique has in the past few years received much attention by bio-medical investigators. The reported findings that malarias can be studied by this method will find utility in a number of laboratories where malaria is being studied. The fact that one stage of an organism was used to stain a different stage of the same organism should facilitate the study by this technique of many parasitic diseases in which the organism undergoes several stages of development.

Proposed course of the study:

As other species of malarias become available to us and experimental infection with these become possible, we shall study immunological cross reactions among the various species. We also intend to use this method to help trace the cycle of the organism through the host. Attempts may be made to partially purify the antigen or antigens involved in the reaction.

Serial No. NIAID-133-D

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Ingram, Robert L., Otken, Luther B., Jr. and Jumper, John R. Staining of malaria parasites by the fluorescent antibody technique. In press. Proc. Soc. Exp. Biol. and Med.

LABORATORY OF IMMUNOLOGY

LABORATORY OF IMMUNOLOGY

LABORATORY OF IMMUNOLOGY
SUMMARY STATEMENT
CALENDAR YEAR 1960

During the past year there has been a tremendous resurgent interest in the field of immunology. This has been brought forcefully to our attention by a variety of events which occurred during the Fall of 1960. A very high percentage of the many Research Associate applicants, which were interviewed over a period of several weeks, chose the Laboratory of Immunology as being the one in which they desired to conduct research for a two year period. The interest of these physicians in immunology stems from the possibilities that they envisage in the application of immunology and its newer techniques to the study of human disease. Many of these exceptionally bright young men have been stimulated by their professors at medical schools such as Harvard, N.Y.U., etc. During the course of their medical studies they have had the opportunity to carry on immunological investigations, which have made them realize the importance of immunology as a fruitful area of research.

For several years the Laboratory of Immunology has conducted a bi-weekly immunology seminar. In the past, this was attended by the members of our staff and a few investigators from several other Institutes. In view of the ever-increasing interest in immunology, and in anticipation of this, the Laboratory scheduled a weekly seminar which is, at the time of this writing, so well attended that it has been necessary to abandon the NIAID conference room and to hold these lectures and discussions in the Clinical Center Auditorium (14th Floor). The investigators attending these seminars are from all of the Institutes and represent a variety of disciplines. It is evident that these scientists have found the need for incorporating immunology in their studies in order that they may attain their research goals. This is another example of the resurgence of immunological research at NIH and points out the necessity for the development of a truly strong and outstanding laboratory. At the present time we possess a small nucleus of competent immunologists and from this nucleus it will be possible to develop a laboratory which potentially can be a national and international leader in the field.

During the year the demands on the scientific staff of the Laboratory for consultation and collaboration have been increasing. Many of the demands on their time center around the performance of the newer techniques and methods of immunological research, such as immuno-electrophoresis, fluorescent antibody, passive cutaneous anaphylaxis, immunization with adjuvants, etc. This almost daily demand has been of a proportion as to interfere at certain times with the scientists' own research program.

Since the activation of the Laboratory of Immunology in 1957, the program has been concerned, principally, with basic research. However, for some time an important need has been felt for the initiation of clinical studies in immunology and allergy. In September 1960 the Clinical Immunology Section was activated with the appointment of Dr. Howard C. Goodman as Head of this

Section. For some years he has been engaged in clinical studies and more recently has been concerned with the importance of auto-antibodies in the pathogenesis of certain disease states. Dr. Goodman spent the year preceding his appointment at the Pasteur Institute where he was engaged in studies on serum proteins involving the use of the technique of immunoelectrophoresis. As space permits, the Section will be expanded with the addition of two investigators at the doctoral level. Dr. Goodman's section will be working in close collaboration with Dr. Vernon Knight, Clinical Director, NIAID, on clinical studies involving the immunological aspects of such diseases as lupus erythematosus, nephritis, chronic thyroiditis, and others in which an auto-immune basis is suspected. In order that clinical studies are executed properly, it has become apparent that the clinician must have intimate contact with the basic scientist and the laboratory methods involved in immunological research. The newly activated Clinical Immunology Section, being an integral part of our Laboratory, affords us a unique opportunity to accomplish first-rate and complete clinical studies.

Due to lack of space since the formation of the Laboratory, it was necessary to place several of our permanent professional staff on contract with universities. Two of these scientists resigned during the year and accepted positions at institutions of medical research. The exact reasons for their leaving is not completely understood but, in part, it was due to better salary opportunities and our present lack of facilities. The loss of these established scientists leaves a gap in certain areas of our research program. Another scientist working on tissue transplantation resigned during the year to enter training in surgery. It seems imperative that this person be replaced in the near future in order that this important segment of immunological research be represented in our Laboratory.

The activities of the staff have resulted in certain significant scientific advances:

Allergic Thyroiditis

Experimental allergic thyroiditis was produced in Strain 13, inbred, histocompatible guinea pigs by immunization with a single dose of guinea pig thyroid extract in complete Freund's adjuvant. Thyroiditis developed as early as 5 days after immunization, was present in all animals at 16 days, and by 7 weeks was consistently present and generally severe. Delayed skin test hypersensitivity was found as early as 5 days after immunization in nearly all animals, and was present in all animals with thyroiditis at 7 weeks. Circulating anti-thyroid antibody was absent at 5 days in animals with thyroiditis and with delayed hypersensitivity. At 7 weeks after immunization, anti-thyroid antibodies were present, and antibody titres correlated with the presence and degree of thyroiditis. This correlation was not found at certain other times after immunization. To intensify the formation of antibody without producing delayed hypersensitivity, guinea pigs were immunized with thyroid extract in Freund's incomplete adjuvant. These animals at 7 weeks showed no thyroiditis and no delayed hypersensitivity, although they did develop low levels of circulating antibody. The presence of delayed hypersensitivity was correlated with experimental allergic thyroiditis, while the presence of circulating antibody did not correlate with thyroiditis. These observations constitute the earliest

production of experimental allergic thyroiditis and the most severe disease at the time intervals studied.

House Dust Allergens

Studies on the chemical and physical properties of house dust extracts that are used clinically for the diagnosis and treatment of house dust allergy have been studied to identify the components responsible for the specific skin reactions produced in house dust sensitive individuals. It has been found that the house dust extracts consist of a heterogeneous mixture of acidic polysaccharides. The heterogeneity has been demonstrated by electrophoretic and ultracentrifuge sedimentation analysis and also by the multiplicity of cross reactions obtained with antisera to the various pneumococcal polysaccharides. The chemical composition of the various fractions has been shown to be roughly 5-20% polypeptide and 80-95% polysaccharide, containing about equal amounts of uronic acid (probably glucuronic acid), D-glucose, D-galactose, D-mannose with lesser amounts of L-rhamnose and L-arabinose. Starch block electrophoresis experiments at low pH and in the presence of 7M urea have shown that the polypeptide components are covalently linked to the polysaccharides. A number of different kinds of experiments have shown that the colored components may be separated from the bulk of the material and the skin test data suggest that the color is not associated with skin reactivity. The skin test data also indicate that more than one allergen specificity is present in the dust extracts. By starch block electrophoresis it has been shown that certain isolated fractions have greater skin reactivity than the original extract; however, it should be appreciated that the skin test assay is exceedingly inaccurate so it is not possible to determine the degree of concentration of activity.

Immunochemistry and Genetics of Gamma Globulin

Agar-gel immunochemical analysis of sera from rabbit litters, with precipitating antibodies prepared in rabbits, has shown that seven antigenic determinants of the gamma globulins are genetically controlled by at least two distinct gene loci with each specificity exhibited when the appropriate allele is present. Since the gamma globulins are soluble proteins which have properties of both an antigen and an antibody, they should be subject to quantitative estimation and cytological localization. This immunogenetic system, therefore, may be uniquely suited for the study of certain basic problems in genetics, embryology, immunology and protein chemistry. Since gamma globulin should be expected to pass through maternal-fetal barriers, of considerable interest would be the study of gamma globulin synthesis in fetal and neonatal rabbits uncomplicated by the problem of distinguishing maternally derived gamma globulin. In other studies, antibodies to human serum proteins were prepared in monkeys since this animal, being a closely related species, might be more discriminating for minor antigenic differences than a distantly related species. Three "slow" gamma globulins were found, instead of the one usually detected with horse or rabbit antibodies. Two of these were shown to be related to myeloma proteins. The quantitative estimation of these gamma globulins in serum should be helpful in the early diagnosis and study of diseases, such as multiple myeloma, which involve qualitative and

antitative changes in the gamma globulins.

Mechanisms of Hypersensitivity

The genetically distinct guinea pigs of inbred Strains 2 and 13 have proved to be a very important immunological tool. After studies established the fact of skin compatibility in the two strains, experiments were conducted to transfer cells with a measurable biological activity. Transfers of tuberculin sensitivity were undertaken by the intraperitoneal injection of living lymphoid cells from compatible donors. The almost quantitative transfers between inbred guinea pigs were a reflection of the continued viability of the active cells in the recipients. It was found that transfers could be made soon after active sensitization of the donor; early transfers merely required a longer waiting period for full sensitivity to appear in the recipient. This contrasted with the need to test as soon as possible after transfer in random-bred animals in face of a dwindling population of transferred lymphoid cells in a non-compatible host. Two models are being developed to study the mechanisms of immediate and delayed hypersensitivity in the inbred guinea pigs; protracted anaphylactic shock and, the massive local hemorrhagic reaction, respectively. It has been shown that there are differences in susceptibility to hypersensitivity reactions. Strain 2 guinea pigs were more resistant to death by bronchospasm and tended toward a protracted syndrome in anaphylactic shock. Both Strain 2 and 13 guinea pigs required more mycobacteria than did random-bred Hartley guinea pigs for inducing "delayed" sensitivity to egg albumin, using Freund's adjuvant.

Characterization of Human Serum Auto-antibodies

Fractions of human serum separated by anion-exchange cellulose column chromatography were studied by immunoelectrophoresis. The conditions for elution of eighteen immunologically distinguishable human serum proteins from the columns were determined. Gamma globulin obtained under the appropriate conditions by this method was found to be pure; rabbits immunized with this fraction made antibodies to none of the other serum proteins. By the use of anion-exchange cellulose columns, it has been found possible to separate the 7S from the 18-19S antibody activities in sera of patients with thyroiditis and lupus erythematosus. Initial results indicate that the addition of immunoelectrophoretic characterization of these and other sera will be extremely helpful in our aim of characterizing the antibody activities found in human serum.

Fluorescent Antibody Staining of Malaria Parasites

The fluorescent antibody staining of the human malaria parasite, Plasmodium vivax, has been recorded for the first time. A globulin fraction of convalescent serum from a patient having a long-standing infection with P. vivax was labeled and the fluorescent antibody applied to thin blood films containing the parasite. The organism was visible by virtue of its specific immuno-fluorescence. If blood films can be stained in such a manner that, essentially, only the parasites are visible, this could provide a method whereby numerous blood films could be examined in a single day by means of an automatic scanning microscope. Fluorescent antibody studies were conducted on P. cynomolgi bastianellii, the monkey malaria parasite which, recently,

has been shown to be transmissible to man. Considerable morphological detail was observed at fluorescence. Preliminary studies on the serological relationships, as based on degrees of fluorescence, indicate that P. vivax and P. cynomolgi bastianellii parasites may have common antigens and that the two species may be closely related. This is of importance in the program of the world-wide eradication of malaria which has become more complex with the possibility that monkeys may serve as reservoirs for human infection.

Submitted by:

John E. Tobie

John E. Tobie, Ph.D., Acting Chief,
Laboratory of Immunology, NIAID

Form No. ORP-2
Nov. 1960

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 140
1. Laboratory of Immunology
2. Natural & Acquired Resistance
3. Bethesda, Maryland

Part A:

Project Title: Studies in the Transplantation of Tissues

Principal Investigator: Dr. Sanford H. Stone

Other Investigators: Dr. Joseph A. Bauer, Jr.

Cooperating Units: None

Man Years: Patient Days: None

Total: 1.25
Professional: 0.75
Other: 0.5

Project Description: Isologous and Homologous Lymphoid Cell Transplants

Objectives:

To compare the relative efficiency of passive transfer of tuberculin sensitivity and antibody producing activity between histocompatible guinea pigs with that between random-bred animals.

Methods Employed:

Inbred guinea pigs are sensitized with egg albumin and sheep erythrocytes in Freund's complete adjuvant (containing mycobacteria). Lymphoid cell transfers are made by intraperitoneal route into inbred and random-bred recipients. Cell recipients are tested for skin-reactivity and circulating antibody.

Major Findings:

1. The passive transfer of delayed hypersensitivity and antibody synthesizing capacity appears to be more effective between inbred guinea pigs than between random-bred animals.

2. Sonication or freezing and thawing of lymphoid cells obliterates their capacity for successful transfer.

Significance to the Program of the Institute:

Passive transfer with viable lymphoid cells represents an important

tool in the study of immune phenomena. In histocompatible animals, the variable of transplantation immunity is eliminated, the transferred cells thrive, and go on to immune maturity in the recipient (if the lymphoid cells are harvested soon after sensitization of the donors, a longer waiting period will intervene before full sensitivity is manifested in the recipient).

Proposed Course of Project:

F_1 generation guinea pigs of the inbred Strain 13 - inbred Strain 2 crosses will be used to study problems of host vs. graft and graft vs. host reactions during transfers of lymphoid cells.

Part B included: Yes X No

(Attachment I)

Serial No. NIAID - 140

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications:

Bauer, J. A., Jr. and Stone, S. H.: Isologous and Homologous Lymphoid Transplants I. The Transfer of Tuberculin Hypersensitivity in Inbred Guinea Pigs. J. Immunol. 85: (in press), 1960.

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Form No. ORP-2
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PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 141
1. Laboratory of Immunology
2. Natural and Acquired Resistance
3. Bethesda, Maryland

Part A:

Project Title: Studies on Antibody Production and Mechanism of Hypersensitivity

Principal Investigator: Dr. Sanford H. Stone

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1.0
Professional: 0.5
Other: 0.5

Patient Days: None

Project Description:

Objectives:

To study the mechanisms of "immediate" and "delayed" hypersensitivity.

Methods Employed:

Inbred histocompatible guinea pigs permit experimentation on genetic factors involved in hypersensitivity. The experimental models: randombred or inbred guinea pigs injected with antigen in complete (for "delayed" hypersensitivity studies) or incomplete (for "immediate" hypersensitivity studies) Freund's adjuvants, and challenged by the subcutaneous route with homologous antigen. Methods of quantitation of the manifestations of "immediate" (survival time in protracted anaphylaxis) and "delayed" (amount of mycobacterium necessary to sensitize) become feasible. The local massive hemorrhagic reaction described in previous reports is mot versatile for manifestation of "delayed" hypersensitivity.

Major Findings:

If quantitative methods are to be applied to the field of hypersensitivity research, it becomes important to know if all guinea pigs react similarly to the conditions set up in an assay. It was found that the NIH inbred Strains 2 and 13 differ from the random-bred Hartley guinea pigs in several significant areas concerned with hypersensitivity. Strain 2 guinea pigs are relatively resistant to the lethal bronchospasm

of anaphylactic shock, and tend toward the protracted type of syndrome involving the gastrointestinal system. Both Strains 2 and 13 are more difficult to sensitize for "delayed" type reactivity (local hemorrhagic reaction), requiring from 10 to 50 times as much mycobacteria in the Freund adjuvant emulsion. Questions of genetic variance in such areas as mast cell concentration, affinity of antibody for tissue, competence of mononuclear cells to sensitize or of the skin to react, etc., come to the fore.

Significance to the Program of the Institute:

The genetic influences on hypersensitivity and its manifestations have been relatively unstudied. The significance of the availability of the N.I.H. inbred strains of guinea pigs is best stated by quoting from Dr. Freund's Summary Statement of 1959: "The most important of these (new methods involves) the introduction of genetically distinct, highly inbred guinea pigs into immunologic research work. The initiation of such studies is of particular importance in the field of delayed type of hypersensitivity. The guinea pig is unique in its suitability...high degrees of local and systemic reactions can be produced."

Proposed Course of Project:

The mechanisms of protracted anaphylaxis and the local hemorrhagic reaction will be investigated using random-bred and inbred guinea pigs.

Part B included: Yes No X

Form No. ORP-2
Nov. 1960

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 142
1. Laboratory of Immunology
2. Applied Immunology
3. Paris, France

Part A:

Project Title: The Immunology of C_3h Mouse High Line Sarcoma

Principal Investigator: Dr. Philip R. B. McMaster

Other Investigators: None

Cooperating Units:

Dr. Pierre Grabar, Laboratorie de Chimie Microbienne, Institut Pasteur, Paris, France

Dr. Georges Barsky, The Hospice Paul Brousse, Paris, France

Man Years:

Total: 0.4
Professional: 0.2
Other: 0.2

Patient Days: None

Project Description:

Objectives:

To attempt to find an antigen in the C_3h mouse high line sarcoma, developed by Earle and others at the NIH, which is not present in the normal mouse tissue, as well as to determine the most effective method of immunization to prevent the growth of subsequently inoculated tumor.

Methods Employed:

To compare by immunoelectrophoretic and other methods, the nature and number of antigens in normal mouse tissue and the above named sarcoma, as well as to try various methods of immunization to determine which is the more effective in the prevention of the growth of subsequently injected tumor.

Major Findings:

Study in its initial stages. No significant findings to date.

Significance to the Program of the Institute:

The continued search for antigens in tumors not present in normal tissue may yield results which would seem to offer diagnostic and

therapeutic possibilities.

Proposed Course of Project:

To be continued along the lines outlined above.

Part B included : Yes No X

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Nov. 1960

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 143
1. Laboratory of Immunology
2. Immunochemistry Section
3. New York City, New York

Part A:

Project Title: Characterization of Substances of Bacterial Origin Affecting Resistance to Infection

Principal Investigator: Dr. Curtis A. Williams, Jr.

Other Investigators: None

Cooperating Units: Dr. Rene J. Dubos, Rockefeller Institute, N.Y., N.Y.

Man Years:

Total: 1.0
Professional: 0.5
Other: 0.5

Patient Days: None

Project Description:

Objectives:

1. Further examination of biologically active materials extracted from tubercle bacilli. Their isolation and composition.

2. To distinguish between the mechanisms of specific and non-specific acquired resistance to infectious diseases.

Methods Employed:

1. Animal protection tests are performed in albino mice. They are studied by bacterial enumeration from infected organs and by survival time.

2. Biologically active substances from tubercle bacilli are compared with bacterial endotoxins from gram negative organisms, which have similar chemical composition and most of the same activities.

3. Clearance of microorganisms from the blood stream of mice.

4. Chromatography, zone electrophoresis, ultracentrifugation, standard immunochemical tests.

Major Findings

1. There are many variables to be carefully controlled in the preparation of active substances from tubercle bacilli. Solvent ratios,

water content of organic solvents, and temperature of importance in terms of recoverable activity in products.

2. Strains of mice differ in their response to these products. C57/6 mice and Rockefeller Swiss behaved roughly as predicted from their respective responses to BCG.

3. Studies with Serratia plymuthica have demonstrated that the use of so-called "non-pathogens" to infectious disease may be very fruitful. The usual serological reactions and immunological responses are operative. The cause of death is apparently toxemia. Survival 24 hours usually leads to complete recovery and a high level of immune resistance.

4. The blood clearance mechanism has been examined with reference to immune and non-immune resistance, to strain of mouse, to physiologic and genetic variants among bacteria. It has become clear that bacterial variation plays a more significant role in the function of this mechanism in resistance than many host treatments or the strain of host.

Significance to the Program of the Institute:

Relating recognized resistance mechanisms in experimental animals to the outcome of infectious diseases is of obvious importance. The study of bacterial variation with respect to these mechanisms, however, could lead to an eventual understanding of the terms pathogenicity, virulence, susceptibility, and resistance.

Proposed Course of Project:

Terminated as NIAID Project June 31, 1960, with the resignation of principle investigator.

Part B included: Yes X No

(Attachment I)

Serial No. NIAID - 143

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications:

Williams, Jr., Curtis A.: Immunoelectrophoresis. Scientific American. 130:140, 1960.

Form No. ORP-2
Nov. 1960

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 144
1. Laboratory of Immunology
2. Clinical Immunology
3. Bethesda, Maryland

Part A:

Project Title: Pathogenesis of Allergic Encephalomyelitis

Principal Investigator: Dr. Philip Y. Paterson

Other Investigators:

Miss Jennifer Bell and Mr. S. Martin Harwin, medical students at N.Y.U. School of Medicine and Mr. N. C. Didakow, laboratory technician.

Cooperating Units: None

Man Years:

Total: 2
Professional: 0.5
Other: 1.5

Patient Years: None

Project Description:

The allergic encephalomyelitis (AE) induced in rats and other animals by injection of normal nervous tissue combined with Freund type adjuvants is under study at New York University College of Medicine under contract with the National Institute of Allergy and Infectious Diseases (Contract Serial #SA-43.PH 3027).

Project Subtitle I: Transfer of Allergic Encephalomyelitis (AE) by means of Lymph Node Cells

Objectives:

To transfer AE from diseased donors to normal recipients using lymph node cells.

Methods Employed:

Adult donor rats are sensitized to nervous tissue by injection of guinea pig spinal cord emulsified in Freund's complete adjuvant (oil, emulsifier and killed mycobacteria). At varying times thereafter, the lymph node cells (LNC) from these donors are collected and used for 2 different types of transfer:

(a) homologous type of transfer--the LNC are injected intracerebrally into rats of a genetically different strain.

(b) isologous type of transfer--the LNC are injected intravenously into rats of the same genetic strain.

Major Findings:

1. Homologous type transfers - AE lesions may be identified in approximately 10% of recipients if the donor cells are injected via the intracerebral route. Transfer of cells by other routes (viz., intravenous, intraperitoneal) has not resulted in transfer.

2. Isologous type transfers - AE lesions may only rarely (less than 5%) be found in recipients.

Significance to the Program of the Institute:

These means of transferring AE by LNC, in addition to the technique described in Annual Report for 1959, provides further support for the cellular nature of the disease and offers a means for ultimately defining the morphogenesis of the AE lesion in precise terms. The information obtained should prove helpful in transfer studies of analogous auto-immune disease states, e.g., thyroiditis, aspermatogenesis.

Proposed Course of Project:

Contract terminated July 1, 1960.

Project Subtitle II: Role of Freund's adjuvant components in induction of Allergic Encephalomyelitis (AE).

Objectives:

To define which component(s) of Freund's adjuvant is required for rapid and regular induction of AE in rats and guinea pigs.

Methods Employed:

Groups of rats and guinea pigs are given a single intracutaneous injection of guinea pig or rat spinal cord alone or combined with Freund's adjuvant components in varying combinations. Occurrence of AE is determined by presence or absence of AE lesions in brains and spinal cords of animals about 3 weeks post-sensitization.

Major Findings:

AE may be regularly induced in the rats and infrequently induced in the guinea pig by injection of nervous tissue combined with Freund's incomplete adjuvant, i.e., paraffin oil, emulsifying agent but no killed mycobacteria. The paraffin oil is the important component of

the adjuvant for rapid induction of the disease in the rat. In contrast, both paraffin oil and killed mycobacteria are essential for rapid and regular induction of disease in guinea pigs. An occasional rat may exhibit lesions of AE if injected with nervous tissue alone; such is not the case in the guinea pig. In both the rat and the guinea pig, heterologous nervous tissue is more active as sensitizing antigen than is homologous nervous tissue.

Significance to the Program of the Institute:

In addition to providing a less complex technique for induction of the disease at least in the rat, these findings provide additional clues to understanding the mode of action of Freund type adjuvants and role of cellular and humoral factors in development of experimental auto-immune disease. This work underscores the long appreciated potential hazard of using any vaccine consisting of nervous tissue for immunization of man.

Proposed Course of Project:

Contract terminated July 1, 1960.

Project Subtitle III: Antibodies against autologous brain in rats with Allergic Encephalomyelitis (AE).

Objectives:

To determine whether rats sensitized to homologous or heterologous nervous tissue-adjuvant produce antibodies specifically directed against their own (autologous) brain.

Methods Employed:

Rats are sensitized to nervous tissue by a single injection of rat or guinea pig spinal cord in adjuvant. About 3 weeks later the rats are bled, sacrificed and their sera tested (by complement fixation) for antibodies using both autologous and homologous brain extracts as the antigens. Appropriate controls are included in every test.

Major Findings:

More than half of the rats tested produce antibodies against their own (autologous) nervous tissue. The antibody appears organ-specific; it does not react with kidney tissue and does not appear in response to sensitization with kidney-adjuvant.

Significance to the Program of the Institute:

These results add weight to the presumed auto-immune theory of AE and analogous forms of experimental tissue damage.

Proposed Course of Project:

Contract terminated July 1, 1960.

Part B included: Yes X No

(Attachment I)

Serial No. NIAID - 144

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications:

Paterson, P. Y.: Transfer of Allergic Encephalomyelitis in Rats by Means of Lymph Node Cells. J. Exper. Med. 111:119, 1960.

Bell, J., and Paterson, P. Y.: Rapid Induction of Allergic Encephalomyelitis in Rats Without the Use of Mycobacteria. Science. 131:1448, 1960.

Harwin, S.M., Paterson, P. Y., and Didakow, N.C.: Antibodies Against Autologous Brain in Rats With Allergic Encephalomyelitis. Nature (in press).

Form No. ORP-2
Nov. 1960

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 145
1. Laboratory of Immunology
2. Clinical Immunology
3. Paris, France and Bethesda, Maryland

Part A:

Project Title: Studies of Auto-Antibodies in Human Disease States and in Experimental Animals.

Principal Investigator: Dr. Howard C. Goodman

Other Investigators: None

Cooperating Units:

Dr. John L. Fahey, GM., NCI, and Dr. Richard A. Malmgren, PA., NCI.

Dr. James H. Baxter, LC., NHI.

Dr. Leonard Laster, A&R., NIAMD.

Man Years: Patient Days: 60

Total: 2.0
Professional: 1.0
Other: 1.0

Project Description:

Objectives:

1. To detect the factors in human sera which react with human tissue antigens.

2. To characterize these factors, i.e. show whether they are actually gamma-globulins and whether the reactions with tissue antigens behave like typical antigen-antibody reactions.

3. To attempt to produce similar auto-antibodies in experimental animals.

4. To determine whether animals with experimentally produced auto-antibodies develop tissue damage.

Methods Employed:

The tannic acid hemagglutination test of Boyden, gel-diffusion antigen-antibody precipitation tests (Ouchterlony and Oudin), immunoelectrophoresis (Grabar and Williams), and DEAE cellulose column chromatography (Sober and Peterson) techniques are used to

detect and characterize the serum factors which react with tissue antigens.

To produce auto-antibodies in animals experimentally, rabbits, guinea pigs and rats are immunized with tissue extracts incorporated in Freund's adjuvant.

Patient Material:

Patients with lupus erythematosus and idiopathic nephrotic syndrome are admitted to the clinical center for these studies. Kidney biopsies are performed and the clinical response to steroid administration is studied; lipid metabolism studies on patients with the nephrotic syndrome have been performed in conjunction with Dr. James Baxter, NHI.

Major Findings:

During the past year at the Pasteur Institute with Professor Pierre Grabar, the technique of immunoelectrophoresis was used to analyze the fractions obtained from normal human serum after DEAE cellulose column chromatography. The information gained about conditions for elution of some 20 of the immunologically distinguishable serum proteins from the cellulose columns, particularly the gamma, beta-2 A, and beta-2 M (the macrogammaglobulins), will be immensely useful this year when we attempt to see into which of these classes of "immune-globulins" auto-antibodies fall. A specific example of what will be a general usefulness of immunoelectrophoresis in characterization of serum proteins in other diseases (not necessarily caused by immune reactions) is the demonstration with Dr. Leonard Laster (NIAMD) and Dr. Donald Frederickson (NHI) of the absence of beta lipoproteins from the serum of their patient, thus confirming the diagnosis of "a-betalipoproteinemia", a newly described disease syndrome.

Attempts to detect auto-antibodies in patients with nephritis and nephrosis and to produce auto-antibodies to kidney tissue and renal disease in animals have not been successful so far, although these studies continue. Auto-antibodies are detectable in patients with lupus erythematosus, chronic thyroiditis, and a few patients with scleroderma and rheumatoid arthritis. In patients with thyroiditis, the serum factors which react with thyroglobulin have been shown to be gamma-globulins and to belong to both the 7S (mol. wt. about 160,000) and 18S (mol. wt. about 1,000,000) gamma-globulins. In the sera of patients with lupus erythematosus, auto-antibodies reacting with components of cell nuclei have been studied. The L. E. cell factor has been shown to be in the 7S gamma-globulins. Some of the other anti-nucleus factors were found to be 7S gamma-globulins, and were demonstrated for the first time to be among the 18S gamma globulins.

Rabbits injected with calf thymus and human liver nucleoprotein extracts have been shown to develop antibodies not only to the injected

nuclear material, but also auto-antibodies to their own cell nuclei. So far no associated tissue damage has been detected.

Significance to the Program of the Institute:

These studies, as well as those to be developed in our own Laboratory of Immunology and with the cooperating units listed, represent the approach of the section on clinical immunology to the study of the role of immune mechanisms in human disease states. The question of the importance of auto-antibodies in the pathogenesis of a great number of diseases deserves critical study. The finding that anti-nucleus auto-antibodies in lupus erythematosus can be macro-globulins raises new questions about the reasons for the production of high molecular weight antibodies. The production of anti-cell nucleus auto-antibodies in experimental animals should enable us to determine whether these antibodies can produce a disease state in these animals, and thus help decide whether the auto-antibodies are important in the pathogenesis of the human disease states where they are found.

Proposed Course of Project:

Attempts to detect auto-antibodies in different disease states will continue (e.g. ulcerative colitis with Dr. Laster). The auto-antibodies detected will be characterized by immunoelectrophoresis and cellulose column chromatography. A logical extension of our studies on serum antibodies in disease states will be to apply, in conjunction with Dr. Wilton E. Vannier, the technique of DEAE cellulose column chromatography and immunoelectrophoresis to the characterization of reagins in serum of patients with clinical allergy.

Animal studies on the production of and characterization of auto-antibodies and production of tissue damage will continue, with emphasis on the antibodies to components of cell nuclei to thyroid tissue and to kidneys. In patients and in experimental animals where tissue damage is associated with auto-antibodies, attempts will be made to determine whether tissue damage is caused by hypersensitivity of the delayed type or due to circulating antibodies. Cell and serum transfer experiments can help decide this question, and we are in a position to do these studies using the histocompatible, pure strains of guinea pigs developed at NIH.

Part B included: Yes X No

(Attachment I)

Serial No. NIAID - 145

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Part B: Honors, Awards, and Publications

Publications:

Baxter, James H., Goodman, Howard C. and Havel, Richard J. Serum Lipid and Lipoprotein Alterations in Nephrosis. J. Clin. Inv. 39:455, 1960.

Fahey, John L., Dutcher, Thomas E. and Goodman, Howard C. Cell Nuclei and Gamma Macroglobulins. Royal Soc. Med. Vol. 53 No. 8, 1960

Fahey, John L. and Goodman, Howard C. Characterization of Anti-Thyroglobulin Factors in Human Serum. J. Clin. Inv. 39:1259, 1960.

Goodman, Howard C., Fahey, John L. and Malmgren, Richard A. Serum Factors in Lupus Erythematosus and Other Diseases Reacting With Cell Nuclei and Nucleoprotein Extracts: Electrophoretic, Ultracentrifugal and Chromatographic Studies. J. Clin. Inv. 39:1595, 1960.

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PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 146
1. Laboratory of Immunology
2. Applied Immunology
3. Bethesda, Maryland

Part A:

Project Title: The Resistance of Fetal and Neonatal Gonads to Damage by Maternal Immunization to Testicular Antigens.

Principal Investigator: Dr. Philip R. B. McMaster

Other Investigators: None

Cooperating Units: None

Man Years: Patient Days: None

Total: 0.4
Professional: 0.2
Other: 0.2

Project Description:

Objectives:

To demonstrate that maternal immunization to testicular antigens is innocuous to the development of the offspring in contrast to the reported results of maternal immunization to lense and brain, and thereby lend support to the hypothesis that delayed hypersensitivity is a requisite for the development of allergic aspermatogenesis, and also to provide a control model for the study of white cell transmission across the placenta.

Methods Employed:

Adult female outbred Hartley strain guinea pigs were immunized with testicular extract in complete adjuvant in two groups, one shortly before mating and the other shortly after mating. These animals were later tested for delayed hypersensitivity and serum antibodies to testicular extract, whereas the offspring were examined for the same serum antibodies as well as for histologic and physiologic abnormalities of the reproductive system.

Major Findings:

All the actively immunized animals developed circulating antibody and delayed hypersensitivity to testicular extract. All the offspring had circulating antibody in their serum, but nevertheless their reproductive organs were normal physiologically and histologically.

Significance to the Program of the Institute:

This study extends the previous work of Dr. Freund, who demonstrated a correlation between aspermatogenesis and delayed hypersensitivity to testicular antigens, and a lack of such a correlation between the disease and serum antibody in adult animals. These experiments on the development of offspring of immunized females show that circulating anti-testicular antibody does not cause detectable damage even when present early in life during the development of barrier membranes and organ differentiation. This study also indicates a lack of a transfer of prolonged nature across the placental membrane of outbred animals, even when a high degree of delayed hypersensitivity is present in the maternal parent.

Proposed Course of Project:

This study may serve as a model control for the study of white cell transfer across the placenta, by comparing the above with a similar study in inbred animals, in which transferred white cells would survive.

Part B included : Yes No X

Form No. ORP-2
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PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 147
1. Laboratory of Immunology
2. Immunochemistry Section
3. Bethesda, Maryland

Part A:

Project Title: Immunochemical and Immunogenetic Studies of the Protein Isoantigens in Serum

Principal Investigator: Dr. Sheldon Dray

Other Investigators: Miss Glendowlyn O. Young

Cooperating Unit:

Project Subtitle I - Drs. Charles W. McPherson and Donald Bailey, Animal Production Section, Serial No.

Project Subtitle V - Dr. T. N. Harris, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

Man Years: Patient Days: None

Total: 3.0
Professional: 1.5
Other: 1.5

Project Description:

Project Subtitle I: Genetic Control of Serum Isoantigens

Objectives:

To establish the genetic basis for the presence of serum isoantigens and to develop lines of animals (rabbits) with genetically defined isoantigens.

Methods Employed:

1. Qualitative tests using primarily agar-gel methods for the identification of isoantigens in families of rabbits and in various rabbit populations.

2. Selective breeding of rabbits to test genetic hypotheses and establish genetically defined lines.

Major Findings:

The genetic control for two gamma-globulin allotypes have been established. A genetic theory for five additional gamma globulin allotypes is

being tested utilizing the serum bank of rabbit families available from earlier studies.

Significance to the Program of the Institute:

To know the genetic mechanism for the control of isoantigens is essential for the usefulness of these isoantigens in studies of basic immunology and hypersensitivity. The molecules having antigenic sites under the genetic control postulated are soluble proteins, namely, gamma globulins. Some of these molecules may also react as antibodies. As antigens, precipitable by antibodies, they should be subject to quantitative estimation. Moreover, these molecules may be expected to pass through maternal-fetal barriers. Thus, this immunochemical genetic system may be uniquely suited for studies of some basic problems in immunology, hypersensitivity, embryology, and protein chemistry.

Proposed Course of Project:

1. To establish genetically defined lines of rabbit gamma-globulin types so that they may be available for other studies.

2. To study the genetic control of new gamma globulin isoantigens or other isoantigens as they are discovered.

Project Subtitle II: Chemical Studies of Serum Isoantigens

Objectives:

1. To identify the antigens in serum which induce isoantibodies.

2. To study the chemical nature of these isoantigens.

Methods Employed:

1. Immunochemical analysis by diffusion in agar-gel; simple diffusion in plates, and immunoelectrophoresis.

2. Passive cutaneous anaphylaxis, tanned-cell hemagglutination and other immunological tests for antibody.

3. Cellulose ion-exchange chromatography and other protein fractionation and purification methods.

4. Enzyme (papain) digestion of isoantigens to produce smaller fragments for study.

Major Findings:

Investigations have shown that components of sera from individual rabbits are antigenic in certain other rabbits and that isoprecipitins can be produced which react with serum antigens having electrophoretic

mobilities corresponding to alpha-, beta-, and gamma-globulins.

Significance to the Program of the Institute:

1. Differences in serum proteins between individuals of the same species may be of genetic and clinical significance similar to that of the blood groups. The serum isoantigens may be implicated in some of the unexplained transfusion reactions in man.

2. Serum isoantigens may be useful in the study of "immune tolerance" and perhaps serve as a somewhat analagous experimental model for the study of supposed tolerance to Rh antigen.

3. Serum protein isoantigens may be particularly suitable for study of some basic problems in immunology concerning the nature of antigen and antibody sites, antigenicity of antibodies, chemical structure of gamma-globulin, the antigen-antibody reaction, etc.

Proposed Course of Project:

1. Chemical characterization of the gamma-globulin isoantigens in rabbits. Development of methods of assay for each of the isoantigens.

2. Studies on the chemical structure of gamma-globulin by papain digestion studies and quantitative assay of antigenic sites.

3. Search for additional gamma-globulin isoantigens and additional alpha- and beta-globulin isoantigens.

4. Specificity of rabbit isoantigens and antigenicity in other species.

5. Isoantigens in other species, particularly mice and man.

6. Isoantigens in other tissues.

7. Antibody production in rabbits of different gamma-globulin types.

Project Subtitle III: Application of serum isoantigen-isoantibody systems to study of "Immune Tolerance."

Objectives:

To investigate the question as to whether the isoantigens would be useful in the study of so-called "immune tolerance."

Methods Employed:

1. Selective breeding of rabbits on the basis of known genetics of gamma-globulin isoantigens.

2. Immunochemical methods to evaluate production of isoantibodies and presence of isoantigens.

3. Quantitative assay of isoantigens by agar-gel methods and labeling with tracers.

Major Findings:

Results of preliminary experiments suggest that "immunologic unresponsiveness" to gamma-globulin isoantigens may be induced in rabbit offspring in the following ways. The offspring may be exposed to the "foreign" isoantigen naturally during pregnancy as a result of the "foreign" isoantigen of the mother passing through the maternal-fetal barrier. The offspring may also be exposed artificially by transfusion of the mother with the "foreign" isoantigen during the last week of pregnancy.

Significance to the Program of the Institute:

"Immunologic unresponsiveness" has been one of the most interesting problems in hypersensitivity during the last few years as a result of new developments in our knowledge of tissue grafting. Should the isoantigen-isoantibody system be effective in the induction of "tolerance," this system may very well be most suited for a study of some of the basic mechanisms involved in "tolerance." The isoantigen-antibody system has the advantage that one may work with soluble proteins (rather than tissue) and with the precipitating antibodies (rather than tissue rejection).

Proposed Course of Project:

Discontinued at present because of lack of sufficient animals and facilities.

Project Subtitle IV: Synthesis of Gamma-Globulins in The Fetus and Newborn

Objectives:

To determine when and where gamma-globulins are first produced by the fetal or neonatal rabbit and the rate at which the gamma-globulin synthesized by the offspring appears in the serum.

Methods Employed:

1. Selective breeding of rabbits on the basis of the known genetics of

gamma-globulin antigens.

2. Immunochemical methods to assay quantity of gamma-globulin isoantigen appearing in the serum.

3. Fluorescent antibody to localize time and site of appearance in tissues.

Major Findings:

Preliminary findings have shown that the six-week neonatal rabbit lacks some of the isoantigens it later produces.

Significance to the Program of the Institute:

1. The isoantigen-isoantibody system in rabbits provides the best means for study of gamma-globulin formation in the newborn animals since the gamma-globulin produced by the offspring can be clearly distinguished from maternal gamma-globulins which pass the placental barriers.

2. This study may lead into an experimental system analagous to the Rh system.

3. Serve as a pilot study for the question of transfusion reactions to plasma.

Proposed Course of Project:

1. Study the production of gamma globulin in offspring of mothers immunized to the gamma-globulin produced by the fetus in analogy to the Rh system.

2. Use of isoantigens as cell markers in connection with studies of skin homograft rejection in cooperation with Dr. Joseph Bauer, Peter Bent Brigham Hospital, Boston, Massachusetts.

3. One of the interesting by-products would be the possibility for the study of the rate of gamma-globulin synthesis per cell.

Project Subtitle V: Use of Isoantigens as Cell Markers in Cell-Transfer Studies.

Objectives:

To determine whether donor white cells from an immunized rabbit produce antibody in an irradiated recipient.

Methods Employed:

1. Use of gamma-globulin typed rabbits as donors and recipients.

2. Fluorescent antibody to identify the source of gamma-globulin antibody found in the recipient.

Major Findings: None

Significance to the Program of the Institute:

The isoantigen-isoantibody system provides the means to definitively determine the above objective, and would demonstrate the applicability of this system to other problems in immunology involving cell transfer.

Proposed Course of Project:

Evaluate the question as to whether one or two gamma-globulin isoantigens may be produced in the same cell. This is a question of considerable theoretical interest with regard to theories of antibody production.

Part B included: Yes X No

(Attachment I)

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Part B: Honors, Awards, and Publications

Publications:

Dray, Sheldon and Young, Glendowlyn, O.: Genetic Control of Two Gamma-Globulin Isoantigenic Sites in Domestic Rabbits. Science, 131:738-739, 1960.

Form No. ORP-2
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PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 147-A
1. Laboratory of Immunology
2. Immunochemistry Section
3. Bethesda, Maryland

Part A:

Project Title: Immunochemical Studies on Human Serum Antigens and Antibodies

Principal Investigator: Dr. Sheldon Dray

Other Investigators: None

Man Years:

Total: 2.0
Professional: 0.5
Other: 1.5

Patient Years: None

Project Description:

Objectives:

1. Identification, fractionation, purification, and chemical properties of antigens in human serum.

2. Significance of serum antigens and antibodies in disease.

Methods Employed:

1. Immunochemical analysis by diffusion in agar-gel; simple diffusion in tubes, double diffusion in plates, and immunoelectrophoresis.

2. Cellulose ion-exchange chromatography, zone electrophoresis, and other methods of protein fractionation and purification.

3. Use of primates for immunization with human serum antigens.

Major Findings:

1. Primates were selected for immunization with human serum antigens with the idea that a closely related species to man might yield antibody to components of human serum not readily obtainable in other ways. Immunization of Rhesus monkeys with various antigens from human serum has yielded precipitating antibody to many components of human serum (albumin, alpha-, beta-, and gamma-globulins). Precipitating antibodies were found which were specific for three normal gamma-globulins of relatively slow electrophoretic mobility instead of the one usually found with rabbit or horse antisera. Furthermore, the immunochemical relationship between the three normal 7S gamma-globulin antigen and two myeloma gamma-globulins

were investigated and it was found that one of the myeloma gamma-globulins corresponded to one of the normal gamma-globulins while a second myeloma gamma-globulin corresponded to the second myeloma gamma-globulin. It was further found that these globulins have antigenic determinants in common.

2. Nine chimpanzees have been immunized with components of human serum. The results thus far have revealed two antisera to human alpha-globulin and several antisera to human gamma-globulin.

Significance to the Program of the Institute:

The availability of precipitating antibodies specific for three normal human gamma-globulins should facilitate many studies of considerable interest concerning these gamma-globulins, such as: quantitative estimation in serum and other body fluids; fractionation and purification; chemical structure, particularly in the analysis of fragments resulting from enzyme digestion; antibody properties in infectious diseases and diseases of supposed immunologic etiology; cytological localization by fluorescent antibody; and possible genetic differences. Of immediate clinical interest, the quantitative estimation of these gamma-globulins in serum should be useful for early diagnosis and study of diseases which involve qualitative and quantitative changes in the gamma-globulins, such as in multiple myeloma.

Proposed Course of the Project:

1. Continue the immunization of primates with various fractions of human serum and to study the antigens which then may be detected.

2. Survey 30 multiple myeloma sera (in collaboration with Dr. John Fahey, Metabolism Branch, NCI) with respect to their reactions with monkey antisera.

3. Immunization of monkeys with myeloma proteins, rheumatoid factor, and other proteins of pathological origin.

4. In cooperation with Dr. Edward C. Franklin (New York University College of Medicine) to investigate the antigenic determinants of papain fragments of human gamma-globulins.

Part B included Yes No X

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PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 148
1. Laboratory of Immunology
2. Applied Immunology
3. Bethesda, Maryland

Part A:

Project Title: Basic Studies on the Cellular Localization of Antigens, Antibodies and other Substances by the Fluorescent Antibody Technique and Fluorescence Microscopy.

Principal Investigator: Dr. John E. Tobie

Other Investigators: None

Cooperating Units:

Project Subtitle II - Dr. G. Robert Coatney, LPC, Serial No. NIAID-130-B

Project Subtitle III - Mrs. Eleanor J. Tobie, LPD, Serial No. NIAID-123-D

Man Years: Patient Days: None

Total: 3.0
Professional: 1.0
Other: 2.0

Project Description:

Project Subtitle I: Antibody Formation in Neonatal Rabbits

Objectives:

To perform studies on the types of cells involved in antibody formation and to determine the capacity of neonatal animals to synthesize antibody.

Methods Employed:

Neonatal and adult rabbits were given a single injection of bovine serum albumen, crystalline egg albumin or killed typhoid organisms, combined with hydrocarbon adjuvants, into the footpad. The animals were bled frequently and tested by hemagglutination or agglutination reactions in an effort to determine the earliest time at which circulating antibodies first appear. In certain animals the regional popliteal lymph node was removed and the node fluid tested for presence of antibody. Fluorescent antibody methods will be employed to determine the lymph node cells involved in antibody synthesis.

Major Findings:

Using the antibody responses after a single injection of bovine serum albumen as an example, neonatal animals (1-3 days old when injected) were very slow in producing antibody. None of the rabbits produced detectable antibody earlier than 3-4 weeks while in individual animals it took as long as 100 days. Certain animals failed completely to respond to a single injection when followed for as long as 400 days. One animal which became positive at 102 days continued to produce antibodies through 481 days, at which time the animal was sacrificed. In contrast to the antibody response in neonatals, antibody could be detected consistently in adult rabbits 7-9 days after a single injection. In several animals no circulating antibody could be detected in spite of the presence of antibody in lymph node fluid.

Significance to the Program of the Institute:

A study of the nature of the antibody response in neonatal animals may lead to a better understanding of the mechanisms involved in "immunological immaturity" in the infant. The influence of age upon antibody formation is an important factor in the capacity of infants to be actively immunized.

Proposed Course of Project:

Fluorescent antibody studies will be conducted on lymph nodes from the neonatal rabbit because the slow antibody formation in the neonatal, as compared with adults, seems to offer a better opportunity to study the cellular aspect of antibody synthesis.

Project Subtitle II: Fluorescent Antibody Studies on Malaria Parasites

Objectives:

To specifically stain human malaria parasites by the fluorescent antibody technique in such a manner that essentially only the malaria parasites are visible on a blood film.

To feed such blood films through an automatic scanning microscope for the rapid detection of parasites in large-scale surveys.

Methods Employed:

Globulin fractions of sera were prepared from inmate volunteers who had long-standing infections with Plasmodium vivax. The globulin was conjugated with fluorescein isothiocyanate and applied to thin blood films which had been dehemoglobinized and the films examined at fluorescence microscopy.

Globulin from a patient infected with the vivax-like malaria parasite (P. cynomolgi bastianellii) of rhesus monkeys was labeled and applied to individual blood films containing either parasites of human origin or those of monkey origin. The cross reactions between these two malaria species were determined by the fluorescent light emitted.

Major Findings:

The first recorded instance of the fluorescent antibody staining of human malaria parasites, Plasmodium vivax, and that of the monkey parasite, P. cynomolgi bastianellii, has been accomplished. Preliminary studies on the serological relationships of these plasmodia, as based on the degree of fluorescence, indicate that the two species may be closely related. The separate application of P. vivax labeled antibody to individual blood films containing the parasites of rodent malaria, P. berghei, or human malaria, P. vivax, resulted in the latter fluorescing approximately 3 times as bright suggesting that these two species probably are not as closely related.

Significance to the Program of the Institute:

The fluorescent antibody studies on malaria could lead to a method whereby hundreds of blood films could be scanned in a single day. A study of the serological relationships between malaria plasmodia of human and animal origin are of great importance in connection with the program of the worldwide eradication of malaria which has become more complex with the possibility that monkeys may serve as reservoirs for human infection.

Proposed Course of Project:

To continue the studies on the serological relationships, explore methods of producing high-titered antisera in rabbits by sporozoite immunization and investigate the application of fluorescent stained malaria films to rapid scanning in an automatic microscope.

Project Subtitle III: Localization of Trypanosoma rangeli in the Insect Host

Objectives:

To determine the organ localization of Trypanosoma rangeli in the arthropod host and to study the developmental relationships between the parasite and the insect vector.

Methods Employed:

The insect vector, Rhodnius prolixus, has been experimentally has been experimentally infected, either artificially, by injection of trypanosomes into the hemocoel or naturally, by allowing the

insect to take blood meals on an infected animal. Fresh-frozen sections of the whole insect were prepared and the sections conventionally stained. Fluorescent antibody methods also will be employed for discrete localization in the arthropod host.

Major Findings:

The trypanosomes have been localized in the salivary glands of the insect after artificial infection. After certain intervals, the developmental stages of the trypanosome also were found in the intestinal tract of the arthropod.

Significance to the Program of the Institute:

The successful localization of Trypanosoma rangeli in fresh-frozen sections of whole insects opens up the possibility of the precise localization of other infectious agents in arthropod hosts. Detailed studies on the developmental relationships between the parasite and the insect vector are now within the realm of possibility.

Proposed Course of Project:

Fluorescent antibody studies have been initiated in an effort to elucidate the mechanisms involved in the insect transmission of this trypanosome.

Part B included: Yes X No

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Part B: Honors, Awards, and Publications

Publications:

Milch, Robert A., Tobie, John E. and Robinson, Robert A.: A Microscopic Study of Tetracycline Localization in Skeletal Neoplasms. Jour. Histochem. Cytochem., In press.

Tobie, John E. and Beye, Henry K.: Fluorescence of Tetracyclines in Filarial Worms. Proc. Soc. Exp. Biol. Med., 104:137-140, 1960.

Tobie, John E., Burgdorfer, Willy and Larson, Carl L.: Frozen Sections of Arthropods for Histological Studies and Fluorescent Antibody Investigations. Experimental Parasitology, In press.

Tobie, John E. and McCullough, Norman B.: Serological Evidence of Leptospira pomona Infections in Meat Inspectors. Jour. Am. Vet. Med. Assoc. In press.

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Individual Project Report
Calendar Year 1960

Serial No. NIAID - 150
1. Laboratory of Immunology
2. Applied Immunology
3. Bethesda, Maryland

Part A:

Project Title: The Relationship of Delayed and Immediate Hypersensitivity to Allergic Thyroiditis in Inbred Guinea Pigs.

Principal Investigator: Dr. Philip R. B. McMaster

Other Investigators: None

Cooperating Units: Dr. Edwin M. Lerner, LPH, Serial No. NIAMD - 4

Man Years:

Total: 1.2
Professional: 0.6
Other: 0.6

Patient Days: None

Project Description:

Objectives:

To determine the relative importance of delayed and immediate hypersensitivity to thyroid extract in the etiology and pathogenesis of allergic thyroiditis.

Methods Employed:

Two groups of strain 13 histocompatible guinea pigs, one immunized with the aid of complete adjuvant, the other with the aid of incomplete adjuvant, were compared with respect to the development of circulating antibody, delayed hypersensitivity to thyroid extract, and thyroiditis.

Major Findings:

A close correlation between the appearance of delayed hypersensitivity to thyroid extract and allergic thyroiditis has been found. The discovery that the disease could appear as early as five days after the start of immunization allowed the investigators to show an inverse relation between serum antibody and the disease could exist as well as a direct correlation between the two, depending on the interval of time after immunization. This suggests the possibility of a similar relationship between delayed hypersensitivity to the thyroid and Hashimoto's disease.

Significance To The Program of The Institute:

As a result of these studies, a close correlation has been demonstrated between delayed hypersensitivity to the thyroid and experimental allergic thyroiditis. This, therefore, provides experimental evidence for the role of delayed hypersensitivity in the pathogenesis of experimental allergic thyroiditis and suggests the possibility of a similar role in the development of allergic thyroiditis in humans.

Proposed Course of Project:

The long term course of this disease in guinea pigs is now being followed in preparation for a study of the effects of desensitization to an auto-antigen upon an auto-allergic disease, for which this particular condition is most admirably suited.

Part B included: Yes X No

(Attachment I)

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Part B: Honors, Awards, and Publications

Publications:

McMaster, Philip R.B.: Autoantibodies and Autosensitivity, in Fundamentals of Modern Allergy, Edited by Samuel J. Prigal, 51-69, 1960.

McMaster, Philip R.B.: Decreased Aqueous Outflow in Rabbits With Hereditary Bupthalmia. A.M.A. Arch. Ophth. 64:388-391, 1960.

McMaster, Philip R.B., Lerner, Edwin M., and Exum, Eurmal D.: The Relationship of Delayed Hypersensitivity and Circulating Antibody to Experimental Allergic Thyroiditis in Inbred Guinea Pigs. J. Exp. Med. (In Press)

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PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 152
1. Laboratory of Immunology
2. Applied Immunology
3. Pasadena, California and Bethesda, Maryland

Part A:

Project Title: The Chemistry of Antibodies

Principal Investigator: Dr. Wilton E. Vannier

Other Investigators: None

Cooperating Units:

Project Subtitle I - Dr. Ernest M. Heimlich of the Department of Pediatrics of the University of California at Los Angeles

Project Subtitle II - Dr. James Miller of the Department of Infectious Diseases of the University of California at Los Angeles

Project Subtitle III - Dr. Anil Saha of the California Institute of Technology and Dr. Jean-Marie Dubert of the Pasteur Institute in Paris

Man Years:

Total: 1.16
Professional: 0.66
Other: 0.5

Patient Days: None

Project Description:

Project Subtitle I: The Chemistry of the Skin Sensitizing Antibodies of Atopic Human Allergy

Objectives:

To study the sedimentation properties of the skin sensitizing antibodies of the sera of individuals with atopic allergies.

Methods Employed:

Sera from allergic individuals were fractionated by sedimentation in an angle rotor in the preparative ultracentrifuge, by sedimentation in the Waugh partition cell in the analytical ultracentrifuge and by sedimentation in salt density gradients in a swinging bucket rotor in the preparative ultracentrifuge. The fractions obtained have been characterized by analytical ultracentrifugation, electrophoresis and passive transfer skin reaction.

Major Findings:

As previously reported, our data show that the skin sensitizing activity cannot be exclusively associated with the S19 component and that probably the S19 component is not active at all. Our data and more recent experiments carried out by Dr. Alec Sehon, Dr. Julius Gordon and Dr. Ladislas Gyenes at Montreal are all consistent with the hypothesis that the skin sensitizing activity is associated with a serum component or components of intermediate sedimentation coefficient, i. e. between 7S and 18S. The salt density gradient studies have shown that the sensitizing antibody appears to be associated with serum proteins of hydrated density of about 1.36 rather than being present in the lipoprotein fractions of density 1.2 or lower. During the course of the salt density gradient studies, it has been observed that there is a considerable loss in antibody activity associated with the fairly high salt concentrations used.

Significance to the Program of the Institute:

The physical properties of the skin sensitizing antibody of atopic allergy are of fundamental importance in understanding allergic reactions. The skin sensitizing antibody is qualitatively different from most ordinary precipitating antibody in its electrophoretic and sedimentation properties and in that it is retained locally when injected intradermally rather than diffusing away. The process of antibody fixation to tissues is an important problem in all allergic reactions and any differences that can be demonstrated in the chemical or physical properties between ordinary precipitating and skin sensitizing antibody may be important in this process.

Proposed Course of Project:

A more detailed study of the salt inactivation of the skin sensitizing antibody will be carried out.

Project Subtitle II: The Chemistry of the Antibodies of Syphilis.

Objectives:

To study the physical properties of some of the antibodies associated with syphilitic infections and to study a macroglobulin antibody system in which a specific purification of antibody may possibly be achieved.

Methods Employed:

Sera from individuals with various stages of syphilitic infection are being fractionated by starch block electrophoresis and ultracentrifugation methods and the fractions tested for VDRL flocculation antibody and for TPI antibody.

Major Findings:

The work has not progressed far enough for any definite conclusions to be reached.

Significance to the Program of the Institute:

There is confusion in the literature with regards to the interrelationship between the antibodies in syphilis as measured by the various tests available. Studies, such as this, involving the preparation of purified antibody fractions may help to clarify the problem. The study of the chemical and physical properties of specifically purified macroglobulin antibody may yield basic information regarding the structure of these antibodies and their role in immune processes.

Proposed Course of Project:

The studies will be continued as indicated. In the future further fractionation will be attempted using ion exchange chromatography.

Project Subtitle III: The Fractionation and Specific Purification of Antibody

Objectives:

1. To explore possible specific methods of antibody purification.

2. To achieve the chromatographic fractionation of specifically purified antibody and study changes in the distribution of specific bonding affinities of antibodies during the course of immunization.

Methods Employed:

Specific antigen-antibody aggregates are dissociated at low pH and the antibody recovered. Cellulose coupled with p-arsanilic acid through tyramine is being used as a solid adsorbent to purify and fractionate antibody directed against the p-arsanilic acid hapten.

Major Findings:

1. Purified antibody fractions have been obtained by the low pH dissociation of BSA antiBSA precipitates and the elution of antibody from the cellulose-hapten adsorbent with simple hapten (sodium p-arsanilate).

2. Cellulose-tyramine-p-arsanilic acid, cellulose-histamine-p-arsanilic acid and cellulose-lysine-p-arsanilic acid adsorbents have been prepared and it has been found that all three will adsorb antibody from sera obtained by immunization with p-arsanilic acid coupled hemocyanin.

Significance to the Program of the Institute:

The general problem of specific purification of antibody is one that is of basic importance for chemical work in immunology. The presence of large amounts of nonspecific protein with antibody complicates or prevents a study of the chemical reactions of antibodies, the structure of antibodies or the physical properties of antibodies. A study of the heterogeneity of antibodies and especially the influence of degree or route of immunization on the distribution of antibodies found will provide additional basic information about the nature of the immune process.

Proposed Course of Project:

The yield, purity and immunochemical characteristics of antibody removed by the specific adsorbents and purified by dissociation of specific precipitates will be further investigated. The preparation of cold and C^{14} labeled haptens for equilibrium dialysis studies with antihapten antibody fractions will be continued.

Part B included : Yes [X] No [ ]

(Attachment I)

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Part B: Honors, Awards, and Publications

Publications:

Heimlich, Ernest W., Vannier, Wilton E. and Campbell, Dan H.: Sedimentation Studies of Skin Sensitizing Antibody. J. Allergy 31:364 (1960).

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Nov. 1960

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NIAID - 153
1. Laboratory of Immunology
2. Applied Immunology
3. Pasadena, California and Bethesda, Maryland

Part A:

Project Title: Basic Studies on the Chemical, Physical and Skin Reactive Properties of Extracts of House Dust

Principal Investigator: Dr. Wilton E. Vannier

Other Investigators: None

Cooperating Units:

Dr. Ernest M. Heimlich of the Department of Pediatrics of the University of California at Los Angeles and Dr. A. M. Targow, a practicing allergist in Los Angeles have tested various house dust fractions for skin reactivity in specifically sensitive individuals.

Man Years:

Total: 0.83
Professional: 0.33
Other: 0.50

Patient Days: None

Project Description:

Objectives:

1. To study the chemical and physical properties of the materials used clinically for the diagnosis and treatment of house dust allergy.

2. To try to identify the components responsible for the specific skin reactivity.

Methods Employed:

1. Fractions of aqueous house dust extracts have been prepared by starch block electrophoresis and evaluated for activity by direct skin test and analyzed chemically.

2. Studies are in progress to determine the effect of chemical or enzymatic modification of the dust fractions on the specific skin reactivity.

Major Findings:

1. Previously it was shown that the dust allergen fractions consist

of a heterogeneous mixture of acidic polysaccharides containing 5-20% polypeptide. Starch block electrophoresis studies at low pH and in 7M urea have indicated that the polypeptide and polysaccharide components are linked through covalent bonds rather than being present as separate components or linked by ionic bonds or multiple hydrogen bonds.

2. Preliminary experiments involving the chemical modification and enzyme treatment of active dust fractions have been carried out. Partial methyl esterification of the free carboxyl groups of the polypeptide portion did not affect the activity; however, the extent of conversion to ester was rather low and it will be necessary to test materials in which a more complete methyl esterification has been achieved in order to be sure that the carboxyl groups are not involved in determining the allergen activity. Treatment of active dust allergen fractions with pepsin has had no influence on their skin reactivity.

Significance to the Program of the Institute:

1. About one-third of all allergic individuals give a positive skin reaction to house dust extracts. These extracts have been used for many years in the diagnosis and treatment of house dust allergy. It is of importance to investigate the chemical nature of these materials and to find out what chemical groups are involved in the skin reactions.

2. A rational standardization of the potency of house dust allergen extracts might well be based on a knowledge of the chemical nature of the active materials.

Proposed Course of Project:

The study of chemically modified and enzyme treated dust fractions will be continued.

Part B included: Yes [X] No [ ]

(Attachment I)

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Part B: Honors, Awards, and Publications

Publications:

Vannier, Wilton E. and Campbell, Dan H.: A Starch Block Electrophoresis Study of Aqueous House Dust Extracts. J. Allergy, in press.

ROCKY MOUNTAIN LABORATORY

ROCKY MOUNTAIN LABORATORY

PHS-NIH
Summary Statement
Rocky Mountain Laboratory
Hamilton, Montana
Calendar Year 1960

The accompanying annual report summarizes, as well as such a report can, the activities of the staff of the Rocky Mountain Laboratory for the year 1960. We have presented our projects in the same manner as we have in previous years. This is necessary because of the frequent cooperation of workers in various sections in the solution of mutual problems and because of the types of problems in which we are engaged.

Our research has continued to be directed toward both basic laboratory investigations and field studies of insect- and animal-borne diseases. In the first category are those projects concerned with the chemistry and surface properties of viruses, the highly intriguing relations of hypersensitivity and humoral immunity, and the basic relation of structural elements of microorganisms to the activity of the agents, both in vivo and in vitro. Field work is the foundation of projects related to studies of Q fever, tularemia, Colorado tick fever, and the ar-bo viruses. In addition, the combined efforts of the staff are directed to many other areas of research. Such projects include Q fever, tuberculosis, influenza, poliomyelitis, and cryptococcosis.

We are also engaged in studies of a cooperative nature with members of the staff of the National Institutes of Health, Communicable Disease Center, University of Minnesota, University of Georgia, Purdue University, University of Michigan, New York University, Buffalo University, and other institutions. The cooperators have sought our aid because of special methods, techniques, or ideas which have been developed at RML. As a result of our past accomplishments, 63 visitors have spent some time here during the last year. I believe this reflects in some measure the solid contributions the staff has made in both the recent and distant past, for these visitors represent scientists interested not only in entomological and biological problems but also those interested in chemical, physical, and immunological problems related to many fields of microbiology.

Hypersensitivity. Studies of hypersensitivity have been directed primarily toward clarification of the relations existing between delayed hypersensitivity and circulating antibodies and determination of the factors responsible for induction of contact hypersensitivity. It has been demonstrated previously that delayed hypersensitivity precedes circulating antibodies. This delayed hypersensitivity is directed toward protein. When circulating antibody appears, as occurs with relatively large amounts of conjugated protein, or when booster doses are administered, the specificity of the antibody response becomes oriented toward smaller configurations on the antigen molecule. Studies of immunity in neonatal animals have revealed that these animals, when injected with an antigen 12 hours after birth, develop circulating antibodies but fail to develop delayed hypersensitivity. Additional experiments suggest that this inability of neonatal animals to express such reactions is due to a deficiency

in a skin reactive factor rather than an inability to respond to a primary injection of antigen.

It was shown that contact hypersensitivity is directed toward a hapten and that simple compounds rather than protein conjugates of these compounds produce contact hypersensitivity when administered to experimental animals. It is considered, however, that the production of this phenomenon is related to the specific proteins present in the host tissues because a conjugate containing soluble guinea-pig skin proteins and a hapten produces in guinea pigs delayed reactions and Arthus reactions to the conjugate and contact hypersensitivity to the hapten.

Poliovirus. Continued studies of poliovirus have yielded considerable fundamental data. In cooperation with the group at the University of Minnesota, it was shown that agents such as octyl alcohol-chloroform and neutral hydroxylamine do not materially change the physical properties of purified infectious RNA of poliovirus but destroy over 99% of the infectivity of this material. The destruction of infectivity by uncoupling of a single link in a large particle (probably an acyl link of an amino acid with a phosphate group at the end of an RNA chain) suggests a possible approach to virus chemotherapy. Other studies have revealed that only 0.1% of RNA infectivity could be accounted for by residual protein, thus strengthening the concept that RNA does indeed constitute the infectious portion of the poliovirus moiety. By the use of chromotographic methods, it was also demonstrated that only certain of the avirulent strains of poliovirus can be differentiated from the virulent strains from which they are derived. This is in direct contrast to work reported by others. In studies of the infectivity of RNA it was found that the bulk of virus particles react with susceptible cells and that the relatively poor correlation between virus particles and PFU is due to the poor efficiency of RNA at entering sites where it can influence virus production. A precipitation test for detection of antibodies against poliovirus has been developed which is more sensitive than the neutralization test presently employed. The antigen used is radioactive virus.

Endotoxins in bacterial fractions. Research on endotoxins derived from Gram-negative organisms has continued to occupy much of our attention. As is so frequently the case, a fresh outlook on an old problem yields results of great value. The ideas held by Dr. Westphal, which attributed the activity of endotoxins to the presence of a firmly bound lipid ("lipid A"), were apparently generally accepted until presentation of the work done at RML. The finding that lipid A was not active and that deproteinized and "delipified" endotoxin was active stirred considerable controversy. In fact, the controversy was so intense that our efforts to develop a vaccine against Salmonella infections have been diverted to settling this issue. Recent studies of the kinetics of inactivation of toxin by hydrolysis with hot acid have given data which should end this discussion. The old concept of "purified endotoxins" must be abandoned since there have been no previous toxins as good as those obtained at RML, and we feel these are to be still further purified. Many of our studies would have been difficult to pursue without the active participation of Dr. Landy and others, especially certain commercial concerns which, because of interest in our results, provided us with mass cultures of organisms grown in commercial lots.

The purification of Vi antigen by curtain electrophoresis is a major advance in the study of this most important antigen. The demonstration that certain labile acetyl groups are responsible for the activity of Vi antigen resulted in production of material which was ten times more active than purified preparations prepared by mild acid hydrolysis. Emphasis should be placed upon the new chemical, physical, and biologic methods that have been devised to solve these problems.

Many of these methods and ideas have been used in studies of other problems raised here and by visiting scientists. Contributions made to the study of immunology of brucellosis and of Q fever will be discussed elsewhere. The fine structure of bacterial spores has been studied in conjunction with Dr. Gerhardt. These studies show that the coat is made up of two-dimensional crystals. Results of a study with the group from Purdue University revealed that the fine structure of the walls of Penicillium chrysogenum contained much chitin and very little glucan in contrast to the previous finding of this group of workers. These studies substantiate the results with Histoplasma capsulatum previously reported from RML.

While these results may be considered to be of only theoretical interest, many questions have been solved by methods applied here. It is believed that these studies are of fundamental importance and will serve as guidelines in future studies of the relation of morphological elements to various in vitro and in vivo activities of microorganisms.

Tuberculosis. The problem of immunity in tuberculosis has been studied intensively. Significant findings include the fact that mice may be satisfactorily immunized to subsequent pulmonary infection with virulent organisms by administration of small doses of avirulent organisms by the aerosol method. The demonstration that resistance is not due to interference strengthens the case for the value of immunization with living attenuated organisms for the prevention of tuberculosis. Since it has been shown that the delayed reactions elicited by protoplasm of various acid-fast organisms are specific in nature, it seems practicable to apply these findings to certain diagnostic problems in man. Use of fractions of tubercle bacilli in producing isoallergic encephalitis in guinea pigs shows that the adjuvant effect lies in the cell walls and in a water-soluble protein prepared from the walls. This latter finding is important since it will allow us to study the adjuvant phenomenon from a molecular level.

Q fever. Our studies on Q fever in many ways have been most productive and in other ways most frustrating. It has been demonstrated that the number of dairy cattle infected with Coxiella burnetii is large and is increasing, yet it is extremely difficult to detect cases of clinical disease in man. In Idaho and Montana we have shown that a greater number of individuals residing on infected premises have antibodies against this organism than do those living on noninfected premises, yet no difference can be detected in the number of individuals who have symptoms compatible with clinical disease. The fact that organisms isolated from cattle have been uniformly of low virulence for experimental animals may account for our inability to find clinical cases of disease in those exposed only to cattle.

More satisfying experiences were noted with other phases of the studies. By the use of skin tests to eliminate allergic individuals from the study group, 190 inmates of the Montana State Prison were safely immunized without producing such reactions as have been previously reported. It is evident from these results, as well as from those previously obtained in laboratory personnel, that human beings can be safely vaccinated against Q fever if the precaution is taken to eliminate reactors by previous administration of specific skin-test antigen.

Methods developed for growing rickettsiae on modified Zinsser tissue cultures yielded relatively large volumes of organisms. These studies led to others involving purification of C. burnetii by sucrose gradients and by continuous-flow centrifugation in molar salt solution. These methods likewise made it possible to obtain certain chemical and physical fractions of these organisms. It was found that dimethyl sulfoxide could extract from Phase II C. burnetii a material which acted only as a hapten, but from Phase I organisms the extract obtained acted as a complete antigen. Lauryl sulfate also extracts complete antigen from Phase I organisms. Physically, the cell walls of these organisms can be separated from the protoplasm, and it has been noted that the cell walls are about 25 times more active in producing immunity than is protoplasm.

In addition, considerable effort was expended in evaluating Q fever vaccines and in assembling data which might be used for promulgating minimum standards for production of Q fever vaccines.

These studies on Q fever are of considerable significance. The laboratory and related studies have yielded information of both scientific and applied interest. It is apparent now that we can safely use our present vaccines for immunization of man and that it is feasible to produce large numbers of organisms which can be purified and used as vaccine or manipulated to give physical or chemical fractions which may be less toxic. The failure to find clinical cases of Q fever in man in the face of a rising incidence of infection in dairy cattle is highly interesting even if disappointing. The lack of virulence of strains of C. burnetii for laboratory animals probably is responsible for this finding.

Other rickettsioses. Studies of rickettsiae other than C. burnetii have been continued. By combining the methods presently used for fluorescent microscopy, we have developed with Dr. Tobie a technique for sectioning arthropods which should be of interest to entomologists working in the field of embryology and anatomy and to medical entomologists, since thin sections in which the organs are not displaced can be obtained routinely. By applying the technique to the study of ticks infected with R. rickettsii it was found that the infection rate in local ticks varies from 15% to 28%. Not all of the ticks found infected by this method are infective for laboratory animals. The value of this type of study has yet to be fully appreciated.

By application of methods developed at RML we have been able to produce a vaccine for immunization against Rocky Mountain spotted fever which is 10 to 100 times more potent than those presently manufactured on a commercial basis.

The use of specific toxins has resulted in clarification of many of the problems related to the taxonomy of rickettsiae and has proved to be useful in ecological and epidemiological studies of this complex group of diseases. In further studies, potent immunogenic extracts have been obtained from certain of the rickettsiae. Their value as diagnostic and prophylactic agents is presently under consideration.

Tick paralysis. Studies of the incidence of ticks capable of producing tick paralysis in hamsters have shown that 24% to 90% of lots from Colorado induce disease; from 0% to 50% of those from the Bitterroot Valley; and 75% from adjoining Missoula County. The incidence of ticks with ability to produce disease does not appear to be related to the number of cases of tick paralysis reported in man.

Pasteurella novicida. Considerable debate has been caused by our description of P. novicida as a new species. Application of the Ovary reaction (passive cutaneous anaphylaxis) has given weight to our description.

Bacterial vaccines. Studies have been continued on vaccines for certain bacterial diseases. It has been found that while live Russian tularemia vaccine is capable of protecting mice more effectively than does ether-extracted vaccine derived from cell walls of P. tularensis, the protection produced by live organisms was not effective for long periods of time. Continued studies, in conjunction with Dr. Foster, have emphasized the value of cell walls in producing immunity to infections with Brucella abortus in laboratory animals. It has also been found that live cells suspended in phosphate buffer and shaken with an excess of ether are killed but not disrupted. These cells constitute an excellent protective antigen which is less toxic (LD_{50} 7.5 mg.) than aqueous ether extracts obtained by conventional methods (LD_{50} 0.9 to 2.0 mg.). It is planned that Dr. Foster will continue these studies in Georgia.

Investigations of other bacterial, fungal, and viral infections common to man and animals have been continued. P. multocida is found as a contaminant of the oral cavity of many species of animals, and this situation is a potential source of human infection. Leptospira pomona has been shown to be present in cattle in western United States, and the organism was obtained from cerebrospinal fluid of cattle for the first time. The organism causing haplomycosis in animals was demonstrated in tissues of animals from a number of different countries and, in conjunction with Dr. Emmons, a new name, "adiaspiromycosis," was applied to this type of parasitism. It has been found that massive doses of this organism are lethal for monkeys and sheep. The possible relation of this agent to hemorrhagic fever has been suggested.

Rabies in bats. The study of rabies in bats has been continued and, although the number of infected bats has not been high, it continues as a problem. Progress has been made in maintaining bat colonies in the laboratory, and it is now practicable to commence laboratory investigations of pathogenesis and other aspects of rabies infections in bats.

Cryptococcosis. An antigen isolated from Cryptococcus neoformans is now being tested in man to determine the incidence of cryptococcosis in New York,

an area in which pigeons have been shown to be heavily infected with this fungus.

Ar-bo viruses. Studies of ar-bo viruses have yielded results of interest and suggest that emphasis on field studies would greatly increase the production of useful data. The California strain, described by Reeves and Hammon, has been isolated from a snowshoe hare in Montana, and serologic studies of hares obtained from Michigan indicate that the majority possess antibodies against this virus. In California it has been demonstrated that, although most infections in man with this agent are of the inapparent type, some infections result in serious disease. A virus closely related to Powassan virus was recovered from ticks from Colorado and is of importance since viruses of this group produce serious illness in man. Studies to date indicate that ticks probably are not the natural vector, but the relation to Powassan virus suggests that mosquitoes would most likely be the vector in nature. In studies of the complex relation of WEE virus with snakes and mosquitoes it has been possible to demonstrate that the virus can be readily overwintered in garter snakes and that mosquitoes can be infected by feeding on such snakes. While we have not been successful in isolating virus from snakes collected in the field, the laboratory data suggest that these or similar animals would constitute a host suitable for overwintering of WEE virus. In Idaho and Oregon, WEE virus was isolated with considerable frequency during the summer season, while in North Dakota the virus did not appear to be active. Isolations of a considerable number of strains of trivittatus and inornata viruses were made.

Considerable research was performed to determine the level of viremia attained in wild and domestic birds infected with ar-bo viruses. After infection with WEE or St. Louis viruses, turkeys, ducks, chickens, and pheasants display levels of viremia which should cause infection in mosquitoes feeding on them. It is of interest, however, that in spite of considerable effort we have been unable to isolate ar-bo viruses from the bloods of vertebrates. Negative results were obtained in examination of 1,074 specimens collected in Montana, North Dakota, Oregon, and Minnesota during the spring of 1960. These studies fail to add weight to the contention that latent infections of birds are a factor in overwintering or of introduction of virus into endemic areas.

Colorado tick fever. Colorado tick fever continues to be a problem in the western United States. Without stimulation of physicians we still received a large number of specimens for examination this year and isolated virus from 49 of them. Our interest in the spectrum of symptoms has continued and we still see severe cases of illness due either to encephalitis or bleeding tendencies. It was found that the complement-fixation reaction developed at the Rocky Mountain Laboratory is the simplest method for diagnosis of Colorado tick fever. Vaccine has been prepared and has been shown to be efficacious in mice. This type of vaccine has been used repeatedly in man without ill effects, indicating that a vaccine prepared from suckling mouse brain is harmless to man when repeated doses are given.

Ticks collected in Estes Park, Colorado, were examined for the presence of Colorado tick fever virus. The incidence of infection was found to vary from 5% to 21%. This high incidence of infection in ticks accounts for the

large number of cases of CTF reported in Colorado annually.

Publications. At the time this report was written we had published 49 papers and had 27 accepted for publication during the calendar year 1960. This compares favorably with those published in previous years, and the diversity of subjects dealt with reflects the varying interests and capabilities of the members of the staff of the Rocky Mountain Laboratory.

Comment. Although the RML has developed a broad research program and a staff capable of dealing adequately with the laboratory problems assigned to it and those arising from public health needs, consideration should be given to certain physical needs of the plant. These needs include a building suitable for housing experimental animals and facilities for housing the library and the administrative staff. These are matters that call for additions to our present facilities or radical changes. The availability of funds to modernize certain of our laboratories would result not only in some consolidation of space, but also would lead to better safeguards for the health of our staff.

Difficulties were experienced again this year in operating the Rocky Mountain Laboratory on a budgetary apportionment of 80% for personal services and 20% for other expenses. Even though some of our allotted positions have not been filled, 20% of our budget has not been adequate to provide essential materials and equipment. In view of the proposed reactivation of the pathology unit and approved addition of personnel in the areas of epidemiology and immunology, a material increase in the amount allotted for other expenses, in addition to that allotted for staff increases, is essential for continued productive research and for the morale of our present staff.

The quality and quantity of our field work is a specific weakness which we hope to correct by the addition of a qualified epidemiologist to our staff. The application of some of our basic findings to limited human experience would establish whether or not the products developed in the laboratory might be useful for control or diagnosis of disease. The recent study of Q fever vaccine in humans, previously skin tested, to detect and eliminate reactors shows that vaccine can be used with impunity if proper precautions are taken. Similar studies with typhoid fever vaccine (based on observations by Ribi and Milner) should surely be attempted on the above basis and followed by challenge studies in cooperation with Woodward's group. Another vaccine that should be studied in man is prepared from B. pertussis and described by Ribi and Munoz. In addition, purified products of C. burnetii should be examined, and the value of various fractions of acid-fast organisms as diagnostic agents should be investigated. Studies of this nature are essential in order to prove the value of basic research and to shorten the temporal gap which exists between discovery in the laboratory and application to man. This is a legitimate field for endeavor in this institute and in no way interferes with the programs of CDC.

It should be emphasized that the scientific stature of the RML has not suffered from the partial shift in the research aims of the group. Exceptional interest and competence have continued to be manifested by those members of the staff who have followed their studies of insect- and animal-borne diseases through field and laboratory investigations. The recent assignment of

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Drs. Brennan and Yunker to MARU to study the role of mites in the transmission of disease is a move in the direction of establishing the necessary type of entomological field investigation.

Increases in our staff during the past few years have allowed us to develop programs of significance in areas new to us. These areas, specifically viral proliferation, allergy, and the relation of morphological elements of microorganisms to their immunologic activity, have been highly productive and even have created international interest. It should be emphasized that, in spite of the relative isolation of this institution, new ideas, new methods, and important scientific results are forthcoming. As a matter of fact, it is my opinion that much of our productiveness may be a result of the relative remoteness of the laboratory.

Carl L. Larson, M.D.
Director
December 1, 1960

NIAID-170 OFFICE OF THE DIRECTOR
C. L. Larson, Director
C. B. Philip, Acting Director
H. G. Stoenner, Assistant to the Director

NIAID-171 RICKETTSIAL INFECTIONS
Principal investigator: C. B. Philip
Others: E. J. Bell, W. Burgdorfer, C. M. Clifford, G. M. Kohls, D. B. Lackman, R. A. Ormsbee, H. G. Stoenner

NIAID-172 Q FEVER
Principal investigators: L. Luoto, H. G. Stoenner
Others: B. H. Hoyer, D. B. Lackman, R. A. Ormsbee, E. Ribi

NIAID-173 DISEASES HAVING A RESERVOIR OF INFECTION IN THE NATURAL ENVIRONMENT (OTHER THAN RICKETTSIOSES)
Principal investigator: J. F. Bell
Others: W. L. Jellison, D. B. Lackman, M. T. McKee, C. R. Owen, H. G. Stoenner

NIAID-174 TRANSMISSION OF DISEASE AGENTS BY CERTAIN VECTORS
Principal investigator: C. B. Philip
Others: J. F. Bell, J. M. Brennan, W. Burgdorfer, C. M. Clifford, W. L. Jellison, G. M. Kohls, D. B. Lackman, V. F. Newhouse

NIAID-175 ALLERGY AND IMMUNOLOGY OF FUNGAL INFECTIONS AND THE MECHANISMS OF ALLERGIC PHENOMENA
Principal investigator: S. B. Salvin
Others: M. B. Gregg, R. F. Smith

NIAID-176 VIRUSES, VIRUS COMPONENTS, AND VIRUS SURFACES
Principal investigator: B. H. Hoyer
Others: R. K. Gerloff, F. G. Jarvis*, R. A. Ormsbee, D. B. Ritter

NIAID-177 IMMUNE PROPHYLAXIS OF MYCOBACTERIAL INFECTIONS
Principal investigator: E. Ribi
Others: W. T. Haskins, C. L. Larson

NIAID-178 IMMUNE PROPHYLAXIS OF SALMONELLA INFECTIONS
Principal investigator: E. Ribi
Others: W. T. Haskins, F. G. Jarvis*, K. C. Milner

NIAID-179 INVESTIGATIONS OF THE ROLE OF MORPHOLOGICAL ELEMENTS OF MICROORGANISMS IN IMMUNITY AND RELATED PHENOMENA

Principal investigator: E. Ribi
Others: R. L. Anacker, J. E. Coe, C. L. Larson, K. C. Milner

NIAID-180 THE ENCEPHALITIDES

Principal investigator: C. M. Eklund
Others: W. Burgdorfer, D. B. Lackman, V. F. Newhouse, L. A. Thomas

NIAID-181 COLORADO TICK FEVER

Principal investigator: C. M. Eklund
Others: W. Burgdorfer, G. M. Kohls, D. B. Lackman, L. A. Thomas

*Resigned July 29, 1960

Serial No. NIAID-170
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Office of the Director

Principal Investigator: C. L. Larson, Director

Other Investigators: C. B. Philip, Acting Director
H. G. Stoenner, Assistant to the Director

Cooperating Units: None

Man Years (calendar year 1960):

Total:	31.5
Professional:	1.5
Other:	30.0

Project Description:

The over-all direction and supervision of the scientific research program of the Rocky Mountain Laboratory; the direction of the administrative aspects of the Laboratory including all fiscal, supply, housekeeping, and maintenance activities; and the providing of scientific services as required by the research staff, including laboratory animal care and breeding, sterile glassware and media preparation, special shop services, library, graphic arts, etc.

Part B included: No

Serial No. NIAID-171
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Rickettsial Infections

Principal Investigator: C. B. Philip

Other Investigators: E. J. Bell, W. Burgdorfer, C. M. Clifford, G. M. Kohls, D. B. Lackman, R. A. Ormsbee, H. G. Stoenner

Cooperating Units: None

Man Years (calendar year 1960):
Total: 11.0
Professional: 3.0
Other: 8.0

Project Description:

Objectives:

This project is concerned with elucidation of problems created by rickettsial agents (exclusive of Coxiella burnetii) causing human and animal diseases. In the main, attention has continued to be directed toward the spotted fever group of diseases, their relationships, and classification.

Methods:

The customary, standardized procedures for study of rickettsial agents have been followed with particular attention to methods of purification, cultivation, and use of specific rickettsial toxins in study of relationships of the spotted fever group.

Major findings:

1. The value of the mouse protection test against specific toxin has been demonstrated for measuring immunogenic activity of spotted fever vaccines and for correlation with other methods of potency assay.

Part B included: Yes

2. By improved standardized procedures, yolk-sac vaccines prepared at RML were 10- to 100-fold more potent than commercial products. It was a surprise to find that some vaccines prepared from infected ticks by the old methods and stored at 5° C. for from 10 to 15 years exhibited immunogenic potency values equivalent to those of the commercial vaccines.

3. Factors affecting optimum toxin production from R. conorii and R. rickettsii grown in fertile hen's eggs have been defined. Temperature of incubation (33.5° C.) before and after death of the embryos is important, and yolk sacs should be harvested from 36 to 48 hours after death. However, a better yield is obtained from eggs injected with rickettsias of Kenya and Siberian tick typhus and South African tick-bite fever if eggs are held 48 to 60 hours after death. Snyder's solution, which inhibits growth of organisms, is contraindicated for use as a rickettsial seed diluent.

4. An agent consistently nonpathogenic for guinea pigs and mice was isolated from local ticks. The identity and relationships of this isolate, which resembles rickettsia, are under investigation. The relationship of an atypical rickettsial isolate from a patient, who died of an undiagnosed disease, is also being studied.

5. Dimethyl sulfoxide will extract a complement-fixing antigen from purified R. rickettsii but not from R. prowazekii.

6. The fluorescent antibody technique was found to provide a reliable specific diagnostic tool for detection of R. rickettsii in sections of infected ticks which had been held for 3 days at 37° C. Conditions were adequately controlled by comparison with preparations of uninfected ticks, by inhibition with unlabeled immune serums, or by use of heterologous conjugates. Furthermore, untreated samples of fluorescent-positive ticks, experimentally infected, caused infection in guinea pigs. As demonstrated by this technique, natural infection rates among 245 ticks from 4 localities in the Bitterroot Valley varied from 15.5% to 28%. However, only a small percentage of ticks representative of the group yielded strains when tested in eggs or guinea pigs.

7. In a study of transovarial transmission, 100% of 585 eggs and 205 larvae from 5 female ticks were fluorescent positive.

8. Sexual transmission of R. rickettsii from infected male ticks to the progeny of noninfected females appears to be through the agency of "contaminated" fluid surrounding the spermophores received by the female, rather than through the medium of rickettsia-bearing sperm.

Significance:

Continued refinement of methods of preparation of potent rickettsial vaccines, particularly in areas of purified antigens and

standardized potency assays, should benefit commercial producers. The more precise characterization of different isolates of R. rickettsii and relatives within the group should provide tools for improved investigation of other rickettsial entities; for example, the rather bewildering variation observed in the tsutsugamushi (scrub typhus) agents. By these methods additional knowledge of variations in the properties of strains from various geographic locations, both here and abroad, should become available.

Likewise, techniques developed for study of R. rickettsii in D. andersoni by fluorescent microscopy should be adaptable to investigations of other rickettsia-vector relationships. A better insight into vector-parasite relationships, for example the difference in ability of acarine vectors and insect vectors to pass rickettsiae through their eggs, should also be facilitated. This new technique revealed that natural infection in ticks from the west side of the Bitterroot Valley was about double the highest infection rate observed following inoculation of ticks into guinea pigs during a 5-year study in this area in the 1930's. However, it should be noted that a considerable proportion of fluorescent-positive ticks failed to cause infection when untreated samples were injected into embryonated eggs and guinea pigs. This raises questions of quantitative or qualitative factors involved.

The occurrence of fluorescing R. rickettsii in 100% of progeny of 5 female ticks is in contrast to former reports of only incomplete transovarial passage.

Proposed course:

Completion of spotted fever assay studies is expected. Lacunae in our knowledge of the relationships of the spotted fever group will continue to be filled by results of studies of rickettsial toxins. Initial observations by fluorescent microscopy on development of R. rickettsii in ticks will be elaborated.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Bell, E. J. and Stoenner, H. G.: Immunologic relationships among the spotted fever group of rickettsias determined by toxin neutralization tests in mice with convalescent animal serums. J. Immunol. 84(2): 171-182. Feb. 1960.

Philip, C. B.: Rickettsial diseases. In A Manual of Tropical Medicine, 3rd ed. W. B. Saunders & Co., Philadelphia, 1960, pp. 61-69, 82-103.

Burgdorfer, W. and Lackman, D.: Identification of Rickettsia rickettsii in the wood tick, Dermacentor andersoni, by means of fluorescent antibody. J. Inf. Dis. 107(2): 241-244. Sept.-Oct. 1960.

Philip, C. B.: Microtatobiotes, Rickettsiae and Tick-borne Diseases. McGraw-Hill Encyclopedia of Science and Technology. McGraw-Hill Book Co., Inc., New York. 1960. Vol. 8, pp. 403-404; Vol. 11, pp. 567-570; Vol. 13, pp. 630-631.

In press:

Kohls, G. M.: Rocky Mountain spotted fever. History of Medical Department of Army in World War II.

Philip, C. B.: Scrub typhus. History of Medical Department of Army in World War II.

Tobie, E. G. and Burgdorfer, W.: Cryostat technique for sectioning arthropods. Exp. Parasitol.

Honors and Awards relating to this project:

Dr. D. B. Lackman

Appointed lecturer in Microbiology, University of Montana, Missoula, Montana.

Dr. H. G. Stoenner

Invited to rewrite chapter on Rocky Mountain spotted fever for Tice's Practice of Medicine with the collaboration of Dr. C. M. Eklund and Glen M. Kohls.

Serial No. NIAID-172
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Q fever

Principal Investigators: L. Luoto, H. G. Stoenner

Other Investigators: B. H. Hoyer, D. B. Lackman, R. A. Ormsbee, E. Ribi

Cooperating Units: None

Man Years (calendar year 1960):
Total: 12.5
Professional: 4.5
Other: 8.0

Project Description:

Objectives:

In view of the wide-spread distribution of Coxiella burnetii among dairy cattle throughout the United States, continued investigations on this problem are warranted. The objectives of these studies are fourfold: (1) to evaluate the public health aspects of Q fever, particularly the role of dairy cattle in the epidemiology of this disease, (2) to determine the means by which C. burnetii spreads among livestock, (3) to reduce the toxicity and improve the immunogenicity of Q fever vaccine, and (4) to develop a practical and safe procedure for immunization of man.

This project will be considered in two categories: "A" deals primarily with field investigations and "B" with laboratory studies.

A. Field Investigations

Methods:

Methods consist of field studies supported by laboratory work, including complement-fixation tests, capillary agglutination tests, and isolation of strains of Q fever in suitable laboratory animals. In Montana these studies were restricted chiefly to Gallatin and Ravalli counties, whereas studies in Idaho were concentrated in an area surrounding Boise.

Part B included: Yes

Major findings:

1. During the past year, the infection rate among herds of dairy cattle in Montana almost doubled. In the two-county study area in Montana, herd infection rate increased from 2% in 1959 to 18% in 1960. Preliminary observations indicate that many factors are involved in dissemination of Q fever rickettsiae among livestock. Although the purchase of infected-herd replacements is responsible for dissemination of Q fever, a significant proportion of newly-infected herds was closed to outside replacements. Hence, the disease might have been spread by arthropods, wind, or mobile fomites.

2. In two studies designed to evaluate the role of dairy cattle in the epidemiology of Q fever, similar results were obtained. In an area in Ada and Canyon counties in Idaho, where very few sheep are raised and approximately half the herds of dairy cattle are infected, 27% of 199 persons living on infected premises had antibodies, whereas 14% of 260 persons residing on Q-fever-free premises possessed antibodies. However, the incidence of illnesses resembling Q fever among seropositives was essentially similar to that observed among seronegative persons. In the study in Ravalli County, Montana, involving 490 persons, 18% of those residing on infected premises possessed antibodies, whereas 4% of those residing on Q-fever-free premises were seropositive. Only 1 of 30 positive individuals had experienced an illness compatible with that of Q fever.

3. In a study involving 190 human volunteers at the Montana State Prison, persons who failed to react to a skin test of 0.02 complement-fixing (CF) units of antigen were safely immunized with 10 CF units administered subcutaneously. In a limited number of volunteers a dose of 20 CF units of vaccine resulted in painful reactions and areas of induration at the sites of vaccination. Antibody response was detected more readily by the capillary agglutination (CA) test than by the CF test. After 1, 2, and 3 doses, 27%, 53%, and 68%, respectively, developed antibodies detectable by the CA test, but only 4.5% of these persons developed antibodies detectable by the CF test. Dermal hypersensitivity of 16.5% of these persons converted from negative to positive after vaccination.

4. Investigation of the feasibility of combining strain 19 Brucella abortus vaccine and Q fever vaccine for immunization of calves revealed that the antibody response in calves given both vaccines was similar to that obtained in calves given only Q fever vaccine or strain 19 vaccine.

5. Mature sheep immunized with varying doses of Q fever vaccine developed high levels of antibody which were still detectable 6 months after vaccination.

Significance:

The failure to associate clinical illness with the presence of antibodies in persons exposed to infected dairy cattle conflicts with observations made earlier in southern California. Most of the strains of C. burnetii isolated from dairy cattle in Idaho and Montana are only mildly pathogenic for guinea pigs and this may explain the relative inability of these organisms to cause disease in man.

The presence of antibodies in a large proportion of rural residents creates a diagnostic problem. A clinical diagnosis of Q fever should be based upon the judicious evaluation of a rise in antibody titer or actual isolation of the organism. Because of the known propensity of C. burnetii to cause human disease, the large reservoir of infection in the dairy cattle population of this country must be considered a potential public health problem. The high incidence of sterile abscesses and tissue reactions among sensitized persons given Q fever vaccine and the absence of such reactions in nonsensitized persons emphasized the need of a skin test before the administration of Q fever vaccine.

Proposed course:

In studies completed to date, minor illnesses attributable to infection with C. burnetii could not be accurately evaluated. As Q fever continues to spread through dairy cattle in Ravalli County, rural populations will be observed closely to detect any minor illnesses attributable to Q fever.

Epizootiological studies will be continued to elucidate the means whereby the organism is disseminated throughout the livestock population. Isolates from man and livestock will be studied and characterized in an attempt to explain the relative inability of these organisms to cause human illness.

B. Laboratory studies:

Methods:

Organisms grown in tissue culture or embryonated chicken eggs are purified and subjected to chemical and physical fractionation. Fractions are then assayed for complement-fixing activity and immunogenic properties by appropriate serologic and biologic tests.

Major findings:

1. A method of purifying rickettsiae by centrifuging 10 percent yolk-sac suspensions in molar salt solution in a continuous-flow centrifuge has been found to be particularly useful for purifying large quantities of Q fever, spotted fever, and typhus rickettsiae.

2. The density-gradient-sedimentation technique was found to be useful for separating cell walls from intact cells.

3. In chemical fractionation studies, dimethyl sulfoxide (DMS) extracts of phase II rickettsiae were CF specific but nonimmunogenic in guinea pigs, whereas DMS extracts of phase I rickettsiae were immunogenic.

Lauryl sulfate extracts react specifically in the CF test and elicit protective antibodies in guinea pigs. This chemical agent appears to extract phase I antigen from mixtures of rickettsiae containing both phase I and II components.

4. In physical fractionation studies, preparations of cell walls and protoplasm were prepared from purified suspensions of C. burnetii. Preliminary studies indicate that the immunogenic and CF activity is situated chiefly in the cell wall. On a weight basis, cell wall preparations showed 25-fold greater protection than did protoplasm. Fractions of C. burnetii were toxic when inoculated intradermally into normal rabbits. The least dose to produce a visible reaction was 0.125 micrograms for whole cells, 0.025 micrograms for cell walls, and 40.0 micrograms for protoplasm. The difference in dermal activity between cell wall and protoplasm is similar to that of cellular fractions of Salmonella enteritidis and S. typhosa.

5. Comparative studies on the capillary agglutination test and the CF test on serums of varied sources indicate that at least three types of antibodies are involved in the immune response of this disease.

6. Growth of C. burnetii in cell-free medium still has not been accomplished.

Significance:

Studies to date indicate that both toxic and immunogenic properties of the Q fever rickettsiae are situated in the cell wall. By proper chemical or physical fractionation it is hoped that a nontoxic, protective antigen can be isolated from this organism. Such a product is needed since the amount of vaccine that can be administered at the present time is limited by its toxic properties.

Proposed course:

Studies on the physical and chemical fractionation of C. burnetii will be continued to obtain fractions which will be particularly useful in immunologic and allergic investigations. Attempts will be made to isolate and identify the toxic component of the rickettsial cell wall since this has direct relationship to other projects dealing with bacterial toxins.

Attempts will be made to correlate the virulence of strains of C. burnetii with the chemical composition of their cell walls. Investigations on the relationship of the phase of the Q fever rickettsia to allergic and protective properties of various cell fractions will be continued.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Stoenner, H. G. and Lackman, D. B.: The biologic properties of Coxiella burnetii isolated from rodents collected in Utah. Am. J. Hyg. 71(1): 45-51. Jan. 1960.

Luoto, L.: Report on the nationwide occurrence of Q fever infections in cattle. Pub. Health Rep. 75(2): 135-140. Feb. 1960.

Stoenner, H. G.: Q fever. In A Manual of Tropical Medicine, 3rd ed. W. B. Saunders & Co., Philadelphia, 1960, pp. 104-106.

Ribi, E. and Hoyer, B. H.: Purification of Q fever rickettsiae by density-gradient sedimentation. J. Immunol. 85(3): 314-318. Sept. 1960.

In press:

Pickens, E. G. and Gaon, J. A.: Growth of Coxiella burnetii in agar tissue culture. Am. J. Trop. Med.

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Rocky Mountain Laboratory
Hamilton, Montana

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Individual Project Report
Calendar Year 1960

Part A.

Project Title: Diseases having a reservoir of infection in the natural environment (other than rickettsioses)

Principal Investigator: J. F. Bell

Other Investigators: W. L. Jellison, D. B. Lackman, M. T. McKee, C. R. Owen, and H. G. Stoenner

Cooperating Units: Dr. C. W. Emmons, LID, NIAID

Man Years (calendar year 1960):
Total: 11.0
Professional: 4.0
Other: 7.0

Project Description:

Objectives:

Studies performed in this project are directed toward an understanding of the ecology of those diseases transmissible to man from animals and the natural environment. As a direct continuation of the ecological studies, attention is paid to the laboratory aspects of the problems encountered with special emphasis on diagnosis, treatment, and control.

Methods:

The methods employed are, in general, straightforward microbiological techniques, with such variations as are required for study of specialized problems, rabies, for example.

Major findings:

1. Since only 2 isolations of bat rabies were made this year and 9 were made in 1959, the idea of an initial upsurge toward epizootic proportions can be discounted.

2. Survival of mice patently infected with isolates from various sources has been found to be a common phenomenon contrary to previous

Part B included: Yes

ideas. The factors responsible are unknown.

3. Continuing presence of Group A streptococci in areas of high vole populations confirms that similar earlier observations were not isolated ones, but the epizoological significance remains obscure.

4. In an initial, long-term experiment designed to compare the duration of protection afforded mice against P. tularensis when vaccinated with ether-extracted antigen (LV) and a Russian living attenuated strain (RV), the role of latent infection in the immune response of the latter group could not be determined. During the ensuing weeks the log protection decreased rapidly or slowly depending on the virulence of challenge material. The slopes of decreasing protection showed considerable similarity between LV mice challenged with 425-F4G strain and RV mice challenged with Schu. Protection in RV mice challenged with 425-F4G remained high through week 20 but decreased to nearly zero after 52 weeks.

5. In continued comparisons of the virulence of P. tularense from different geographic areas, three strains from 2 patients and a dead hare in Japan were strikingly lower in virulence for guinea pigs and rabbits than 3 isolates from hares in France and 4 from D. variabilis wood ticks from eastern Montana.

6. Results of the use of Ovary's passive cutaneous anaphylaxis test lend weight to the specific rather than subspecific differentiation of P. tularensis and P. novicida. Cross-vaccination studies still leave a question as to their relationship.

7. Isolations of Type A_2 influenza virus were made from 15 of 30 throat washings collected during a local epidemic. One isolation on 22 January was earlier than usual for this disease. These isolates were forwarded to the International Influenza Center for the Americas.

8. P. multocida was isolated from mouths of 32 of 47 domestic cats, one bobcat, and one of 5 dogs. None of the 27 other animals, including grizzly bears, woodchucks, skunks, and snakes, were positive.

9. The fungal organism causing haplomycosis in animals has been found in one South American and 4 European countries, and 3 new rodent hosts were discovered. In conjunction with Dr. C. W. Emmons, the new name, "adiaspiromycosis," was applied to this parasitism because of the peculiar lack of cellular multiplication of organisms in the animal host. Massive doses from cultures given intravenously have been lethal for monkeys and a sheep.

10. Reciprocal cross-immunity in guinea pigs and mice was found between Brucella suis and Br. neotomae when care was taken to use only smooth-phase cultures. The explanation for inconsistencies in earlier work was found to be due to undetected colonial dissociation.

11. Leptospira pomona was isolated for the first time from the spinal fluid of a bovine animal, a local dairy cow that showed signs of meningitis prior to death.

12. The absence of HI antibodies against swine influenza virus in serums from members of the Alaskan National Guard confirms epidemiologic evidence that the 1918-19 pandemic of influenza failed to reach some areas of Alaska. Survivors of another isolated population severely affected at that time, still retain significant levels of such antibodies.

Significance:

Additional laboratory data progressively confirm recent notions that rabies virus does not cause an invariably fatal disease as it was once thought to do. The concern that rabies might progress to epizootic proportions, as suggested by the large number of isolations last year, was fortunately not justified.

Though human infection with P. multocida has not been observed locally, isolation from the mouths of cats and dogs suggests this source as of potential local health concern.

A reassessment of the epidemiology of epidemic nephroso-nephritis may provide a new viewpoint for a fresh attack on this puzzling Old World entity. Dr. Jellison has made the novel suggestion that some 2,000 cases of "trench nephritis" in troops during World War I may have been this type of hemorrhagic fever.

Studies have revealed that Br. suis and Br. neotomae are closely related immunologically but are not completely similar. Br. neotomae has not been found to persist in tissues of swine. Therefore, if results of studies in mice and guinea pigs are applicable, this organism could conceivably be used as an effective vaccine for control of swine brucellosis.

The application of results of research to field problems is one of the main accomplishments of the serology laboratory. The identifications of infections in human and animal populations during epidemiologic study are largely the result of serologic testing. When it is possible to isolate the causative agent, serology plays an important part in its final identification and characterization. While such work progresses, improved techniques and diagnostic reagents are sought. This in turn gives rise to improved prophylactic vaccines and better methods for their evaluation and standardization. Results of serologic testing are also utilized for the identification of problems in infectious disease which may exist in the natural environment of the west.

Pandemic influenza is a continuing threat to man. Therefore, it is important to detect any antigenic variation in strains causing epidemics.

To do this it is necessary to have some laboratories, especially ones located in isolated areas, which will forward new isolates to the WHO Center. Also of importance is the continual investigation of the influenza experience of isolated populations because it provides information as to what strains should be included in vaccine. Our results suggest that consideration should be given to the inclusion of swine influenza virus in vaccine because there are people who have not been exposed to this virus and it may still be present among swine.

Proposed course:

Steady progress in laboratory maintenance of bats suggests that experimental infection can now be instituted with confidence.

Studies on the efficacy of vaccination of domesticated beaver against tularemia are under way.

Attempts will be continued to elucidate now obscure factors in the epizoology of Group A streptococci and "M" organisms (Toxoplasma microti) in Microtus mouse populations.

If facilities become available, further work is planned on differentiation of P. tularensis and P. novicida and on possible protection of swine by Br. neotomae against Br. suis.

Studies also will be continued on virulence and cultural patterns among strains of P. tularensis of diverse origins and on factors favoring persistence of the organism in natural waters.

Bird tissues collected in Alaska for test for arbor viruses will be screened in the serology unit for viruses of the psittacosis group.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Bell, J. F. and Moore, G. J.: Rabies virus isolated from brown fat of naturally infected bats. Proc. Soc. Exp. Biol. & Med. 103(1): 140-142. Jan. 1960.

Owen, C. R., Meis, A., Jackson, J. W., and Stoenner, H. G.: A case of primary cutaneous listeriosis. New Eng. J. Med. 262(20): 1026-1028. May 19, 1960.

Emmons, C. W. and Jellison, W. L.: Emmonsia crescens sp. n. and adiaspiromycosis (haplomycosis) in mammals. Ann. N.Y. Acad. Sci. 89: 91-101. Aug. 27, 1960.

Jellison, W. L., Glesne, L., and Peterson, R.: Emmonsia, a fungus, and Besnoitia, a protozoan, reported for South America. Bol. Chilena de Parasitol. XV(3): 46-47. July-Sept. 1960.

Jellison, W. L., Vinson, J. W., and Holager, E.: Haplomycosis in Norway. Acta Pat. et Microbiol. Scandinavica 49(4): 480-485. 1960.

Esplin, D. W., Philip, C. B., and Hughes, L. E.: Impairment of muscle stretch reflexes in tick paralysis. Science 132(3432): 958-959. Oct. 7, 1960.

In press:

Jellison, W. L., Helminen, M., and Vinson, J. W.: Presence of a pulmonary fungus in rodents in Finland. Ann. Med. Exp. et Biol. Fenniae 38(3):

Jellison, W. L.: Sodoku. Rat-bite fever due to Spirillum minus Carter. Diagnostic Procedures and Reagents, 4th ed. American Public Health Assoc., New York, N.Y.

Larson, C. L.: Tularemia. Diagnostic Procedures and Reagents, 4th ed. American Public Health Assoc., New York, N.Y.

Serial No. NIAID-174
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Transmission of disease agents by certain vectors

Principal Investigator: C. B. Philip

Other Investigators: J. F. Bell, J. M. Brennan, W. Burgdorfer, C. M. Clifford, W. L. Jellison, G. M. Kohls, D. B. Lackman, V. F. Newhouse

Cooperating Units: Laboratory of Immunology, NIH, NAMRU 3

Man Years (calendar year 1960):
Total: 13.5
Professional: 4.5
Other: 9.0

Project Description:

Objectives:

This group directs its studies toward problems of the intimate relationships between infectious agents and their actual or potential invertebrate vectors. Occasionally, symbiotic organisms in arthropods come under scrutiny. Efforts during the past year have been directed primarily toward continuation of experimental tick paralysis, completion of studies on transovarial tick transmission of Pasteurella tularensis, intensification of studies on relationships of Rickettsia rickettsii and Colorado tick fever virus in vectors, and a reconsideration of evidence on the epidemiology of the hemorrhagic fever known as epidemic nephrosonephritis. An intensive attack on potential disease relationships of chigger mites in Panama was organized.

Methods:

Field and laboratory methods are mainly those developed at the Rocky Mountain Laboratory, including more recent fluorescent microscopy as applied to vertebrate-invertebrate cycles of disease agents.

Major findings:

1. A successful cryostat technique for the thin-sectioning of

Part B included: Yes

fresh-frozen tissues of hard and soft ticks was developed in conjunction with Dr. J. E. Tobie of the Laboratory of Immunology, NIH. This was a necessary antecedent to refined studies of certain tick-borne agents by fluorescent microscopy. In comparison with older techniques this method is much more rapid and is a marked improvement for sectioning and staining both nymphs and adults of these ticks in various states of engorgement.

2. The incidence of tick paralysis in man is not necessarily a reflection of the relative ability of indigenous ticks to produce the disease in hamsters. From 24% to 90% of test lots of ticks (48 lots) from 3 areas in Colorado caused experimental tick paralysis though only 3 cases were reported in the entire state up to 1950; whereas only from 0% to 50% of similar lots (39) from 3 areas in a single county (Ravalli) in western Montana were paralytic though 7 local cases have been reported in the same period. On the other hand, 75% of 16 lots from adjoining Missoula County were positive. If ecologic factors were responsible for these differences, they have so far eluded solution.

3. Species of ticks of the genus Argas, which are virtually indistinguishable in the adult stage, have been separated by taxonomic characters present in the larval stage. This has resulted in clarification of a subspecies which infest bats in Egypt and in the discovery that a species found in cliff swallow nests in western North America and another from birds in Chile are new. Present classification of other species, particularly parasites of migratory birds and bats, probably requires clarification as a prerequisite to investigations of the disease-carrying potential of the ticks.

4. A new funguslike agent which causes death in white mice and embryonated chicken eggs has been isolated from Argas found in nests of local cliff swallows. Studies are under way to assess its systematic relationship.

Significance:

New data confirm the utility of fluorescent microscopy in study of arthropod-borne agents as applied particularly to rickettsial agents (see No. 171). Special tissue tropisms are now more readily detectable, and a tool is provided that may enable estimation of growth in different stages and periods of a given cycle. The new techniques for tick sectioning will be applicable to other fields and right now mosquitoes are being subjected to similar techniques.

Previous advances in knowledge of the chigger-mite fauna of Central and South America will now pay dividends in facilitating the testing of species in Panama for presence of pathogenic agents. This illustrates the advantages of fore-knowledge of parasitic arthropods in a given fauna over information obtained after a health problem has arisen. In Korea, for example, a hurried survey of medically important parasites lagged

behind the emergency need for epidemiologic studies of hemorrhagic fever in military forces.

Several faunal studies of this nature on parasites in various parts of the world were completed or were under way during the year by appropriate staff specialists; the significance of these studies may only become apparent when some future need arises.

Proposed course:

As opportunity arises, the relationship of acarine and insect-borne pathogens will continue to be investigated. Strains of supposed Rickettsia prowazekii reported to have been isolated from tick and domestic animal sources in Abyssinia are now under study. Attempts to purify the salmon-poisoning agent for antigenic purposes were not fruitful during the year, but the problem will be attacked by a new technique. Some initial promising fluorescent antibody studies on this agent will be elaborated.

The Panama chigger-mite unit may be expected to have some results from initial field and laboratory tests.

A revision of the world systematics of the important tick genus, Argas, in collaboration with Dr. Harry Hoogstraal of NAMRU 3 in Cairo, Egypt, should be well advanced by the end of next year.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Brennan, J. M.: Ectonyx, a new Neotropical genus of chiggers (Acarina: Trombiculidae). Acarologia II(1): 88-91. 1960.

Brennan, J. M. and Dalmat, H. C.: Chiggers of Guatemala (Acarina: Trombiculidae). Ann. Ent. Soc. Amer. 53(2): 183-191. March 1960.

Philip, C. B.: New North American Tabanidae. XI. Supplemental notes pertinent to a catalog of Nearctic species. Ann. Ent. Soc. Amer. 53(3): 364-369. May 1960.

Brennan, J. M. and White, J. S.: New records and descriptions of chiggers (Acarina: Trombiculidae) on bats in Alabama. J. Parasitol. 46(3): 346-350. June 1960.

Kohls, G. M.: Records and new synonymy of New World Haemaphysalis ticks, with descriptions of the nymph and larva of H. juxtakochi Cooley. J. Parasitol. 46(3): 355-361. June 1960.

Newhouse, V. F.: Birds of selected irrigated river valleys of west-central Idaho. Murrelet 41(1): 1-6. 1960.

Philip, C. B.: Further records of Neotropical tabanidae (Diptera) mostly from Peru. Proc. Calif. Acad. Sci., 4th Series XXXI(3): 69-102. July 8, 1960.

Brennan, J. M. and Maki, W. A.: An inexpensive and effective indoor insectary. J. Econ. Ent. 53(4): 685-688. Aug. 1960.

Hoogstraal, H. and Kohls, G. M.: Observations on the subgenus Argas (Ixodoidea, Argasidae, Argas). 1. Study of A. reflexus reflexus (Fabricius, 1794), the European bird argasid. Ann. Ent. Soc. Amer. 53(5): 611-618. Sept. 1960.

Kohls, G. M. and Hoogstraal, H.: Observations on the subgenus Argas (Ixodoidea, Argasidae, Argas). 2. A. cooleyi, new species, from western North American birds. Ann. Ent. Soc. Amer. 53(5): 625-631. Sept. 1960.

Philip, C. B.: Another holarctic species of Tabanidae (Diptera). Canad. Ent. XCII(9): 697-699. Sept. 1960.

Kohls, G. M.: Ixodides. McGraw-Hill Encyclopedia of Science and Technology. McGraw-Hill Book Co., Inc., New York. 1960. Vol. 7, pp. 298-299.

Kohls, G. M.: Ixodes (Endopalpiger) zaglossi, n. sp. from the long-beaked echidna of New Guinea (Acarina, Ixodidae). Acarologia 2(4): 447-452. Oct. 1960.

Brennan, J. M.: Eight new species of Pseudoschöngastia from Mexico and Panama with a revised key to species (Acarina: Trombiculidae). Acarologia 2(4): 480-492. Oct. 1960.

Brennan, J. M. and Jones, E. K.: Chiggers of Trinidad, B.W.I., (Acarina: Trombiculidae). Acarologia 2(4): 493-540. Oct. 1960.

Philip, C. B.: Malaysian Parasites. XXXV. Description of some Tabanidae (Diptera) from the Far East. Malaysian Parasites No. 29, pp. 1-32. 1960.

Philip, C. B.: Malaysian Parasites. XXXVI. A summary review and records of Tabanidae from Malaya, Borneo, and Thailand. Malaysian Parasites No. 29, pp. 33-78. 1960.

In press:

Philip, C. B. and Mackerras, I. M.: On Asiatic and related Chrysopinae (Diptera: Tabanidae). Philippine J. Sci.

Philip, C. B.: Additional records of Tabanidae (Diptera) from the West Coast of South America. Pan-Pacific Ent.

Fairchild, G. B. and Philip, C. B.: A revision of the Neotropical genus Dichelacera, subgenus Dichelacera, Macquart (Diptera, Tabanidae). Studia Entomologica, Brazil.

Philip, C. B.: Three new Tabanine flies (Tabanidae, Diptera) from India. Ent. J. India.

Hoogstraal, H. and Kohls, G. M.: Observations on the subgenus Argas (Ixodoidea, Argasidae, Argas). 3. A biological and systematic study of A. reflexus hermanni Audouin, 1827 (revalidated), the African bird argasid. Ann. Ent. Soc. Amer.

Brennan, J. M. and Jones, E. K.: New genera and species of chiggers from Panama (Acarina: Trombiculidae). J. Parasitol.

Brennan, J. M. and Jones, E. K.: Chiggers of Peru (Acarina: Trombiculidae). Acarologia.

Kohls, G. M. and Clifford, C. M.: A new species of Ixodes (Lepidixodes) from bats in Malaya, North Borneo, and the Congo (Acarina-Ixodidae). Acarologia.

Philip, C. B. and Burgdorfer, W.: Arthropod vectors as reservoirs of microbial disease agents. Ann. Rev. Ent. 6: 1961.

Philip, C. B.: Arthropod vectors in relation to the reservoir mechanism of microbial agents of animal diseases. Presented at 11th Internat. Ent. Cong., Vienna, Aug. 1960. Acta Tropica.

Philip, C. B.: Proposal to validate under the plenary powers the specific name Akamushi (Trombidium) Brumpt, (Class Acarina). Z.N.(S) 400. Internat. Bull. Zool. Nomenclature.

Philip, C. B.: Proposal to validate under the plenary powers the specific name Dermacentor andersoni Venustus. Internat. Bull. Zool. Nomenclature.

Philip, C. B.: New North American Tabanidae. XIII. Change of name for a well-known species of Chrysops. Ent. News.

Honors and Awards relating to this project:

Glen M. Kohls

Continued as an Assistant Editor for the Journal of Parasitology.

Invited by the Editor of the Annals of the Entomological Society of America to prepare a review of Dr. D. R. Arthur's monograph on several genera of ticks published by the Cambridge University Press.

Vice President and member of the program committee of the International Northwest Conference on Diseases in Nature Communicable to Man.

Dr. C. B. Philip

Invited to serve on the "Research and Engineering Advisory Panel on Biological and Chemical Defense" under the office of the Director of Defense Research and Engineering. (May 6, 1960)

Received the Outstanding Achievement Award of the University of Minnesota, which is reserved for former students of the institution who have attained "high eminence and distinction." (June 4, 1960)

Invited to be a Visiting Lecturer in the program of the Academic Year Institute for High School Teachers of Science and Mathematics, 1960-61.

Continued as lecturer, CDC Training Course, PHS, Atlanta, February 1960-61.

Serial No. NIAID-175
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Allergy and immunology of fungal infections and the mechanisms of allergic phenomena

Principal Investigator: S. B. Salvin

Other Investigators: M. B. Gregg, R. F. Smith

Cooperating Units: None

Man Years (calendar year 1960):
Total: 13.25
Professional: 4.25
Other: 9.00

Project Description:

Objectives:

Further efforts to uncover the mechanisms responsible for delayed allergy have been stimulated by relationships recently established between delayed allergy and homograft rejection and collagen or "auto-immune" disease. Three main objectives of this project are: (1) to study the biologic mechanisms responsible for the development and expression of delayed hypersensitivity in experimental animals; (2) to relate delayed allergy to other immunologic phenomena such as antibody formation, contact hypersensitivity, or tolerance; and (3) to apply such knowledge of delayed hypersensitivity to its possible role in the pathogenesis of "auto-immune" diseases such as lupus erythematosis and rheumatoid arthritis, as well as to certain infectious diseases, such as rheumatic fever, tuberculosis, or histoplasmosis.

Methods:

Although biologic and chemical procedures typical of immunologic investigations are used, emphasis has been placed on some quantitative immunochemical procedures for analyses and study of allergy and antigen-antibody reactions. Highly purified proteins, such as diphtheria toxoid, egg albumin, and bovine gamma globulin, are used as antigens, either singly or conjugated with a wide variety of haptenic groupings and carbohydrates. A new technique involves the use of radioactive conjugates to

Part B included: Yes

trace the progress of antigen-antibody reactions within the cell by autoradiographic procedures. Also, electron opaque metals conjugated to conventional proteins are used to trace the course of the antigen within the cell.

Major findings:

Previous studies have shown that delayed allergy in guinea pigs is superseded by circulating antibody and Arthus reactions if a large antigenic dose is given. When guinea pigs are sensitized with a small dose, only delayed hypersensitivity develops. Animals given a small dose of antigen develop maximum anamnestic responses when a second injection is given at the time of maximum delayed hypersensitivity. These and other observations strongly indicate that delayed hypersensitivity is an immature form of the immune response.

1. In studies on the anamnestic response to conjugated diazotized proteins, guinea pigs developed delayed hypersensitivity to the protein moiety, but developed specific circulating antibody directed toward small chemical configurations present only in the booster antigen.

2. Carbohydrates, when conjugated to proteins, act as haptens and can be used to detect circulating antibody. Since, however, the specificity of delayed allergy is directed toward the protein, the carbohydrate can neither induce nor detect delayed hypersensitivity.

3. Contact hypersensitivity, which is believed to be analogous to delayed allergy, may be induced in experimental animals by repeated application of a simple chemical (2,4-dinitro-1-flurobenzene, DFB) on the skin or by intradermal injection of the chemical in Freund's adjuvant. Animals sensitized with a simple chemical develop circulating antibody and Arthus reactions to a conjugate composed of hapten and a protein such as guinea pig serum and react to a surface application of the hapten only. In contrast, animals sensitized with the conjugate (DFB guinea pig serum) develop Arthus reactions and circulating antibody directed toward the hapten and produce typical delayed allergy to subsequent intradermal injection of the conjugate, but do not react to surface application of the simple chemical. This inconsistency, that synthetic conjugates fail to induce contact skin hypersensitivity to the hapten, but that hapten induces Arthus reactions to the conjugate, may be explained by one of two possible hypotheses: (a) When the host animal is injected with simple chemicals, these substances may enter intracellular areas inaccessible to extracellular proteins. Subsequent conjugation leads to conjugates which exist in intracellular sites inaccessible to conjugates prepared in vitro. Several workers have provided evidence that this phenomenon may be functional. (b) The protein members of in vivo conjugates are unique and have not been duplicated by proteins chosen for in vitro preparations. The observation that intradermal injection of an in vitro conjugate of soluble guinea pig skin protein with DFB produces contact hypersensitivity

to surface application of the hapten as well as delayed and Arthus reactions to skin tests with the conjugate supports this hypothesis. Thus, the production of contact hypersensitivity is directly related to a particular protein or proteins in the host tissue and the specificity of contact hypersensitivity is analogous to that of delayed reactions.

4. In further substantiating the role of delayed allergy in the immune process, immunologic studies were conducted in newborn guinea pigs because they may show immaturity in the formation of delayed allergy and circulating antibody. When these animals are sensitized within 12 hours after birth, typical delayed responses cannot be evoked for about 2 weeks. By this time, however, circulating antibody has appeared, and animals develop typical Arthus reactions when challenged. If sensitization of neonatal animals is postponed for 12 to 14 days after birth, delayed allergy is not apparent, but circulating antibody appears about 11 days after sensitization. When the neonatal animal is sensitized later than 14 days after birth, allergic and antibody responses are similar to those of the adult. These observations suggest either that delayed hypersensitivity cannot be produced by the neonatal animal but conventional antibody can, or that the basic mechanism of delayed hypersensitivity is present but cannot be made manifest by the host animal because of some inherent deficiency. The following observations suggest that the latter hypothesis is true: (a) delayed hypersensitivity cannot be transferred passively to newborns with lymph-node cells from highly sensitized adults, (b) delayed hypersensitivity, however, can be transferred passively to normal adults with lymph-node cells from highly sensitized neonatal guinea pigs which themselves do not show delayed hypersensitivity, and (c) contact hypersensitivity can be exhibited in neonatal animals although it is somewhat slow in onset.

5. Guinea pigs sensitized to purified ultraviolet-killed Type II poliovirus develop an anamnestic response to a second injection of antigen.

6. Through chemical and physical fractionation procedures, a specific skin-test antigen has been prepared from Cryptococcus neoformans, a pathogenic fungus frequently isolated from pigeon excreta in the vicinity of New York City. This antigen produces large and impressive delayed-type skin reactions in guinea pigs previously infected with C. neoformans. The active fraction of this antigen has been purified and its biochemical properties determined.

Significance:

To date, experimental data support the hypothesis that delayed hypersensitivity is an immature phase of the classical immune response. Studies on contact sensitivity induced by simple chemicals indicate that its specificity and dynamics are similar to those of conventional delayed hypersensitivity. The inability to invoke typical delayed responses in a neonatal animal appears to be attributable to an inherent deficiency

which prevents the young host from manifesting an established hypersensitive state. Hence, even in the young animal, delayed allergy is an early phase in the antibody response.

In view of the prevalence of C. neoformans in pigeon excreta in urban areas, large numbers of people are exposed to this fungus. Therefore, cryptococcosis in a mild, unrecognized form may be much more prevalent than the relatively infrequent occurrence of this disease would indicate. The preparation of a specific skin-test antigen creates a new tool for conducting epidemiologic studies of this disease.

Proposed course:

Studies on the immune mechanism in the neonatal animal will be continued. The possibility exists that a cellular or humoral factor is necessary or supplemental for manifestation of delayed hypersensitivity in an infant or in a newborn animal. Experiments are in progress to examine this hypothesis. In addition, skin grafts from adults to newborn and vice versa are being used to determine whether maturity of the skin is essential for delayed reactions. Since gamma radiation of newborn guinea pigs delays the appearance of Arthus reactions, this technique is also being used to postpone the appearance of antibody and thus aid in the determination and examination of delayed reactions.

Studies with live bacteria such as Mycobacterium tuberculosis have been initiated to determine if the response relative to hypersensitivity and immunity of the newborn differs from that of the adult.

Further investigation will be made on the effect of age and the role of skin and other cellular elements in the allergic response.

To clarify the part delayed allergy plays in antibody formation, antigen should be traced through the cell, and its particular activity within the cell should be correlated with the gross reaction of the host. Two techniques will be used. One involves the injection of guinea pigs with the electron-opaque protein conjugate and the determination of the exact locus and relationship of the antigen within the cell by examination of ultrathin sections under the electron microscope. The other method involves a study of the cellular disposition of injected radioactive proteins and the position of antigen within the cell. Radioactive antigens and subsequent antibody combinations are traced by autoradiographic techniques.

Experiments on sensitization of guinea pigs with purified poliovirus will be continued in order to learn whether sensitization with Types I or III will produce an anamnestic response on later introduction of Type II virus.

Standardization of the skin-test antigen for detecting cryptococcosis in man and collaborative epidemiologic studies of this disease are contemplated.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Salvin, S. B.: Resistance of animals and man to histoplasmosis. In Histoplasmosis. Chas. C Thomas, Springfield, Ill., 1960, pp. 99-112.

Salvin, S. B. and Smith, R. F.: Specificity of allergic reactions. I. Delayed versus Arthus hypersensitivity. J. Exp. Med. 111(4): 465-483. April 1960.

Salvin, S. B. and Smith, R. F.: Delayed hypersensitivity and the anamnestic response. J. Immunol. 84(5): 449-457. May 1960.

Salvin, S. B. and Smith, R. F.: Specificity of allergic reactions. II. Azoproteins in the anamnestic response. Proc. Soc. Exp. Biol. & Med. 104(4): 584-590. Aug.-Sept. 1960.

Honors and Awards relating to this project:

Dr. S. B. Salvin

Invited to contribute paper in "Progress in Allergy," S. Karger, Basel, Switzerland, publisher.

Invited to present series of lectures and seminars on allergy and immunology at the University of Montana, Missoula, Montana.

Serial No. NIAID-176
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Viruses, virus components, and virus surfaces

Principal Investigator: B. H. Hoyer

Other Investigators: R. K. Gerloff, F. G. Jarvis, R. A. Ormsbee, and D. B. Ritter

Cooperating Units:

Man Years (calendar year 1960):

Total:	11.5
Professional:	3.5
Other:	8.0

Project Description:

Objectives:

Elucidation of the mechanisms involved in the synthesis of virus protein and RNA and clarification of fundamental relationships between virus protein and RNA continue to be major objectives. The finding of others that poliovirus protein and RNA are synthesized simultaneously at similar rates required us to make changes in our investigations. Current interest in live poliovirus vaccines has stimulated studies on the behavior of surface proteins of virulent and avirulent poliovirus in column chromatography. A sensitive precipitation system for detecting antibody against poliovirus is being developed.

Methods:

Quantitative and qualitative methods appropriate for the study of virus proliferation and cell metabolism are employed. Cellular and virus fractions are separated and characterized by chemical and physical means. Radioactive labels, P^{32} and S^{35}, are used in following certain biologic processes and in studying biophysical properties of viral components.

Part B included: Yes

Major findings:

1. Previous studies have shown that P^{32}-labeled type 3 Sauckett virus, type 2 MEF-1 virus, and type 1 Mahoney virus can be separated by column chromatography. In continued investigations, Leon and Sauckett type 3 viruses could be separated from each other by elution from the DEAE column at different pH (7.7 vs. 7.1) values.

2. CHAT poliovirus, an avirulent type 1, was separated from the virulent parent by elution in a salt or pH gradient, but FOX and Wi-1 could not be separated from their virulent counterparts. CHAT and Wi-1, both avirulent variants, could be separated from each other by virtue of different elution properties.

3. After prolonged storage, avirulent poliovirus binds more firmly to cellulose ion exchange columns than does its virulent counterpart. This property is probably due to a more rapid change in avirulent virus protein and indicates a less stable surface.

4. Because of the large ratio (1:10 to 1:500) between plaque-forming units (PFU) and physical particles, virus proteins could not be characterized without knowledge of the proportion of total virus that reacts with the cell. After S^{35}- or P^{32}-labeled polioviruses were mixed with KB cells in a virus-cell ratio of 20 to 1, samples were removed at intervals and examined in CsCl or RbCl equilibrium-density gradients. Radioactivity and infectivity disappeared at the same rate from the supernatant medium, and the virus shifted from the density zone characteristic of whole virus. Most of the radioactivity that had left the virus-density zone was concentrated in a well-defined region in the upper level. Alteration of the density of original virus particles indicates that most of the virus actually reacts with susceptible cells.

5. Infectious RNA was prepared from highly purified poliovirus by phenol extraction. RNA preparations of fairly uniform physical properties had an $S_{20,w}$ of 37. Also, a 30% increase in ultraviolet absorption after treatment with RNAase indicates an initial high degree of polymerization. Both octyl alcohol-chloroform and neutral hydroxylamine (0.1 M) destroyed more than 99% of RNA infectivity after 10 minutes at room temperature, but the molecular properties of RNA, as determined by ultracentrifugation patterns, were not affected.

6. In a preparation of RNA yielding 1% of the PFU present in whole virus, the S^{35} content was only 0.001% of that of the whole virus. Thus, residual protein cannot account for more than 0.1% of the RNA activity.

7. The method originally used for preparation of purified poliovirus was modified by treating crude DEAE filtrates of tissue-culture fluids with 0.2% DEXTRAN 500, 6.45% Carbowax 6000 and 0.3 M NaCl (final concentration). After standing for 24 to 48 hours at 4° C., liquids separate

into two phases, the lower of which contains all the virus. From 2,700 ml. of culture fluid, all the virus will be present in the lower 20 ml. of liquid.

8. An extremely sensitive and specific precipitation test for detecting antibody against poliovirus has been developed. A serum with a neutralizing antibody titer of 1:256 possesses a precipitation titer of 1:16,384. Serum dilutions are mixed with P^{32}-labeled poliovirus and incubated. Antihuman gamma globulin is added, followed by further incubation. The precipitate formed is then removed by centrifugation. If a significant amount of radioactivity is present in the sediment, precipitation of virus has occurred.

Significance:

The behavior of derived avirulent poliovirus in column chromatography indicates some alterations in surface protein differing from that of parent strains. Although other workers claim that avirulent poliovirus can be separated uniformly from virulent virus because the avirulent strains are absorbed more firmly to cellulose columns, our findings indicate that generalizations regarding the binding properties and the elution profiles of avirulent strains cannot be made.

In spite of the well-established high ratio between the PFU and virus particles, the bulk of virus in an inoculum actually binds to the cells and forms material of lower density. Thus, this wide ratio is probably explained by the limited ability of RNA to enter sites where virus production can be influenced. Other workers have proposed that RNA infectivity may be destroyed if only a small group of the long molecular chain is inactivated. Our findings indicate that major molecular changes do not occur when RNA is inactivated by hydroxylamine or octyl alcohol-chloroform. Neither of these should harm RNA, and it is entirely possible that infectivity of the relatively large RNA molecule can be destroyed by uncoupling of one link. If this is true and if the link is unique, an approach to virus chemotherapy is indicated.

The case for infectivity of RNA per se was considerably strengthened by demonstrating that only 0.1% of the RNA infectivity could be accounted for by residual protein.

Proposed course:

The protein composition of polioviruses will be studied by protein finger printing of virulent-avirulent combinations which will indicate possible qualitative differences in their proteins. Column elution properties and biophysical tools such as electrophoresis, ultracentrifugation, and equilibrium-density centrifugation may indicate differences in folding if qualitative differences do not exist. Our ability to label both protein and nucleic acid of the poliovirus will facilitate analytical work in

regard to these components of the viruses.

Investigations will be continued on the use of labeled viruses as fundamental and diagnostic reagents. Collaborative studies will be continued on the anamnestic response elicited in guinea pigs against purified poliovirus proteins. Investigations are currently under way to determine if "hybrid" animal cells can be produced and if animal cells exposed to bacterial RNA will form bacterial protein in the same manner that these cells form virus protein when exposed to virus RNA.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Perrine, T. D.: Method for attaching glass water aspirators to water lines. J. Chem. Education 37: 481. Sept. 1960.

Oertli, E. and Perrine, T. D.: Magnetically stirred separatory funnel. Chemie-Ingenieur-Technik 32(8): 554-555. 1960. Also in Chemie für Labor und Betrieb 8: 1960.

Holland, J. J., Hoyer, B. H., McLaren, L. C., and Syverton, J. T.: Enteroviral ribonucleic acid. I. Recovery from virus and assimilation by cells. J. Exp. Med. 112(5): 821-839. Nov. 1, 1960.

Holland, J. J., McLaren, L. C., Hoyer, B. H., and Syverton, J. T.: Enteroviral ribonucleic acid. II. Biological, physical and chemical studies. J. Exp. Med. 112(5): 841-864. Nov. 1, 1960.

Reinhard, K. R. and Gerloff, R. K.: Immunity towards poliovirus among Alaskan natives. II. Am. J. Hyg. 72(3): 298-307. Nov. 1960.

Reinhard, K. R., Gerloff, R. K., and Philip, R. N.: Immunity towards poliovirus among Alaskan natives. III. Am. J. Hyg. 72(3): 308-320. Nov.1960.

Honors and Awards relating to this project:

Dr. B. H. Hoyer

Invited to be Visiting Professor in the Department of Bacteriology and Immunology, The Medical School, University of Minnesota, 1960-61. Invitation not accepted.

Invited to present series of lectures in Virology at the University of Washington, May 1960.

Invited to participate in Animal Virus Symposium, Berkeley, California, 1960.

Appointed Lecturer in Microbiology 1960-61, Montana State University, Missoula, Montana.

Dr. R. A. Ormsbee

Appointed lecturer in Microbiology, Montana State University, Missoula, Montana.

Serial No. NIAID-177
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Immune prophylaxis of mycobacterial infections

Principal Investigator: E. Ribi

Other Investigators: W. T. Haskins and C. L. Larson

Cooperating Units:

Man Years (calendar year 1960):
Total: 4.75
Professional: 0.75
Other: 4.00

Project Description:

Objectives:

The primary objective of this program has been to investigate the role of live or dead tubercle bacilli or fractions thereof in the production of resistance to infection with virulent tubercle bacilli. First, it was considered essential to develop a test based upon a more natural challenge with virulent organisms than had been used previously and, as a consequence, the present method of challenge with a small number of organisms given by aerosol was developed. The objectives also include the application of this method as a control procedure in the production of vaccine and the use of the method to determine the mechanism upon which immunity is based.

The role of physical and chemical components of tubercle bacilli and other acid-fast organisms in producing or eliciting delayed reactions and circulating antibodies has also been investigated.

Methods:

The methods used have been in large measure those developed at the Rocky Mountain Laboratory. Fractionation of organisms is accomplished by means of mechanical disruption. Vaccines are evaluated by all means available, but special emphasis is placed upon aerosol infection of immunized mice.

Part B included: Yes

Major findings:

1. Animals (guinea pigs or rabbits) infected with live cells or sensitized with killed whole cells or cell walls of various acid-fast organisms develop specific sensitivity to protoplasm derived from these organisms.

2. Mice immunized with live cells of H37Ra administered either intravenously or by aerosol develop significant resistance to subsequent aerosol infections with H37Rv. Animals immunized with H37Ra by any other route fail to show similar resistance.

3. Resistance engendered by H37Ra does not appear to be induced by interference, for administration of this organism either 24 hours before or after administration of H37Rv does not influence the course of infection. In these studies both organisms were given as an aerosol.

4. Continued experience demonstrated that C. burnetii, Br. neotomae, or P. tularensis infections do not affect the course of pulmonary tuberculosis in mice. These findings establish the specific nature of this test for studying immunity against tuberculosis.

5. Live or dead acid-fast organisms (M. tuberculosis, M. phlei, M. butyricum, M. smegmatis, and atypical acid-fasts) produce lesions in the lungs of guinea pigs only if the organisms are suspended in Freund's adjuvant prior to subcutaneous injection into the animals.

6. Antibodies were not detected in serums from persons ill with tuberculosis when tested by the double diffusion technique of Parlett and Youmans or by Ovary's method.

7. Previous treatment of animals with BCG organisms produces hyperreactivity to subsequent injections of Salmonella toxins. Relatively large doses of cell walls are required to produce such hyperreactivity.

8. Previously, it was shown that the production of isoallergic encephalitis in guinea pigs was enhanced when 0.05 mg. of Mycobacterium cell walls was added to a subcutaneously administered dose of brain antigen mixed with Freund's adjuvant. As much as 5 mg. of protoplasm mixed with the antigen failed to do so. Further studies have shown that 0.05 mg. of a water-soluble fraction of cell walls has the same enhancement potential as that possessed by an equal weight of cell walls.

Significance:

Since small amounts of cell walls produce hypersensitivity but fail to produce hyperreactivity to toxins, it would appear that these two phenomena are distinct. So far, cell walls and the cord factor have been the only fractions of tubercle bacilli capable of causing hyperreactivity in mice.

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Studies of hypersensitivity show that specific delayed reactions are induced by cell walls or whole cells of various organisms and that the delayed reactions may be specifically elicited by protoplasm of these organisms. These studies indicate that it is feasible to employ protoplasmic fractions of such organisms as the Battey strain to determine the role of this and other atypical acid-fast bacilli in the production of nonspecific tuberculin reactions in man. As Palmer and his group have emphasized, the problem of determining the role of these organisms in producing disease (symptomatic or asymptomatic) in man has yet to be evaluated.

Our recent findings that mice can be immunized against infections with virulent tubercle bacilli when both organisms are administered by aerosol also have shown that the test we have developed is specific and that the resistance noted is not based on interference. To date, only live organisms have been found to engender resistance, but the resistance noted is of such character that it indicates the usefulness of live organisms in the prevention of tuberculosis under experimental conditions.

Proposed course:

It is hoped that a study can be initiated to determine the role of acid-fasts in producing disease and/or hypersensitivity in a normal rural population in the western United States.

Studies will be continued on immunity against infections with virulent tubercle bacilli. Plans are being made to examine lots of BCG vaccine produced in various laboratories to determine the value of our mouse test as a control for manufacture of BCG vaccines.

Studies of antibody production by various fractions of tubercle bacilli will be expanded.

Studies of isoallergic encephalitis will be continued. Our results indicate that it now may be possible to study this phenomenon at a molecular level.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

In press:

Larson, C. L., Ribi, E., Wicht, W. C., and List, R.: Skin reactions produced in rabbits by cell walls and protoplasm of Mycobacterium tuberculosis and M. butyricum. The Amer. Rev. of Respiratory Diseases.

Serial No. NIAID-178
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Immune prophylaxis of Salmonella infections

Principal Investigator: E. Ribi

Other Investigators: W. T. Haskins, F. G. Jarvis, and K. C. Milner

Cooperating Units: Dr. Maurice Landy, NCI, Dr. Emanuel Suter, U. of Florida, and Dr. Erwin Neter, U. of Buffalo.

Man Years (calendar year 1960):

Total:	5.5
Professional:	2.5
Other:	3.0

Project Description:

Objectives:

Since immunogenic properties of Gram-negative bacteria are closely related to potent antigens located in the cell wall, studies are directed toward the acquisition of a more precise knowledge of the chemical structure of such antigens and the correlation of such structure with biologic function. Particular attention has been given to a correlation of chemical composition with biologic activity, particularly that reported to be attributable to the so-called "lipid A" fraction.

Methods:

Bacterial fractions isolated by physical and chemical methods are characterized by appropriate biophysical, biologic, and immunologic assays. In addition to the use of electron microscopy, complete chemical and physical analyses are made to verify purities of fractions.

Major findings:

Endotoxins, which are generally regarded as combinations of lipid, polysaccharide, protein, and peptidelike substances, are so complex that precise relationships between chemical constitution and biologic function are still unknown and are subject to controversy. German workers contend

Part B included: Yes

that the biologic properties of these complexes are attributable to the firmly bound lipid content, so-called "lipid A." Isolated lipid A is relatively nontoxic, but these investigators believe that this component is toxic when conjugated with polysaccharide of the original complex.

Last year it was demonstrated that most, but not all, of the protein, peptide, and lipid portions could be removed from aqueous-ether extracts without altering the whole array of in vivo and in vitro biologic activities of the original complex. In comparison of a Boivin-type extract with a 19.2% lipid content and an aqueous-ether extract with a 1.2% lipid content, large but not significant differences in biologic properties were disclosed. Since firmly bound lipid had been determined by the Freeman method and not in terms of lipid A, additional investigations were conducted to delineate the biologic properties of bound lipid.

1. Endotoxin of low lipid content prepared from Salmonella enteritidis by the aqueous-ether method has been treated further for the removal of bound lipid by nonhydrolytic procedures. Such endotoxin, containing 2% to 3% lipid A, was as potent as that prepared by the well-known phenol-water or Boivin procedures, which yield products of high lipid A content (20% to 30%). To verify differences in lipid content of aqueous-ether preparations and other types of endotoxins, three different methods of lipid analysis were employed. These three methods were in accordance in demonstrating the magnitude of differences in Freeman lipid (chloroform-soluble material released by hydrolysis with acetic acid), lipid A (material released with hydrochloric-acid treatment), and esterified fatty acid.

2. With modified methods, endotoxins having a nitrogen content of less than 0.5% and a fatty acid ester content of 1.7% were prepared. The analytical values for these potent endotoxins did not differ appreciably from those of the classic haptenic polysaccharide in respect to nitrogen, phosphorus, aminohexose and fatty acid ester content.

3. The chemical analyses of a series of endotoxins prepared from different bacterial species indicate that firmly bound lipid (lipid A) varies according to the strain of bacteria and to the extracting agent.

Introductory information for the next three findings.

German workers have contended that the biologic activity of lipid A would be comparable to that of endotoxin if it were possible to disperse the free lipid A in water in a manner similar to the original state whereby it is bound to the polysaccharide carrier. These workers have reported that lipid A, when dispersed in detergent or when coupled to inert protein, has 1/10 and 1/5, respectively, of the activity of the intact endotoxin. However, in making these comparisons, endotoxin and lipid A were not derived from the same source. In view of our findings, which were at variance with these reports, and in view of the scarcity of

pertinent biologic data, comparative studies on the biologic effects of lipid fractions and the endotoxins from which they were prepared were made by well-established dose-response assays. Bioassays were based on the following host responses: fever, resistance to infection, tumor damage, primary inflammation of the skin, and toxic death.

4. Without exception, preparations of lipid A had only a small fraction of 1% of the biologic activity of the endotoxins from which they were derived.

5. Lipoidal fractions were dissociated from endotoxins by nonhydrolytic procedures without appreciable reduction in potency of the endotoxins. These lipids exhibited biologic effects of the same low order as lipid A isolated by acid hydrolysis, a method which is known to be destructive to the potency of endotoxin.

6. Lipid A is a material of a heterogeneous nature. For example, hexosamine-free lipids have been prepared which are of comparable potency to lipid A containing 18% to 20% hexosamine. The German workers consider this substance to be an essential component of lipid A.

7. In order to obtain information on the effect of the hydrophilic carrier to lipid A activity, the capacities to evoke various host responses of original endotoxin and of artificial lipoprotein prepared from it were determined in parallel-dose responses. Although artificial lipoprotein had a biological potency at least 100-fold lower than endotoxin, it was greater than that of lipid A. However, less severe acid treatment is used to prepare artificial lipoprotein than is used to release lipid A. The less severe acid treatment, rather than increased solubility of lipid A, was thought to be responsible for the increased activity of lipoprotein. To prove this interpretation, a kenetic study was made of the rate of reduction of biologic activity and the rate of release of firmly bound lipid by acid hydrolysis. Biologic activity of endotoxin, as measured by 5 quantitative biologic assays, disappeared before appreciable amounts of firmly bound lipid were released. The loss of biologic potency cannot, therefore, be explained by the separation of the water-insoluble lipid from the endotoxin complex. At the stage where acid treatment in the kenetic study was comparable to that used for the preparation of artificial lipoproteins, the biologic activity of residual endotoxin was about equal to that of artificial lipoprotein.

8. A method which includes 2 cycles of curtain electrophoresis was developed for the quantitative separation and recovery of both Vi and O antigens. Because this method does not involve hydrolysis, certain labile acetyl groups are not affected, and the recovered antigen is about ten times more active than preparations heretofore available. The biologic activity of Vi antigen was shown to be dependent upon the presence of these labile acetyl groups.

Significance:

The injection into animals or man of minute quantities of endotoxins extracted from Gram-negative bacteria gives rise to an array of striking physiological effects. Among those which have been the subject of extensive study, the following are especially noteworthy: stimulation of resistance to infection with both homologous and heterologous organisms, enhancement of antibody production, protection against radiation injury, pyrogenicity, and induction of a state of tolerance to endotoxins.

Even though they have been studied intensively, endotoxins are sufficiently complex that precise relationships between chemical constitution and the capacity to elicit characteristic reactions in mammals are still unknown and are subject to controversy. The consensus is that these complexes, as ordinarily isolated, consist of lipid, polysaccharide, protein, and peptidelike substances.

Although we had shown that the major portion of the lipid may be removed while retaining the entire array of biological properties, these results are at variance with the widely accepted view that firmly bound lipid is the actual toxic principle of endotoxins and that such lipids could be split off by acids (lipid A) and their toxic activity restored by suitable dispersion in water.

A major contribution from this laboratory involves the use of a number of dose-related quantitative assays to determine in parallel tests the relative potencies of endotoxins and the lipid fractions derived from them. The use of these quantitative measurements revealed, to a degree previously not suspected, that endotoxins are far more potent than their corresponding lipids.

The study of the kinetics of acid hydrolysis of endotoxin showed a progressive destruction of potency which was virtually complete before any separation of firmly bound lipid. It is believed that the small amount of activity exerted by lipid fractions is of a special kind, unrelated to the major activity of the complete endotoxin. This conclusion is supported by the finding that lipids which had been dissociated by nonhydrolytic means, without appreciable alteration of the activity of endotoxin, were at least as potent as lipid A recovered by acid hydrolysis, the remainder of which is inactive.

Proposed Course:

Despite considerable progress in stripping away nonfunctional parts of endotoxic extracts, much more can be done toward reducing complexes to pure active principles. This aspect of the work will be continued in an effort to define the minimum constitution of an endotoxin or of an O antigen. It is sufficiently clear that, up to this point, all announcements of "purified" endotoxins have been premature.

Studies on stimulation of specific immunity to infection will be resumed, particularly investigation of leads reported earlier concerning the possibility of producing effective prophylactic agents of modified toxicity. To implement these aims, enzymatic studies of the endotoxin complex will be added to our chemical approach to the problem. Physiological studies of endotoxin shock and passive protection to it, assays for pyrogenicity in rabbits, and perhaps other tests will be added to the biological work at this laboratory. It is expected that collaborative projects with Drs. Suter, Neter, Landy, and Nowotny will also go forward during the coming year, and that a joint project, involving an exchange of personnel, will be organized with Drs. Malmgren and Hedén of the Karolinska Institutet, Stockholm, Sweden.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Ribi, E., Hoyer, B. H., Milner, K. C., Perrine, T. D., Larson, C. L., and Goode, G.: Physical and chemical analysis of endotoxin from Salmonella enteritidis. J. Immunol. 84(1): 32-47. Jan. 1960.

Jarvis, F. G., Mesenko, M. T., and Tibbs, K. E.: Production of Vi antigen on a chemically defined medium by a coliform bacterium. J. Bact. 80(5): 673-676. Nov. 1960.

Jarvis, F. G., Mesenko, M. T., and Kyle, J. E.: Electrophoretic purification of the Vi antigen. J. Bact. 80(5): 677-682. Nov. 1960.

Honors and Awards relating to this project:

Dr. K. C. Milner

Invited to spend a year in 1961-62 at the Karolinska Institutet in Stockholm, Sweden.

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Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Investigations of the role of morphological elements of microorganisms in immunity and related phenomena

Principal Investigator: E. Ribi

Other Investigators: R. L. Anacker, J. E. Coe, C. L. Larson, and K. C. Milner

Cooperating Units: Dr. J. W. Foster, U. of Georgia; Dr. Philip Gerhardt, U. of Michigan; Dr. Henry Koffler, Dr. John Stasny, and Dr. F. L. Crane, Purdue U.

Man Years (calendar year 1960):

Total:	4.0
Professional:	1.0
Other:	3.0

Project Description:

Objectives:

Studies of the fine structure and chemical composition of a variety of organisms and the correlation of such findings with certain immunologic and biologic properties of microbial cells or fractions thereof comprise the activities of this section. In general, these projects represent collaborative efforts with other investigators who wish to utilize Dr. Ribi's biophysical methodology to fulfill desired objectives. This year, studies have been concerned chiefly with Brucella, Penicillium chrysogenum, Bacillus terminalis and B. subtilis.

Methods:

In addition to the usual microbiologic techniques, special methods, such as X-ray diffraction, the pressure cell system for rupturing bacterial cells, and linear continuous sucrose or glycerol gradients are utilized. The electron microscope is routinely used to observe and evaluate induced morphologic alterations. Immunogenic substances are released from bacterial cells by mild chemical treatment.

Part B included: Yes

Major findings:

1. Continued studies confirmed initial findings that immunogenic and toxic properties of Br. abortus are located in the cell wall. The limited activity of protoplasm was thought to result from contamination with cell-wall materials. Aqueous-ether extracts of cell walls or whole cells possessed far greater immunogenic activity than did other fractions or whole cell preparations. When extraction is performed in the presence of phosphate buffer (pH 7.0), a more potent though less toxic soluble antigen is released.

2. The problem of removing large lipoidal granules from washed aqueous suspensions of bacterial spores was solved by sedimenting washed spore cultures through a linear continuous glycerin gradient. By this technique, dormant spores also could be separated from vegetative spores.

3. In view of the known resistance of spores to heat, drying, and disinfectants, the fine structure of the spore coat was studied in order to explain the protection afforded the spore body. When a purified fraction of spore coats was examined under the electron microscope, these coats appeared to be comprised of multiple layers or lamellae whose thicknesses approached the limit of resolution of the electron microscope. The shape of fractured edges suggested that these layers were composed of laminar crystals, and subsequent X-ray diffraction patterns confirmed the character of the spore coat as a perfect crystalline substance.

4. X-ray diffraction patterns of purified cell walls of Penicillium chrysogenum contained only reflections typical of chitin and not of glucan, a related polysaccharide thought by collaborative investigators to be present in the cell wall. The fine structure of chitinous cell walls was shown to be similar to that of Histoplasma capsulatum, but crystallites of the latter were smaller. The morphologic arrangement of fibrils and amorphous regions explains such properties as rigidity, elasticity, and permeability of the cell walls of these fungi. Chitin crystallites isolated from fibrils of P. chrysogenum are now being purified by Drs. Koffler and Stasny, collaborators on this project.

5. The significance of "double membranes" in osmium-stained thin sections of cells, nuclei, and other morphologic structures observed under the electron microscope has not been resolved. This "double membrane" appears as 2 parallel lines of osmium but the cellular components with which osmium is associated have not been determined. Similar structures have been seen when high speed supernates of bacterial cytoplasm were desiccated in the presence of sodium chloride upon plastic specimen-supporting membranes. It was determined that these same structures appeared in transparent films obtained by simply evaporating aqueous solutions of sodium or potassium chloride on plastic membranes.

Significance:

The intricate association of toxic and immunogenic substances in the cell wall of Br. abortus complicates the development of a nontoxic vaccine. However, the effect of buffer (pH 7.0) on the toxicity of substances released by ether extraction suggests that some selective separation of toxic and immunogenic fractions is possible. To date, ether is the only known agent that will kill Brucella without destroying its immunogenic properties.

The ability to separate various components of washed spore cultures by sedimentation in a linear, continuous glycerin gradient enables further definitive characterization of the coat and body of bacterial spores. Additional information thereby obtained may elucidate the role of the spore coat in protecting the body from detrimental effects of physical and chemical energies.

Concurrent with the use of thin-sectioning technique for the preparation of tissues for study by electron microscopy, numerous reports on "double membranes" associated with various cellular structures have appeared in the literature. The significance of these structures has remained controversial. The results of present studies, however, indicate that these membranes may be formed by some colloidal physical forces which are induced during solidification of inorganic ions on a surface.

Proposed course:

Collaborative studies on spore coats of B. subtilis and characterization of cellular fractions of B. abortus and P. chrysogenum will be continued.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Larson, C. L., Ribi, E., Milner, K. C., and Lieberman, J. E.: A method for titrating endotoxic activity in the skin of rabbits. J. Exp. Med. 111(1): 1-20. Jan. 1960.

Ribi, E., Brown, W., and Goode, G.: Preparation of microorganisms for electron microscopy. J. Bact. 79(1): 142-144. Jan. 1960.

Honors and Awards relating to this project:

Dr. Edgar Ribi

Invited to give lecture on endotoxins for Department of Bacteriology, University of Florida, Gainesville, Florida.

Participated in panel discussion of bacterial endotoxins at meeting of Society of American Bacteriologists, Philadelphia, as representative of endotoxin group.

Invited to participate in a symposium on bacterial endotoxins sponsored by the Society of American Bacteriologists in Chicago, Illinois, April 1961.

Serial No. NIAID-180
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The encephalitides

Principal Investigator: C. M. Eklund

Other Investigators: W. Burgdorfer, D. B. Lackman, V. Newhouse, and L. A. Thomas

Cooperating Units: None

Man Years (calendar year 1960):
Total: 15.5
Professional: 2.5
Other: 13.0

Project Description:

Objectives:

The objectives of this program are to determine the incidence of arthropod-borne viruses in the western United States and to develop better methods of diagnosis of these diseases, a detailed knowledge of the ecology and epidemiology of this group of diseases, and general and specific methods of control.

Methods:

The methods employed are in general those used in field studies of arthropod-borne infections and virological and serological techniques suitable for this type of study.

Major findings:

1. A virus, stored since its original isolation from Colorado ticks in 1952, was recently characterized and found to be closely related to Powassan virus obtained from a patient in Ontario, Canada, and studied at RML last year. These viruses belong to the Russian spring-summer encephalitis group, but they are not as closely related to this group as they are to each other. The tick isolate was neutralized by RSSE immune serum, but RSSE virus was not neutralized by Powassan or tick-virus

Part B included: Yes

immune serum. Failure to isolate this agent in tests of many other field-collected ticks and other considerations suggest that this virus isolated from Colorado ticks was an accidental infection of D. andersoni.

2. In contrast to a very low incidence of WEE virus in C. tarsalis in 1959, a marked increase in number of isolations (22) from Idaho and Oregon was reported this year. One strain also was isolated from Culex pipiens which, like a previous one, shows some biological differences from WEE isolated from C. tarsalis. There were also 4 isolations of St. Louis virus from Idaho C. tarsalis.

3. Three isolations of Culiseta inornata virus were made, perhaps a reflection of an unexpected increase of this species in North Dakota. Isolations had not been made since 1952. This virus belongs to the African Bunyamwera group and is related to the Cache Valley virus in Utah. A colony of C. inornata has been established for transmission studies with these strains.

4. A virus isolated from a local snowshoe hare has been identified as closely related if not identical to so-called California virus. This is the first isolation from a vertebrate, though antibodies have been detected in Bitterroot horses. Domestic rabbits have been refractory to experimental infection, and tests of the susceptibility of native hares and rabbits from other states are inconclusive. The related trivittatus virus was not recovered from mosquitoes this year.

5. Transmission studies showed survival of the snowshoe hare strain in C. tarsalis and A. aegypti for 25 days, but only one unrepeated passage by bites of the latter. D. andersoni, O. parkeri and O. turicata did not acquire infection when fed on experimentally infected hamsters. This virus had not been recovered at RML in tests of 153,000 C. tarsalis collected from various western states during the last 10 years.

6. To check for presence of possible latent WEE virus in bloods of migratory birds arriving before advent of the mosquito season, 976 serums of different species collected from 4 localities in Minnesota, North Dakota, Montana, and Oregon were tested but none contained virus. An additional 148 serums from mammals and snakes were free of WEE or SLE virus.

7. Of 50 garter snakes of 2 species injected intraperitoneally with WEE virus last fall, virus was isolated from 23 (16 were proved WEE) after leaving hibernation. Virus circulated in some snakes up to 70 days. The complete experimental overwintering cycle was confirmed by passage of virus to other snakes through the bites of mosquitoes infected on the above snakes.

8. In garter snakes injected with WEE virus and held at 4°, 22°, and 31° C., viremia was observed to start latest (about a month) at the lowest temperature of storage, but to last longest (at least 25 days) at 22° C.

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9. Preliminary tests suggest that gopher snakes and rattlesnakes may not be as susceptible as garter snakes.

10. On the possibility that immunologic tolerance might influence the overwintering mechanism of WEE virus, mice in various states of pregnancy were infected. Up to the 10th day of pregnancy, mothers and subsequent litters survived. Deaths among mothers infected after the 10th day occurred at varying intervals after inoculation; litters born to mothers surviving until parturition occurred died within 2 to 3 days after birth. However, virus was recovered only from the dead mothers, not from the litters. Pregnancy therefore has a marked effect on the course of the disease.

Significance:

The survival of WEE virus in hibernating garter snakes, the long viremia, and demonstrated snake-to-snake transmission by the bites of C. tarsalis (the important known natural vector) offer at least one explanation for the overwintering of the virus.

Ecologic data being accumulated on the natural occurrence of WEE and SLE viruses were reviewed in last year's report and are still being augmented.

Because of the increasing need for identification of various viral agents isolated in field studies or referred to the virus unit by others, a collection of 37 identified agents is now available. This collection will not only speed systematic reference but will provide data fundamental to an understanding of the relationships of these so-called ar-bo viruses.

The isolation of California virus for the first time from a vertebrate should forge a significant link in the epidemiologic chain of the natural cycle of this virus which has caused some human infection in California and is probably mosquito borne.

The isolation from Colorado ticks of a strain of virus related to Powassan virus of human origin, suggests that a tick-borne entity with some resemblance to the important Russian spring-summer encephalitis of Eurasia may be present in North America.

Proposed course:

Ecologic observations with intensified study of the laboratory features of the relationships among ar-bo viruses of western United States will be continued. Further data on various aspects of the role of snakes are expected to add to epidemiologic information. Comparative studies will be continued on the pathogenesis of infection, including duration of viremia in mice, chickens, and other fowls. The susceptibility of vertebrates to California virus will be investigated.

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Further efforts will be made to correlate HI, CF, and neutralizing antibodies and to determine stability of neutralizing antibodies in serums during storage. Additional techniques will be used to refine certain mouse-brain vaccines.

The reference collection of ar-bo viruses will facilitate collaborative studies with the Greeley laboratory of CDC and other institutions on the characterization of unclassified viruses.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Eklund, C. M.: Insect-borne and animal-borne virus diseases of man. Minnesota Med. 43(3): 184-189, 193. March 1960.

Thomas, L. A., Kennedy, R. C., and Eklund, C. M.: Isolation of a virus closely related to Powassan virus from Dermacentor andersoni collected in Cache la Poudre Canyon, Colorado. Proc. Soc. Exp. Biol. & Med. 104(2): 355-359. June 1960.

Thomas, L. A. and Eklund, C. M.: Overwintering of Western equine encephalomyelitis virus in experimentally infected garter snakes and transmission to mosquitoes. Proc. Soc. Exp. Biol. & Med. 105(1): 52-55. Oct. 1960.

In press:

Olson, T. A., Rueger, M. E., Price, R. D., Schlottman, L. L., Kennedy, R. C., and Eklund, C. M.: Evaluation of activity of western equine encephalomyelitis virus in Minnesota by antibody response of sentinel pigeons. Am. J. Trop. Med. and Hyg.

Honors and Awards relating to this project:

Dr. C. M. Eklund

Appointed lecturer in Microbiology, Montana State University, Missoula, Montana.

Invited to participate in a symposium on the biology of viruses of the tick-borne encephalitis complex in Smolenice, Czechoslovakia.

Serial No. NIAID-181
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Colorado tick fever

Principal Investigator: C. M. Eklund

Other Investigators: W. Burgdorfer, G. M. Kohls, D. B. Lackman, and L. A. Thomas

Cooperating Units: None

Man Years (calendar year 1960):
Total: 18.0
Professional: 2.0
Other: 16.0

Project Description:

Objectives:

The objectives of this program are to develop a knowledge of the clinical syndromes which CTF virus may produce in human beings and to understand the ecology of this agent, especially the role of tick and rodent reservoirs. In addition, studies are under way to check possible interference with concomitant infections of R. rickettsii in tick vectors. Development of a prophylactic vaccine remains a primary objective.

Methods:

The methods employed do not differ greatly from those used in laboratories studying field and laboratory aspects of arthropod-borne viruses except as they are adapted to our local situations.

Major findings:

1. Though CTF had been reported from ticks on Long Island, investigations to date at RML indicate that human infection is limited to areas where D. andersoni is found. Presence of the virus in adult tick vectors depends on a natural cycle between the active immature stages and their small animal hosts, because transovarial maintenance has never been confirmed in exhaustive studies.

Part B included: Yes

2. The disease in human beings varies from preponderant subclinical and benign febrile syndromes to infrequent involvement of the central nervous system or severe bleeding. Although serum samples were not especially solicited from physicians, almost twice as many isolations were made this year as last year. Two-thirds of the blood specimens were received from Idaho, Oregon, and Wyoming.

3. In tests of ticks from several areas in Colorado, infection rates varied from zero to more than 21%.

4. Members of the Trappist Monastery at Snowmass, Colorado, were vaccinated again without ill effect. Danger from repeated use of this inactivated mouse-brain product appears to be minimal because untoward effects were not observed in guinea pigs receiving multiple injections.

5. Three new lots of vaccine prepared from infected suckling mouse brains again provided marked protection, as shown by differences in resistance between vaccinated and nonimmunized control mice.

6. It has now been shown that the simplest diagnostic test for CTF is the complement-fixation technique. Antibodies are readily detected in serums from patients convalescent from CTF; they first appear 20 days after onset of illness and may remain as long as 260 days.

Significance:

As with other tick-borne human diseases, the number of annual cases of CTF fluctuates. Hence, the marked increase in number of isolations may not represent a real increase in disease. Physicians in the Rocky Mountain area have undoubtedly become more aware of the prevalence of this tick-borne disease through our efforts to obtain more data on severe types of CTF. This year one 9-year-old Oregon boy had encephalitis accompanied by increased cell count in the spinal fluid and other aggravated symptoms.

Since a high percentage of ticks in Estes Park carry CTF virus, transient visitors are likely to contract infection but symptoms do not appear until these persons have returned home and it is probable that some cases remain undiagnosed.

In certain areas of considerable occupational incidence, such as in stock handlers in northern Nevada, a suitable vaccine would be useful.

Proposed course:

As in the past, continued efforts will be made to prepare a refined and potent vaccine.

We are continuing to accumulate data on possible interference between infections of R. rickettsii and CTF virus in the same ticks since this is one possible explanation for the observed low incidence of the former in endemic localities of CTF-infected ticks. Study by fluorescent microscopy of the CTF agent in vertebrate and acarine hosts is continuing.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Burgdorfer, W. and Lackman, D.: Identification of the virus of Colorado tick fever in mouse tissues by means of fluorescent antibodies. J. Bact. 80(1): 131-136. July 1960.

Thomas, L. A. and Eklund, C. M.: Use of the complement-fixation test as a diagnostic aid in Colorado tick fever. J. Inf. Dis. 107(2): 235-240. Sept.-Oct. 1960.

In press:

Eklund, C. M., Kohls, G. M., Jellison, W. L., Burgdorfer, W., Kennedy, R. C., and Thomas, L. A.: The clinical and ecological aspects of Colorado tick fever. Proc. Intern. Cong. Trop. Med. and Malaria, Lisbon, Sept. 1958.

Burgdorfer, W. and Eklund, C. M.: I. Colorado tick fever ecological studies in western Montana. J. Inf. Dis.

Burgdorfer, W.: Colorado tick fever. II. The behavior of Colorado tick fever virus in rodents. J. Inf. Dis.

Eklund, C. M., Kennedy, R. C., and Casey, M.: Colorado tick fever. Rocky Mtn. Med. Jour.

LABORATORY OF BACTERIAL DISEASES

SUMMARY OF ACTIVITIES
LABORATORY OF BACTERIAL DISEASES
December 1960

The research program of the Laboratory of Bacterial Diseases has continued in the same general areas as last year with the change of emphasis noted in last year's report.

A greater proportion of the research effort has been directed to the studies on intracellular parasitism. These studies deal with possible changes in characteristics of infected and immune cells as the result of parasitism, and the effect of intracellular growth on the parasite. One such notable change of course is the production of specific antibodies by certain cells of immune animals. During the current year a considerable effort has been directed toward the study of antibody production by cells in vitro. The macrophage was selected as a multipotential cell for such study. Macrophages obtained from the peritoneal cavity of immune guinea pigs (immunized with egg albumin) have been found to release antibody in vitro for a period of several days. This provides a system for further study of the nutritional or other requirements for continued antibody production in vitro, or even in serial cultures. Cells derived from macrophages have been carried in serial tissue culture for several months, retaining their phagocytic ability. This promising line of research will be intensively continued during the coming year.

Studies on brucellosis are conducted at a reduced tempo. There is continuing need to collaborate with other brucellosis research centers throughout the world to try and settle problems of classification and epidemiology of the Brucella. Currently we are doing some laboratory testing of Brucellosis Vaccine for Human Use prepared in Russia. There is present interest in this vaccine by the World Health Organization for its possible use in occupational and otherwise continually exposed groups.

Studies on the Staphylococcus are directed toward determining the factors responsible for pathogenicity, and toward development and standardization of tests for measuring relative pathogenicity of strains.

Some of Dr. Verder's past studies on the genus Pseudomonas are being prepared for publication.

There are other areas within the province of this Laboratory which should be developed were space, personnel, and financial support available. Among these are the diarrheal diseases and the basic field of PPLO and L form research.

There remains a continuing need for certain additional equipment and increased monetary support to pursue the basic studies mentioned.

Serial No. NIAID-200
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Brucellosis

Principal Investigator: Norman B. McCullough, M. D.

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2
Professional: 1/2
Other: 1-1/2

Project Description:

Objectives:

The objectives of this project are broad and studies are conducted on all aspects of brucellosis, including the nutrition, metabolism, and enzymatic make-up of the organism, mechanism of action of antibiotics on the organism, factors determining virulence of the organism, the development and efficacy of prophylactic immunizing agents, field studies to determine the occupational hazard of the disease, epidemiology, diagnosis and therapy of the disease in man.

Clinical studies concern diagnostic criteria for the disease in man, especially in borderline cases of chronic illness in which present cultural and serological techniques fail to provide reliable evidence of infection; studies of the pathogenesis of the disease; and the evaluation of current therapeutic regimens.

Part B included.

Methods Employed:

The Warburg technique is employed in enzymatic and metabolic studies; growth in a synthetic medium is used for nutrition experiments; guinea pig inoculation for virulence and vaccine studies; occupationally exposed groups for epidemiological studies; human patients for studies on diagnosis and therapy.

Major Findings:

The study of antigenic relationships among the Brucella has continued. Br. abortus and Br. suis constitute a single antigenic group as previously held, whereas Br. melitensis is antigenically more variable. The latter species consists of three antigenic patterns, only one of which is identified by the use of currently prepared mono-specific typing serum. The recognition of this antigenic variance allows the proper classification of most of the so-called "intermediate" strains, and should clarify many problems in the classification and epidemiology of the Brucella.

Significance to the Program of the Institute:

In terms of world-wide significance, brucellosis ranks in incidence and importance with malaria, tuberculosis, and parasitic diseases, in relation to the welfare of mankind. Even in the United States, by reported incidence, it is the most important disease of animals transmissible to man, is the cause of serious economic loss to the livestock industry, is an occupational disease, and one which presents many problems in the diagnosis and therapy of the infection in man. The organism readily lends itself to basic studies outlined elsewhere in this report.

Proposed Course of Project:

Continue studies under way.

Continue to collaborate with other Brucella research centers throughout the world in the development and standardization of laboratory tests for species identification, and for diagnosis of Brucella infection.

Study of Brucella bacteriophage as regards basic phenomena of bacteriophagy and potential use of phage for preparation of enzymatic and subcellular fractions of Brucella for biochemical studies, and as possible immunizing agents. Investigate the utility of specific phages as an aid in identification of Brucella species and strains.

Continue to use Brucella as a basic tool in the study of mechanism of development of acquired resistance to antibiotics and in studies of intracellular parasitism.

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Dolan, T. F., Jr.; McCullough, N. B.; and Gibson, L. E.: Nocardiosis. Report of Two Cases in Children. A.M.A. Jour. of Dis. of Child. 99: 234-237, 1960.

2. Dolan, T. F., Jr.; McCullough, N. B.; and Gibson, L. E.: Hypogammaglobulinemia. Report of an Unusual Variation. Pediatrics 26: 817-821, 1960.

Honors and Awards relating to this project:

Principal investigator elected to serve as a member of the Executive Committee and as General Chairman of the Annual Brucellosis Research Conference (1960).

Serial No. NIAID-201
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Basic Studies on Staphylococcus

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1-1/12
Professional: 8/12
Other: 5/12

Project Description:

Objectives:

1. To develop an objective means of measuring virulence of Staphylococci.

2. To determine the factors responsible for virulence.

3. To develop more satisfactory methods for the identification of individual strains used in measuring virulence.

Methods Employed:

The use of experimental animals and tissue cultures in defining overall virulence, and in study of individual fractions or products of the Staphylococcus.

Part B not included.

Use of physical and chemical methods in fractionation of the organism and its products to allow assessment of individual factors in pathogenicity and in cell injury *in vitro*.

Nutritional studies on the production of factors concerned in pathogenicity.

Gel diffusion techniques using both tubes and plates, agglutination, hemagglutination and precipitin tests are being employed. An attempt is being made to correlate mouse protection properties of human sera with certain characteristics of *in vitro* reactions.

Major Findings:

Numerous serological procedures have been explored in an effort to develop a technique for more satisfactory identification of individual strains without immediate success.

Significance to the Program of the Institute:

The number of antigenic components produced by a strain of staphylococcus varies with the virulence. Quantitative estimation of some of these components and also some of the products of the more invasive strains may be very helpful in characterizing strains.

Proposed Course of Project:

As described above.

Serial No. NIAID-202
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Identification of Pseudomonas

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigator: None

Cooperating Units: Dr. Carl Millican, NIAMD

Man Years (calendar year 1960):
Total: 1/12
Professional: 1/12
Other: 0

Project Description:

Objectives:

a) Identification of strains for other scientists.

b) Orientation of scientists in methods used for study of biochemical characteristics of various species and for serological typing.

c) Distribution of cultures of strains of various species and of representative serological types of Ps. aeruginosa on request to research scientists.

d) To determine whether therapeutic antisera contains identifiable group specific antibody.

Part B not included.

Methods Employed:

The methods which we have employed are ones developed or modified in the course of our studies on the classification of Pseudomonas; the studies were initiated during work on our project on diarrheal disease of the newborn and have been carried on for eight years. A proposed scheme for the antigenic analysis of strains of Ps. aeruginosa was worked out and has been employed successfully in identifying strains by serological typing.

Dr. Carl Millican has succeeded in guiding a commercial biological company to the production of an antiserum that has been used successfully in the treatment of children with Ps. aeruginosa infections. He has asked us to collaborate in studies on protection of animals infected with various serological types to determine whether or not a group specific antibody is responsible for these protective properties.

Standard antigen-antibody techniques will be employed in the study. Gel diffusion studies will be done with various gamma globulin and serum preparations and several antigenic fractions of Pseudomonas.

Major Findings:

Methods have been established for use in the identification of individual strains of Pseudomonas so that epidemiological surveys in relation to the spread of resistant strains may be carried out successfully.

Significance to the Program of the Institute:

Methods have been developed for studies on the spread of a group of antibiotic resistant organisms of medical importance, particularly in infections in individuals with minimal resistance such as newborns, the aged, children with leukemia, individuals with extensive or deep burns, etc. With the increasing importance of Pseudomonas infections the findings in these studies are receiving recognition in clinical applications.

Proposed Course of Project:

Collaborative studies with Dr. Millican as outlined above. This will be limited because of time-consuming nature of staphylococcus studies.

Serial No. NIAID-203
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies on Bacteria Characterized by the Production of Reproductive Filterable Granulea

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigator: Dr. Alexis Shelokov was co-investigator on the project at time the organisms were isolated and any reports will be made jointly.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1/12
Professional: 1/12
Other: 0

Project Description:

Objectives:

a) To learn more about 1) the classification of bacteria capable of producing reproductive filterable granules, 2) the environmental changes that induce the formation of granules, 3) the potentialities of these incomplete filterable organisms in the establishment of infectious processes and 4) the factors influencing the development of more complete cells from filtered particles.

b) Distribution of cultures on request to other scientists.

Part B not included.

Methods Employed:

Through the use of special media, organisms of this type have been isolated from the peripheral blood of several patients with illnesses with an indefinite diagnosis. Methods commonly employed in the study of organisms producing L bodies and the pleuro-pneumonia-like (PPLO) group were utilized.

Major Findings:

More detailed studies must be carried out before significant findings on these organisms may be reported. During the past year attempts to produce L forms of several strains of staphylococci have been successful. No detailed physiological studies have yet been carried out.

Significance to the Program of the Institute:

Organisms with similar characteristics have been recognized as important etiological agents of animal disease for many years. Their presence in extracts of tissue and blood serum collected from infected embryo or animals and used inadvertently in the preparation of tissue culture media has increased interest in the development of more satisfactory methods for their detection and identification. Their importance in infectious processes in man has not been adequately assessed. Since several species of Bacterioides are present in large numbers in the intestinal canal of man and animals, some of these organisms may belong to closely related species possessing filterable granules under certain circumstances and be present in the peripheral blood frequently. The importance of the group to medical scientists is emphasized by the three day conference sponsored by the N. Y. Academy of Sciences held in January, 1959, for discussion of various phases of studies on this group of organisms; Drs. Klieneberger-Nobel and Edwards of London and Dr. Freund of Copenhagen were among the invited participants.

Proposed Course of Project:

No additional work will be carried on with these organisms until the studies on the staphylococcus have been well established.

Serial No. NIAID-204
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Intracellular Parasitism

Principal Investigator: Norman B. McCullough, M. D.

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2
Professional: 1/2
Other: 1-1/2

Project Description:

Objectives:

To study host-parasite relationships in intracellular metabolism for the purpose of increasing our understanding of disease and immunity.

1. To determine the effect of infecting organisms on the metabolism of the infected cell.

2. To determine the effect of certain bacterial products, such as Staphylococcus toxin and bacterial fractions, on cells maintained as explants and in tissue culture, and upon certain subcellular particles.

3. To determine the contribution of cells, and of subcellular particles, extracts, and enzyme systems to the nutrition of and their effects on the characteristics of intracellular bacteria.

Part B not included.

4. To study protein synthesis (such as antibody production) by cells from normal and immunized animals.

Methods Employed:

The study employes various pathogenic agents such as Brucella, Staphylococcus, Pasteurella tularensis, and certain viruses. It will entail the use of standard tissue culture techniques, chemically defined media for bacteria, the Warburg technique, standard chemical procedures, phase microscopy, fluorescent antibody technique, vital dyes, electrophoresis, and chromatography.

Major Findings:

Using hemagglutination of tanned red cells as indicator, macrophages derived from the peritoneal cavity of guinea pigs immunized with egg albumin have been found to release antibody in vitro for a period of several days. Production of antibody appears correlated with conditions promoting the best growth of cells. However, after a few days there is a change in the morphology of the cells and antibody production ceases. Primary stimulation in vitro has not yielded antibody.

Cell cultures derived from guinea pig macrophages have now been maintained in serial culture for several months.

Significance to the Program of the Institute:

This study concerns the basic interactions between host cell and infecting agent and hence any contribution to knowledge would be of significance to the entire field of infectious diseases and to the program of the Institute.

Proposed Course of Project:

As stated above.

Serial No. NIAID-205
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Role of Infection in the Delayed Deaths of Mice Following Extensive Burn Injury

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigator: None

Cooperating Units: Drs. Sanford Rosenthal and Carl Millican

Man Years (calendar year 1960):
Total: 2/12
Professional: 2/12
Other: 0

Project Description:

Objectives:

a) To review literature dealing with increased susceptibility to infection following traumatic shock, radiation and extensive thermal injury.

b) To evaluate findings in extensive studies, carried out in 1943, on incidence of bacteria in tissues of burned mice and their role in infectious processes causing delayed deaths in mice following extensive burn injury.

Methods Employed:

Standardized techniques for the isolation and identification of bacteria from infectious processes were employed.

Part B not included.

Major Findings:

Bacteria were isolated from 65% to 90% of the cultures of heart, liver, spleen and kidneys of 43 mice sacrificed two to nine days after extensive burn injury. It appears that bacteria of low virulence and invasiveness may spread, from previously localized foci of latent infections or from areas of colonization on damaged surfaces, and establish generalized infections.

Significance to the Program of the Institute:

Septicemias and other fatal infections are of increasing importance as the cause of death following extensive burn injury in spite of advances in treatment.

Proposed Course of Project:

Study will be terminated in 1960 with the completion of a paper discussing our findings.

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