ANALYSIS OF PROGRAM ACTIVITIES
NATIONAL INSTITUTES OF HEALTH
1955

NATIONAL CANCER INSTITUTE
VOLUME II

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

U.S. National Cancer Institute.
Report of program activities.

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Office of the Chief
SECTION OR SERVICE

4.
LOCATION (OTHER THAN BETHESDA)

5. 400
SER. NO.

6. Biological Studies on the Factors Involved in the Development and Growth of Tumors in Experimental Animals.
PROJECT TITLE

7. H. B. Andervont
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Testicular Tumors

The preceding report summarized an investigation with strain C mice designed to utilize testicular tumors to study a dependent tumor. The study is now virtually completed and will be published as soon as time is available for writing.

Findings reported last year remain unchanged with the exception of those dealing with transplantation. This phase of the study is still in progress but the results to date are as follows.

Sixty-nine tumors were transplanted to ascertain whether they were able to grow in estrogenized hosts only or whether they were no longer dependent upon the hormone. Of 20 tumors transplanted from mice that were carrying stilbestrol pellets, 8 failed to grow in either estrogenized or nonestrogenized hosts, 2 were dependent and 10 became independent.

Of 49 tumors that continued to grow after removal of pellets, 5 failed to grow, 2 were dependent and 43 were independent.

It is seen that as transplantation proceeds more tumors become independent. Considerably more work is necessary to analyse the data but the results reported here are sufficient to establish clearly that earlier reports in the literature concerning the high degree of dependency of this type of tumor were probably the result of transplanting tumors before they had achieved independence in their original hosts. This finding with testicular tumors raises two questions. First, have other types of dependent tumors been transplanted too early or do they remain dependent in their original hosts? Second, what factors are involved in the relatively slow development of autonomy in testicular tumors? Many skin tumors of mice are known to evolve from papillomas, but the difference between papilloma and carcinoma can be detected histologically. Thus far no such difference is detectable between dependent and independent testicular tumors. The problem is complicated further by the observation that during a 16 weeks sojourn in mice a stilbestrol pellet produces histologic changes in testicles which persist for many months after removal of the pellet. Tumors may even develop in a few of these altered testicles.

Apparently in some tumors the development of malignancy is a slow process and such tumors may be used to test the effi... c- of preventive procedures.

The preceding report mentioned three experiments started to study testicular tumors. These are still in progress, but have progressed sufficiently to supply results. The first was performed to ascertain the susceptibility of various F_1 hybrids derived from strain C and other inbred strains. None has proved to be as susceptible as strain C but (C X DBA/2)F_1 and (C X Y)F_1 hybrids are developing testicular tumors in sufficient numbers to indicate their value as test animals. Hybrids between strain C and strains C57BL, I, or RIII, are developing few tumors.

The second experiment consisted of giving stilbestrol pellets to other inbred strains to compare their susceptibilities to induced testicular tumors. None has aproached strain C in this respect. Of the strains tested strain C exhibited a unique susceptibility. All strain C animals developed testicular tumors before a tumor arose in any other strain. A few tumors have occurred in DBA/2 males and one in a strain C3H male. Strains RIII, y, I and C57 BL are too susceptible

PROJECT DESCRIPTION (Cont.)

to the toxic effects of stilbestrol.

The third study was designed to ascertain the influence of age upon susceptibility. Results between young (2 months old) and older mice are not available but mice less than one month of age were very susceptible to toxic effects of stilbestrol and those that survived have not developed tumors. The chief objective of this experiment, however, is to determine the susceptibilities of 2, 6 and 12 month old animals.

Breast Tumors.

But one experiment gave sufficient findings during the year to report at this time. This experiment consisted of administration of stilbestrol and progesterone to agent-free strain C3H females to ascertain their influence upon the occurrence of breast tumors. Our untreated breeding females show a breast tumor incidence of less than 5%.

Group	No. of mice	No. with subcutaneous tumors	Average age in months	No. living
10% stilbestrol pellet	41	10	24	18
10% stilbestrol pellet plus progesterone	39	24	20	9
20% stilbestrol pellet	37	10	19	11

PROJECT DESCRIPTION (Cont.)

Living mice are between 21 and 26 months of age. Tumors are called subcutaneous tumors because they have not been diagnosed histologically.

It is seen that thus far both 10% and 20% stilbestrol pellets have increased the incidence of tumors. Also, progesterone plus 10% pellets produces more tumors than do 10% pellets alone.

This experiment was performed to determine the influence of progesterone when administered to agent-free mice. The aim of the work was to apply the findings to our colony of wild house mice which are known to carry the mammary tumor agent but develop few breast tumors when bred or given pellets of stilbestrol. It is considered essential to obtain breast tumors in these animals and to test the tumors for the presence of the agent. Exploratory findings with agent-free strain C3H mice suggest the use of both stilbestrol and progesterone in the wild mice.

PROJECT REPORT FORM (Cont.)

10. 400
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH X ADMINISTRATION

REVIEW & APPROVAL TECHNICAL ASSISTANCE

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont.)

14. 400
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Andervont, H. B.: Biological background for experimental work on tumors. In: Canadian Cancer Conference, Vol. I: 2-24. Academic Press, Inc., New York, N. Y. 1955.

Andervont, H. B. and Dunn, T. B.: Transplantation of hepatomas in mice. J. Nat. Cancer Inst., 15: 1513-1524, 1955.

1

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

H. B. Andervont, President, American Association for Cancer Research.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. — LOCATION(IF OTHER THAN BETH.)
5. 401 — SER. NO.

6. Sedimentation behaviour and homogeneity of enzymes and other compounds in crude solution (continued project). — PROJECT TITLE

7. Drs. G. H. Hogeboom and E. L. Kuff — PRINCIPAL INVESTIGATOR(S)

8. Drs. W. C. Schneider, B. R. Hill, J. L. Irvin; Miss M. F. Embrey — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

This project is a collaborative effort and includes several phases, some of which are not yet completed.

Methods employed: As described previously, the method employed involved centrifugation under convection free conditions and subsequent sampling and analysis of the fluid column at successive levels from top to bottom. The techniques were devised in this laboratory (G. H. Hogeboom and E. L. Kuff, J. Biol. Chem. 210, 733-751 (1954)).

Major Findings and Proposed Course of Project:

(a) In collaboration with Dr. Borroughs R. Hill of the City of Hope Medical Center, an investigation was made of the sedimentation behaviour of the lactic dehydrogenase present in the serum of normal and leukemic patients. (It had been previously shown that the lactic acid dehydrogenase content of leukemic serum was greatly elevated). The centrifugation studies conducted in this laboratory demonstrated clearly that the enzyme present in leukemic serum behaved in exactly the same fashion (within the limits of experimental error) as did lactic dehydrogenase obtained from normal serum. It is our understanding that Dr. Hill will continue the work, using electrophoresis and other methods of fractionation, and that the results will be published when this latter phase is completed.

PROJECT DESCRIPTION (Cont.)

(b) Several years ago, it was shown in this laboratory that over 50 per cent of the total nitrogen of isolated liver mitochondria was in the form of soluble proteins. It was also shown that the major component of the mixture (apparently a monodisperse globulin) was absent from the mitochondria of a hepatoma. A number of attempts to determine the significance of the latter finding, using standard techniques of protein fractionation, have not met with sufficient success to warrant publication. It is also true that liver mitochondria contain a number of enzymes that can be obtained in the soluble phase. With the new technique of analytical centrifugation mentioned above, initial studies have been made of the DPN-cytochrome c reductase and glutamic dehydrogenase released from isolated mitochondria by treatment with sonic oscillations. The results thus far indicate that DPN-cytochrome c reductase is polydisperse and thus is probably firmly bound to the structural fragments of mitochondria. At the centrifugal forces thus far employed, glutamic dehydrogenase has not been sedimented far enough to warrant any firm conclusions as to its characteristics.

During the next year, it is planned to make a further study of the soluble fraction obtainable from liver mitochondria, with respect both to the enzymes present (e.g., ribonuclease and desoxyribonuclease) and, if possible, to the protein that is absent from hepatoma mitochondria but present in large amounts in liver mitochondria.

(c) It is also planned to investigate the sedimentation behaviour of a number of enzymes localized in the soluble fraction of the cytoplasm, including several of those which are involved in purine metabolism and have not been obtained in a pure state (cf. Schneider, W. C., and Hogeboom, G. H., Intracellular distribution of enzymes IX. Certain purine metabolizing enzymes, J. Biol. Chem. 195, 161-166 (1952)).

(d) In collaboration with Dr. J. L. Irvin, a study will be made of the heterogeneity of desoxyribose nucleoprotein isolated from the nuclei of liver cells. Some work on the rate of incorporation of labelled amino acids into the nucleoprotein (in collaboration with Dr. Stetten) is also contemplated.

Significance to Cancer Research: It is hoped that the work outlined above will provide a rational background to future similar studies of neoplastic tissues.

PROJECT REPORT FORM (Cont'd.)

10. 401
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		TECHNICAL ASSISTANCE	

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. 401
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

E. L. Kuff, G. H. Hogeboom, and M. J. Striebich, the Sedimentation behaviour of alcohol dehydrogenase and urease in crude solutions, J. Biol. Chem. 212, 439-448 (1955).

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

For honors and awards, see Annual Report of Kuff, Hogeboom, and Dalton.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Cellular Biology Section — SECTION OR SERVICE
4. — LOCATION(IF OTHER THAN BETHESDA)
5. 402 — SER. NO.

6. The relation between cell chemistry and cell structure
PROJECT TITLE

7. E. L. Kuff, G. H. Hogeboom, A. J. Dalton
PRINCIPAL INVESTIGATOR(S)

8. M. F. Embrey
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: (a) To determine the distribution of enzymes and other compounds of biochemical importance among the particulate structures released by mechanical disruption of normal and malignant cell; (b) to characterize morphologically the intracellular structures thus released; and (c), thus to arrive at an interpretation of the fine structure of the cells in terms of biochemical function. Results obtained by the use of more precise and sensitive methods for investigating the sedimentation properties of cytoplasmic particulate material have strengthened the conclusion that these particles fall into a relatively limited number of distinct classes, each of which exhibits a peculiar array of biochemical properties. Simultaneously, electron microscopy has greatly broadened our knowledge of the fundamental structural units of the intact cell. Correlation of these two fields of information is an obvious necessity.

Methods employed: The following methods, developed in this laboratory, have been employed for the observation and separation of groups of cytoplasmic particles from homogenates of tissue: (1) size distribution analyses of the particles based upon the rates of sedimentation of their specific biochemical characteristics (see the last annual report for description of method); (2) differential centrifugation according to schedules based upon the size distribution analyses; and (3) density gradient fractionation. In the last method, which is currently under investigation, a small volume of homogenate is layered over a previously prepared continuous sucrose density gradient, and the particles centrifuged (in the SW-39 rotor of the Spinco Model E ultracentrifuge) to their iso-density levels. Fractions obtained by any

of the above methods, and characterized biochemically, have been prepared for electron microscopy by fixation with osmic acid and embedding in methacrylate. Structures observed in thin sections of the fixed fractions were compared with those found in the whole homogenates and in the intact cells.

Results: As described in the last annual report, size distribution analyses of the cytoplasmic particles in rat liver homogenates had demonstrated three major classes of particles. Of these, one undoubtedly corresponded to the mitochondria. Electron microscopy has now revealed that the mitochondria are well preserved during the process of homogenization in 30% sucrose, a fact of some importance in interpreting the many results hitherto obtained by differential centrifugation. The nature of the other two groups of particles is not yet understood. One of these, rich in acid phosphatase and uricase, has been tentatively identified with elements of the ergastoplasm of the intact cell. There is some evidence that the other group, containing the non-mitochondrial DPN-cytochrome c reductase activity of the cell, may be made up at least in part by structures derived from the Golgi complex.

Considerable attention has been devoted to the question of the intracellular distribution of ribonucleic acid (RNA) in view of the mounting evidence for the participation of this material in protein synthesis. RNA has been found to be associated with all three of the above-mentioned particle groups in liver homogenates: about 10% with the mitochondria, 30% with the "acid phosphatase particles", and 50% with the group of smaller particles rich in DPN-cytochrome c reductase. The remainder is not sedimentable in 30% sucrose at the speeds attainable with the SW-39 rotor. Upon severe starvation, the RNA associated with the acid phosphatase particles disappears almost completely, while that of the smaller particles remains (it is known that starvation results in loss of the ergastoplasmic elements of the liver cell as examined in situ). In the very rapid growth situation provided by Strain L fibroblasts grown in tissue culture (by the method of Dr. Harry Eagle), preliminary studies have shown that most of the cytoplasmic RNA is in the non-sedimentable fraction.

The sedimentation characteristics of liver glycogen as it occurs in sucrose homogenates has also been studied. If a density of 1.55 gm./cm.^{3} is assumed, the relatively polydisperse glycogen particles show an average "molecular weight" of about 20,000,000, a value from 5 to 10 times those reported for liver glycogen isolated by standard chemical methods.

Significance to Cancer Research: It is felt that a combination of the techniques of cellular fractionation and electron microscopy will result in a considerable extension of our knowledge of the spatial and functional arrangement of intracellular materials. This information, particularly with regard to components such as ribonucleic acid, may be helpful in understanding the processes of cellular growth, and in particular, in differentiating between normal and abnormal mechanisms of protein synthesis. In addition, the ability to correlate appearance under the electron microscope with known biochemical properties would greatly facilitate the investigation of many situations not now accessible to study - for example, the very early stages of malignancy where changes might be restricted to too few cells to analyse by presently available methods.

Proposed course of the project: During the coming year it is proposed to investigate in more detail the sequence of events which occur at the time of disruption of cells during the preparation of homogenates. Preliminary observations of unbroken and partially broken cells in homogenates indicates that this approach may be the best means of determining the intracellular sites of origin of many now unidentified particulates. This aspect of the project will be carried out with the help of a new microtome for thin sectioning and in collaboration with Dr. F. S. Sjostrand who will be visiting here from the Karolinska Institute of Sweden.

With the more precise methods of cell fractionation now available, it it proposed to continue the study of the major ribonucleic acid containing fractions of normal cells and to compare them with the corresponding fractions derived from rapidly growing cells, both normal and malignant. An interesting situation is provided by the liver cells of animals which have been subjected to severe fasting. As mentioned above, the ergastoplasmic elements are lost from such cells, and there is a great reduction in the volume of cytoplasm. Upon refeeding, there is a rapid resynthesis of cytoplasm without cell division, together with characteristic evolution of the reforming ergastoplasm. It is proposed to compare the cytochemical changes occurring during this period with the findings in the more usual situations where growth is accompanied by cell division.

PROJECT REPORT FORM (Cont'd)

10. 402
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH ADMINISTRATION

REVIEW & APPROVAL TECHNICAL ASSISTANCE

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

Dr. F. S. Söstrand, Karolinska Institutet, Sweden

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE, (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. 402
SERIAL NO.

15.
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Hogeboom, G. H., and Kuff, E. L., Relation between cell structure and cell chemistry, Federation Proceedings 14: 633-638 (1955).

16.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

(a) G. H. Hogeboom: By invitation, addressed the Open Scientific Session of the Federation of American Societies for Experimental Biology, San Francisco, April, 1955. (See ref. 15)

(b) E. L. Kuff, by invitation, presented paper (with G. H. Hogeboom as co-author) at Fourth International Symposium on Enzymes: Units of Biological Structure & Function, on "Sedimentation and biochemical characteristics of cytoplasmic particulates." (To be published in Proceedings of the Symposium)

(c) G. H. Hogeboom acted as chairman of the Session on Enzymes and Cell Structure at the above-mentioned Fourth International Symposium held at the Henry Ford Hospital.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Cellular Biology Section — SECTION OR SERVICE
4. — LOCATION(IF OTHER THAN BETHESDA)
5. 404 — SER. NO.

6. Oxidative metabolism in various cellular structures of normal and tumor tissues. — PROJECT TITLE

7. Ruth K. Kielley — PRINCIPAL INVESTIGATOR(S)

8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Title: Oxidative metabolism in various cellular structures of normal and tumor tissues. Xanthine Oxidase.

Methods employed: Xanthine oxidase and DPNH dehydrogenase enzymes were isolated from the supernatant fraction of liver cells by protein fractionation methods described in a previous annual report. The behaviour of these enzymes in response to changes in environmental conditions was studied by quantitative measurement of their activities. Xanthine oxidase activity was measured spectrophotometrically by following the rate of uric acid formation from the oxidation of xanthine at 290 mu. DPNH dehydrogenase activity was also measured spectrophotometrically by following the decolorization rate of dichlorophenolindophenol at 600 mu. The extent to which these enzymes couple with various nitrogen compounds was determined by estimation of either the reduction products formed or the reactants disappearing. The methods were chemical, spectrophotometric or enzymatic depending upon the nature of the product analyzed. Molybdenum in trace amounts was determined by a special modification of the colorimetric thiocyanate method.

PROJECT DESCRIPTION (Cont.)

Objectives: (1) To obtain information on the mechanism of reduction of various nitrogen compounds by xanthine oxidase and by other as yet unidentified enzymes of liver and tumor and the relation of molybdenum to the reduction process, (2) to identify and characterize other enzymes in liver and tumor tissues which catalyze similar reduction reactions and (3) to study ways and means by which knowledge gained in these investigations can be applied to cancer problems.

Major Findings: It has been found that the xanthine oxidase of liver can be coupled to the reduction of various types of nitrogen compounds representing a wide range of oxidation-reduction levels. The mechanism of electron transfer to these nitrogen compounds appears to require molybdenum as a specific metal activator. Pyrophosphate inhibited these reductions indicating that the reduction process is metal catalyzed. It was shown that the molybdenum present in the enzyme is very tightly bound. All attempts to remove it reversibly have so far been unsuccessful. Further evidence that the reduction of nitro, nitroso and probably other related groups including the azo group proceeds through an intermediate step between the dehydrogenase and the final transfer of electrons to the N acceptor was obtained by the demonstration that the rate of reduction was not directly proportional to the total dehydrogenase activity (xanthine and DPNH). In the reaction of the enzyme with oxygen or dyes such as methylene blue or dichlorophenolindophenol on the other hand, the total dehydrogenase activity was the rate-limiting reaction. Examples of the types of nitrogen compounds reduced by liver xanthine oxidase and their relative reduction rates are as follows: Inorganic Series: nitrate 18, nitrite 24, hydroxylamine 54. Organic Series: p-nitrophenol 35, p-nitrosophenol 253, p-dimethylaminoazobenzene (DAB) 20, 2,5-dinitrophenol 108, 2,4-dinitrophenol 65, 2,6-dinitrophenol 50. In general, it may be said that for the oxidation-reduction levels they represent, the organic compounds were considerably more reactive than the inorganic. The azo group of DAB was significantly reduced although at a relatively slow rate. With the dinitrophenols, the position of the substituents in the ring greatly influenced the reactivity of the compound. 2,5-dinitrophenol containing nitro groups para with respect to each other was more active than its isomers.

Further studies on nitro and nitroso reductases of liver confirmed previous suggestions that a second enzyme was present in the supernatant fraction of liver cells. The enzyme was found to be a flavoprotein specifically catalyzing the oxidation of DPNH in the presence of suitable electron acceptors, but unlike the great bulk of diaphorase activity found in the liver, this flavoprotein has the

unique property of being able to couple with nitro and nitroso compounds (other N-containing groups have not yet been tested). Although xanthine was not oxidized by this enzyme, there is a suggestion that xanthine oxidase is closely associated with this second flavoprotein enzyme since the latter is released from crude xanthine oxidase preparations by heat treatment.

The effect of TSPA (N, N', N'' -triethylene thiophosphoramide), an anti-cancer agent, on the aerobic oxidase activity and on p-nitrosophenol reduction catalyzed by liver xanthine oxidase was tested to determine whether either or both of these electron transferring pathways of the enzyme might be affected. The results were entirely negative showing neither inhibition nor activation.

Preliminary work on the determination of xanthine oxidase levels in blood showed that the activity was limited to the plasma component and that it could be conveniently measured spectrophotometrically provided the sensitivity of the instrument were stepped up sufficiently with a photomultiplier. The feasibility of determining xanthine oxidase levels in blood was looked into because there is some interest in examining the blood levels in cancer patients.

Significance to Cancer: Xanthine oxidase as one of the principal enzymes of purine catabolism is of special interest in the cancer field because of its relation to nucleic acid synthesis and breakdown. Its presence in excess or absence, its inhibition or stimulation might possibly reflect on the chemistry of normal or abnormal growth. Haddow et al. found for example, that xanthopterin, a potent inhibitor of xanthine oxidase in vitro, often induced hypertrophy of the kidney in rodents. He also observed that xanthine oxidase concentrates from cow's milk when injected into mice with spontaneous mammary tumors produce apparently, anti-tumor effects.

Recent developments in the chemotherapy of cancer and leukemia with various purine analogs point to the importance of xanthine oxidase as one of the controlling factors in the effectiveness of the treatment. Dietrich and Shapiro found that flavotin, a riboflavin analog, potentiates the carcinostatic action of 8-azaguanine through inhibition of tumor xanthine oxidase (755 mammary carcinoma). This inhibition results in the accumulation of xanthine which in turn inhibits guanase, an enzyme destroying 8-azaguanine by deamination. The over-all effect of flavotin then, is apparently one of preventing the destruction of the carcinostatic purine. In this connection one

might consider the possibility of limiting the synthesis or activity of xanthine oxidase by antagonism of the molybdenum component rather than the flavin component of the enzyme. The toxicity experienced with flavotin may very well be due to inhibition of a large number of flavoprotein enzymes whose activities are more directly and vitally concerned with the energy metabolism of cells than is xanthine oxidase. As far as it is known, the only flavoproteins containing molybdenum in mammalian tissues are xanthine oxidase and possibly aldehyde oxidase.

Another aspect of the relationship of xanthine oxidase to cancer is the ability of the enzyme to reduce the azo group of carcinogens. After the results on the reduction of DAB were reported, it was learned that similar observations were being made at the Chester Beatty Cancer Institute where an extensive program of research on xanthine oxidase is being conducted. Furthermore, their observations indicate that there is an inverse relationship between rate of reduction and carcinogenicity (private communications). Mueller and Miller have shown that DAB is also reduced by an enzyme in liver microsomes, presumably TPN-cytochrome c reductase, a flavoprotein. It is highly probable that other metallo-flavoproteins of the xanthine oxidase type such as liver aldehyde oxidase and the DPNH dehydrogenase described in this report can also participate in reductive detoxications of azo carcinogens. The relationship between carcinogenesis caused by DAB and the riboflavin level of the diet becomes clearer when it is learned that the enzymes involved in the reduction of DAB, as far as we know, are flavoproteins.

Proposed research: On the hypothesis that tumor growth may be influenced by changes in the balance and activities of critical enzymes in these tissues, it is proposed that investigations be made on the effect of mimosine, an unnatural amino acid, on animals bearing spontaneous tumors. Studies on the physiological effects of this compound are limited to observations on rats in which the symptoms were those of a deficiency disease including marked alopecia, diminished growth rate and in many cases of long duration, cataract. The mode of action in terms of enzyme systems affected is unknown, but on the basis of its known structure (3-hydroxy, 4-Oxy, (4H), 1-pyridine alanine), several possibilities can be considered. The most likely sites of antagonism would appear to be in enzymatic reactions in which pyridoxine is implicated. There is already work by Dietrich and Shapiro on the carcinostatic properties of deoxypyridoxine, a pyridoxine analog and antagonist. Other possibilities are that mimosine may interfere with protein synthesis or amino acid metabolism involving phenylalanine or tyrosine. At the present time, arrangements are being made with Dr. Evan Horning to obtain the compound in quantity from imported plant material.

PROGRESS DESCRIPTION (Cont.)

Some investigations on the xanthine oxidase levels in blood plasma of normal, non-cancerous and cancerous subjects are being considered in collaboration with Dr. F. M. Kalckar. The purpose is to determine whether xanthine oxidase levels can be correlated with the degree of differentiation typical of the tumor.

From the standpoint of cellular structure and function, it is of some interest to know whether enzymes occurring in one part of the cell can be coupled to enzymes in another structure of the cell. In the intact cell where enzymes are acting in heterogeneous systems, such a possibility does not seem to be out of reason. The xanthine oxidase system would appear to be an appropriate model for experimentally testing out such a possibility. There is evidence that in mammalian tissues, "xanthine ocidase" consists of two components, a dehydrogenase and a terminal oxidase system. The purified xanthine oxidase of rat liver is an enzyme no longer associated with its natural terminal oxidase system, but in the process of separation and purification, has become an oxidase reacting directly with atmospheric oxygen. The nature of the natural terminal oxidase system of xanthine oxidase has not been determined. It would be of great interest and significance if it were localized in the mitochondria for the linkage would then provide a possible means for the utilization of a large amount of energy resulting from the oxidation of xanthine and hypoxanthine for useful cellular work through the mechanism of oxidative phosphorylation. With these ideas in mind, it is proposed that experiments be tried to determine whether enzymatic coupling can be achieved between the xanthine dehydrogenase of the supernatant fraction and the electron transport mechanism in mitochondria.

PROJECT REPORT FORM (Cont'd)

10. 404
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH X ADMINISTRATION

REVIEW & APPROVAL TECHNICAL ASSISTANCE

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 404
SERIAL NO.

15.
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Kielley, R. K., Purification of liver xanthine oxidase. Federation Proc., 14: 235 (1955).

Kielley, R. K., Purification of liver xanthine oxidase. J. Biol. Chem., 216: 405 (1955).

Kielley, R. K., Reduction of 2,4-dinitrophenol by liver xanthine oxidase. Third International Congress of Biochemistry, page 55 (1955).

16.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Cellular Biology Section — SECTION OR SERVICE
4. — LOCATION(IF OTHER THAN BETH.)
5 406 — SER.NO.

6. Electron microscopy and phase contrast microscopy of normal and malignant cells — PROJECT TITLE

7. A. J. Dalton and Marie D. Felix — PRINCIPAL INVESTIGATOR(S)

8. J. Bronte Gatenby, Harry Eagle, Clifford Grobstein and W. Ray Bryan — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A comparative study of the Golgi complex with the electron microscope

The objective of this project is to determine the essential variations in the fine structure of the Golgi complex in representative cell types in several phyla of the animal kingdom.

The methods employed are fairly well standardized now - fixation in chrome-osmium, double embedding in formol-agar and n-butyl methacrylate followed by thin sectioning for study with the electron microscope.

The major findings have been the demonstration that (1) the double membranes and small vesicle components of the Golgi comples were regularly present in all vertebrate cell types studied but the large vacuolar component occurred regularly only in highly specialized cell types. (2) The fine structure of the Golgi complex of vertebrate and invertebrate cells is basically similar, the double membrane systems and the small vesicle component being present in both. (3) The walls of contractile vacuoles of protozoa and sponges possess double membrane systems and small vesicles similar morphologically to the Golgi complex of vertebrate and invertebrate cells.

PROJECT DESCRIPTION (Cont.)

The significance of these findings is related to the over-all concept which activates much of the work of the unit - the concept that in order to understand the mechanisms involved in the development of neoplastic cells, much greater knowledge of the structure and function of normal cells is needed. The results obtained from this study suggest first that the Golgi complex is universally present in animal cells and second that the Golgi complex is possibly involved in control of water balance in cells generally since its structure is so similar to that of the walls of the contractile vacuole of many protozoa.

It is proposed to study a series of somatic cells of invertebrates and the contractile vacuoles of several more protozoa to make the above generalizations somewhat more secure.

A comparison of normal and malignant cells with the electron microscope

The object of this project was to determine whether any significant differences in the fine structure of normal and neoplastic cells could be detected.

The major findings have been that while variations in the amount of various cell components were noted, neoplastic cells, as might be expected possess all the essential components of normal cells. The fine structure of chromatin, nucleoplasm and nucleolus was found to be the same in both. Both normal and neoplastic cells possess ergastoplasm in variable amounts but there was a tendency in what might be termed anaplastic tumor cells for the small granule component of cytoplasm (thought to be responsible for cytoplasmic basophilia) to lie free in the cytoplasm rather than to be associated with the membranes of the ergastoplasm (the usual situation in normal cells). The Golgi complex in tumor cell types which retain some degree of function possesses all three components, (double membrane system, small vesicles and large vacuoles) but the Golgi complex of more anaplastic tumor cell types possessed only the double membrane system and small vesicles.

While it was not expected that a significant qualitative difference between normal and neoplastic cells would be found, such a study was indicated since if such a difference were found it would be of considerable importance in diagnosis. If a real and consistent correlation is found between variations in the fine structure of the ergastoplasm and Golgi complex and the degree of anaplasia of malignant, such a correlation could eventually be of some value to the cytopathologist in diagnosis.

In the future it is proposed to study a series of tumor cell types of varying degree of anaplasia to determine whether the variations described above are consistently associated in a general way with the degree of anaplasia.

PROJECT DESCRIPTION (Cont.)

Phase contrast and electron microscopy of tumor cells developing nutritional deficiencies in tissue culture

The objective of this project is to determine whether there are any specific alterations in the fine structure of tumor cells during the development of a deficiency state resulting from the absence of a specific amino acid or vitamin.

In addition to the usual methods for electron microscopy of tissue sections, a method of double embedding, first in formol-agar and then in methacrylate, was developed. This double embedding method makes possible the accurate orientation of cells, tissues and organs and also greatly improves the preservation of cells on free surfaces and in tissue culture. During the development of the deficiency, living cells have been studied with phase contrast and comparable cells preserved for study with the electron microscope.

The major findings to date are: (1) Modification of the fine structure of cells appear to be specifically related to a deficiency in a particular amino acid. For example, changes in mitochondria and the ergastoplasm occur in tyrosine deficiency which have not been noted in dying cells in control cultures nor in cells developing glutamine or folic acid deficiency. Cells developing tyrosine deficiency have been studied in detail but the studies on glutamine, folic acid and glocuse deficiency are still in progress.

The significance of these results, which are meager at present, is in the suggestion that specific changes may occur under these conditions and that these changes may be pinpointed in relation to biochemical and radio-isotope analyses. While these changes may in many cases be an "end result" and thus not necessarily indicators of the site of utilization or metabolism of a particular amino acid within a tumor cell, the very real possibility remains that the combination of electron microscopic, biochemical and radio-isotope studies may eventually give us this information.

It is proposed to continue this study with a more detailed analysis of the changes induced by glutamine, folic acid and glucose deficiencies. This material is already fixed and embedded but not sectioned or examined.

PROJECT DESCRIPTION (Cont.)

A study of the cells native to the peritoneal fluid

The objective of this project is to develop a greater understanding of the structure, function and origin of cells native to the peritoneal fluid.

In this study living cells were examined with the phase contrast microscope both as an aid to identification and as a prerequisite for study with the electron microscope.

The major findings have been that the primary cell components of the peritoneal fluid are lymphocytes and macrophages. The introduction of foreign particulate matter to the peritoneal cavity is followed by an early rise in neutrophilic granulocytes which is followed in turn by a more prolonged rise in the number of macrophages. Under these conditions numerous transitions between lymphocytes and macrophages were found. There was no evidence that mesothelial cells transformed into macrophages. Neither was these any evidence that mesothelial cells contribute significantly to the cell population of the peritoneal fluid.

There has recently been a great increase in the number of papers published which described work using ascites tumors as tools in cancer research. It is surprising that in none of these has any significant attention been paid to the cells native to the peritoneal fluid. On the contrary, the idea that the majority of these cells are derivatives of mesothelium appears to have been accepted by many investigators in spite of the lack of evidence for such a derivation. In the study of the development of ascites tumors and in the associated problem of identification of tumor cells, the information obtainable by the application of the newer methods is important. Our work has made a beginning in this direction.

It is proposed to develop this project further by determining the reaction of normal cells of the peritoneal fluid to the introduction of tumor cells and to study the early stages in the development of ascites tumors, with both the phase contrast and electron microscopes.

Other projects involving electron microscopy but which will be covered in detail by the investigators responsible for preparing the materials are:

(a) Electron microscopy of tissue fractions. Drs. Hogeboom and Kuff.

(b) Electron microscopy of the process of embryonic induction. Dr. Grobstein

(c) Electron microscopy of the Rous tumor. Dr. Ray Bryan

10. 406
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH X	ADMINISTRATION
REVIEW & APPROVAL	TECHNICAL ASSISTANCE

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

In cooperation with Prof. J.Bronte Gatenby, University Zoological Department, Trinity College, Dublin, Ireland.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 406
SERIAL NO.

15.
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Gatenby, J. Bronte, Dalton, A. J. and Felix, Marie D.: The contractile vacuole of parazoa and protozoa and the Golgi apparatus. Nature, 176: 301 (1955).

Dalton, A. J.: A chrome-osmium fixative for electron microscopy. Anat. Rec. 121: 281 (1955). (Note. While this is of abstract length it is the definitive article on this subject).

Felix, Marie D. and Dalton, A. J.: A phase-contrast microscope study of free cells native to the peritoneal fluid of DBA/2 mice. J. Nat. Cancer Inst. 16: 415-445 (1955).

16.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Invited to take part in a symposium on "Mitochondria and other cell inclusions" sponsored by the British Society of Experimental Biology at Oxford, England, Sept. 19-23. At this symposium I was asked to serve as chairman of a section on the Golgi apparatus.

Invited to take part in Symposium on "Cancer Cytology and Cytochemistry" sponsored jointly by the N. Y. Academy of Sciences and the Damon Runyon Memorial Fund for Cancer Research.

Invited to serve as chairman of section on cytology (electron microscopy) American Association of Anatomists Annual Meeting, Philadelphia, 1955.

Invited to take part in symposium on cancer research, Harvard Univ. Medical School, Harwich, Mass., June, 1955.

PROJECT REPORT

1. National Cancer Institute 2. Laboratory of Biology

3. Cellular Biology Section 4. 5. 407
SECTION OR SERVICE LOCATION(IF OTHER THAN BETH.) SER. NO.

6. Precursors of Deoxyribonucleic Acid (DNA) in normal, Regenerating and Tumor Tissues.
PROJECT TITLE

7. Walter C. Schneider
PRINCIPAL INVESTIGATOR(S)

8. Mrs. Leona W. Brownell

9. PROJECT DESCRIPTION

Objective: To determine whether precursors of DNA exist in normal and tumor tissues and if so, to isolate and identify the compounds present and determine the metabolic pathways in which they are intermediates.

Methods: Tissues, obtained under anesthesia and frozen immediately in liquid nitrogen, are extracted with ice cold perchloric acid. Deoxyribosidic compounds are isolated from such extracts using both ion exchange and paper chromatography. The compounds are detected, identified and quantitated with the organism lactobacillus acidophilus R-26 used in conjunction with spectrophotometric and chemical tests.

Major findings: Using more refined methods of isolation it has been possible to show that deoxycytidine is present in rat liver and accounts for essentially the entire deoxyribosidic activity of extracts of this tissue.

In contrast, extracts of the Novikoff hepatoma showed three striking differences in the type of deoxyribose compounds they contained. The deoxyriboside fraction accounted for only about 50 per cent of the microbiological activity of extracts of this tissue and contained, in addition to deoxycytidine deoxyuridine and thymidine. The remainder of the microbiological activity has not yet been identified but, judging from its behavior on ion exchange resins, probably contains either nucleotides, small polynucleotides, or phosphorylated derivatives of nucleotides.

PROJECT DESCRIPTION (Cont.)

Studies of rat liver undergoing regeneration have shown that the total amount of deoxyribosidic compounds present is approximately doubled as early as 24 hours after partial hepatectomy. Furthermore, in contrast to normal liver 25-50 per cent of the total deoxyribosidic compounds appear to be the unidentified nucleotide type.

Significance to cancer research: The fundamental problem of cancer research is to determine why cancer cells are able to multiply without restriction whereas the multiplication of normal cells occurs at a controlled rate. Deoxyribonucleic acid (DNA), more than any other cellular constituent now known, must be intimately involved in the solution of these problems for several reasons. In the first place, DNA is not only a major component of the chromosomes, but also occurs in the cell in a constant amount directly proportional to the number of chromosomes present. Furthermore, before cell division can occur, the entire DNA of the cell must be reduplicated to insure that each daughter cell contains the full complement of DNA. In addition, DNA alone or in combination with protein has been found capable of transforming cells of one genotype to another and even of inducing cancer.

The fact that DNA occurs in such constant amounts and is capable of inducing heritable changes makes it of the greatest importance to determine how this compound is formed in the cell, in terms of the precursor compounds and enzymes involved. Until such information is obtained, attempts at blocking DNA synthesis, and hence at controlling cell division, must be entirely haphazard. The results obtained so far in this project represent a considerable advance toward the achievement of this goal since they demonstrate that specific nucleosides, hitherto unrecognized as precursors of DNA, are widely distributed as tissue constituents. Since these compounds are qualitatively different in the normal and tumor tissues, the synthesis of DNA in the latter may proceed along a different pathway. If this proves to be the case, the possibilities of controlling cell multiplication in tumors would be greatly increased. The continued study of DNA precursors by means of their isolation and identification as well as by the elucidation, through enzymatic and isotopic methods, of the metabolic pathways involved is consequently of utmost importance for cancer research.

Proposed course: The identification of the nucleotide like compounds will receive the greatest emphasis during the ensuing year. A survey of the occurrence of these compounds in various tissues is also planned and it is hoped that it will also be possible to begin work on the metabolism of the deoxyribose compounds.

10. 407
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH X	ADMINISTRATION
REVIEW & APPROVAL	TECHNICAL ASSISTANCE

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 407
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

1. Schneider, W. C.: Deoxyribosides in animal tissues. J. Biol. Chem. 216, 287 (1955).

2. Hogeboom, G. H. and Schneider, W. C.: The Cytoplasm. In The Nucleic Acids, Vol. 2. Edited by Chargoff and Davidson, New York, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Laboratory of Biology (LABORATORY OR BRANCH)

3. Cellular Biology Section (SECTION OR SERVICE) 4. (LOCATION(IF OTHER THAN BETHESDA)) 5. 408 (SER.NO.)

6. Studies on the morphology and physiology of two lymphocytic tumors
PROJECT TITLE

7. Emma Shelton
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To correlate differences in the morphology and physiology of two lymphocytic tumors with the differences in their behavior in the host.

Methods: In order to test the ability of the cells of lymphocytic ascites tumors to pass through membranes of known pore size, measured numbers of cells are sandwiched between two pieces of Schleicher and Schuell membrane filter. The cells are enclosed by sealing the membrane filters to a circle of lucite. The sandwich is placed in the peritoneal cavity of a mouse. By making biopsy punctures of the abdomen and counting differentially the cells in the ascites fluid it is possible to tell with considerable accuracy the time at which the cells escape from the chambers.

Major Findings:

1. Cells of Lymphoma #2 (diploid tumor, highly invasive) survived and grew in chambers (10 mice) for a period of 30 days. The cells did not escape from the chambers. When the contents of these chambers were injected subcutaneously into mice, tumors were produced in every case. Similar results were obtained when chambers were placed in 17 additional mice for periods ranging from 2 to 28 days.

PROJECT DESCRIPTION (Con't.)

2. Cells of Lymphoma #1 (tetraploid, relatively non-invasive) escaped from the chambers and produced ascites tumors in four out of a group of 10 mice. In five of the remaining mice killed after 35 days, a few viable cells were present in the chambers. Inoculation of the chamber contents has produced a subcutaneous tumor in the host mice in only two cases. In the last mouse, killed aftef 59 days, no viable tumor cells were observed in the chamber and no tumor has appeared at the site of the inoculation of the chamber contents.

The final outcome of the project cannot be foreseen as yet, since the results are not clear-cut. The question of leaks in the chambers due to incomplete sealing of the filter is raised. The indications are that the cells of L#2 have a greater capacity to survive and multiply in the chambers than do the cells of L#1.

Significance to cancer research: The nature of the invasive properties of tumor cells is little understood. These experiments are being carried out in the hope that they will shed some light on this phenomenon.

Proposed course: Additional experiments with chambers constructed of membranes of graded porosity are under way. It is planned to apply this technique to a greater variety of ascites tumors than the two mentioned above.

Cinematographic studies on the locomotion of these cells in vitro are planned.

PROJECT REPORT FORM (Cont'd)

10. 408
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH X ADMINISTRATION

REVIEW & APPROVAL TECHNICAL ASSISTANCE

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Cellular Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. 408 — SER. NO.

6. Studies on the morphology and physiology of two lymphocytic tumors — PROJECT TITLE

7. Emma Shelton — PRINCIPAL INVESTIGATOR(S)

8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To correlate differences in the morphology and physiology of two lymphocytic tumors with the differences in their behavior in the host.

Methods: In order to test the ability of the cells of lymphocytic ascites tumors to pass through membranes of known pore size, measured numbers of cells are sandwiched between two pieces of Schleicher and Schuell membrane filter. The cells are enclosed by sealing the membrane filters to a circle of lucite. The sandwich is placed in the peritoneal cavity of a mouse. By making biopsy punctures of the abdomen and counting differentially the cells in the ascites fluid it is possible to tell with considerable accuracy the time at which the cells escape from the chambers.

Major Findings:

1. Cells of Lymphoma #2 (diploid tumor, highly invasive) survived and grew in chambers (10 mice) for a period of 50 days. The cells did not escape from the chambers. When the contents of these chambers were injected subcutaneously into mice, tumors were produced in every case. Similar results were obtained when chambers were placed in 17 additional mice for periods ranging from 2 to 28 days.

PROJECT DESCRIPTION (Con't.)

2. Cells of Lymphoma #1 (tetraploid, relatively non-invasive) escaped from the chambers and produced ascites tumors in four out of a group of 10 mice. In five of the remaining mice killed after 35 days, a few viable cells were present in the chambers. Inoculation of the chamber contents has produced a subcutaneous tumor in the host mice in only two cases. In the last mouse, killed aftef 59 days, no viable tumor cells were observed in the chamber and no tumor has appeared at the site of the inoculation of the chamber contents.

The final outcome of the project cannot be foreseen as yet, since the results are not clear-cut. The question of leaks in the chambers due to incomplete sealing of the filter is raised. The indications are that the cells of L#2 have a greater capacity to survive and multiply in the chambers than do the cells of L#1.

Significance to cancer research: The nature of the invasive properties of tumor cells is little understood. These experiments are being carried out in the hope that they will shed some light on this phenomenon.

Proposed course: Additional experiments with chambers constructed of membranes of graded porosity are under way. It is planned to apply this technique to a greater variety of ascites tumors than the two mentioned above.

Cinematographic studies on the locomotion of these cells in vitro are planned.

PROJECT REPORT FORM (Cont'd)

10. 408
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		TECHNICAL ASSISTANCE	

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

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NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Cellular Biology Section — SECTION OR SERVICE
4. — LOCATION(IF OTHER THAN BETHESDA)
5. 409 — SER. NO.

6. Studies on liver carcinogenesis in the mouse — PROJECT TITLE

7. Emma Shelton — PRINCIPAL INVESTIGATOR(S)

8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To study the changes occurring in the parenchymal cells of the liver of the mouse prior to the formation of a tumor, and to develop methods for studying these changes.

Methods: Nutritional, biochemical, histochemical

Major findings: A study was made of the tetrazolium method for measuring succinic dehydrogenase in order to answer the questions:

1. Do tetrazolium salts accept electrons directly from succinic dehydrogenase?

2. How sensitive is the tetrazolium method in comparison with oxygen uptake as a measure of enzyme activity?

3. Can the histochemical tetrazolium method be used as a reliable quantitative test for succinic dehydrogenase?

It was found that:

1. Since tetrazolium reeuction is inhibited by antimycin, the tetrazolium salts do not accept electrons directly from succinic dehydrogenase but require the mediation of cytochrome b.

2. The tetrazolium method is a relatively insensitive method for measuring succinic dehydrogenase, being in the neighborhood of twenty-five times less than oxygen uptake.

3. In spite of the lack of sensitivity of the method and the place in the enzymatic chain of electron transfer where the enzymatic activity is measured, ratios of activity of liver to kidney to brain are identical using both tetrazolium and oxygen uptake. Thus the tetrazolium method can be used as a valid and accurate quantitative measure of succinic dehydrogenase.

The very small tumors appearing in the livers of mice fed o-aminoazotoluene have a higher succinic dehydrogenase activity than do the cells of the surrounding parenchyma as measured by both the tetrazolium method and by oxygen uptake.

Significance to cancer research: In order to study the earliest changes taking place in livers undergoing the malignant alteration, histochemical methods of known accuracy must be developed. In future experiments, the tetrazolium method can be relied upon to give accurate estimates of the succinic dehydrogenase activity of cells at the microscopic level.

Proposed course of project: Since the strain A mouse is a poor breeder, and is thus not too readily available for use, experiments are under way to ascertain whether a high percentage of tumors may by produced in the more readily available strain C mouse.

A quantitative cytological and histochemical study of the early stages of tumor formation in the livers of these mice is planned.

PROJECT REPORT FORM (Cont'd)

10. 409
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		TECHNICAL ASSISTANCE	

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
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14. 409
SERIAL NO.

15.
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Shelton, E.: Hepatomas in mice. I. Factors affecting the rapid induction of a high incidence of hepatomas by o-aminoazotoluene. J. Nat. Cancer Inst. 16: 107-127, 1955.

16.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Laboratory of Biology (LABORATORY OR BRANCH)

3. Cellular Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION(IF OTHER THAN BETHESDA)) 5. 410 (SER.NO.)

6. Transparent Chamber and Diffusion Chamber Research (PROJECT TITLE)

7. Glenn H. Algire (PRINCIPAL INVESTIGATOR(S))

8. Doctors C. C. Congdon, V. J. Evans, L. W. Law, R. M.Merwin and J.M. Weaver

9. PROJECT DESCRIPTION

No. 1

Research program in Transparent Chamber Project

Problem: Developments in Transparent Chamber Methods

Objectives: To modify transparent chamber designs to attain increased simplicity of construction, and longer useful duration of the chambers.

Methods employed: Transparent chambers are constructed about an internal splint of plastic which provides better support for the skin fold than earlier designs of chambers.

Major findings: These new forms of internal splint chambers are being utilized in current work, in order to determine their applicability to problems in which observations must be made over periods of several months.

Significance: It is hoped to extend the useful duration of transparent chambers, so that observations of cellular and vascular reactions to carcinogens may be carried on for longer periods of time than has been possible heretofore.

Proposed course: To utilize these chambers in long term studies of cellular and vascular reactions to chemical carcinogens.

PROJECT DESCRIPTION (Cont.)

No. 2

Problem: Factors affecting the survival of heterografts in diffusion chambers.

Objectives: From preliminary experiments reported last year, it was found that human (HeLa) cells survived for at least 50 days in diffusion chambers in mice, but after two weeks extensive areas of cellular degeneration were found. Current studies are directed toward a study of immune factors in the survival and growth of human cells in diffusion chambers. Dr. Virginia Evans has furnished the cultures of HeLa cells used in these studies.

Methods: Human cells (HeLa strain) from tissue culture were placed in diffusion chambers using filters of estimated porosity 0.4 microns. Observations were made either in vivo using transparent chamber methods, or as fixed and stained specimens after intraperitoneal implantation and later removal.

Major findings: HeLa strain of human cells proliferate rapidly in diffusion chambers in normal mice for about 2 weeks, after which time areas of cellular degeneration are noted, but many surviving cells are still present for at least 50 days. Extensive destruction of HeLa cells occurs within 7 days if placed within diffusion chambers in mice which have been immunized by subcutaneous inoculation of this cell strain, two weeks prior to implantation of the diffusion chambers, indicating that circulating antibodies pass through the filters.

Significance to Cancer Research: This is a study of the reaction of an animal to grafts of human cells, and the possibility of eliminating destructive effects in order to obtain growth of human tumors in experimental animals. Possible application to diagnostic problems of cancer will be considered, as well as studies on the mechanism of action of drugs capable of affecting tumor cells.

Proposed course: Experiments will be undertaken on the effect of the porosity of membranes on survival of heterografts in diffusion chambers. This will be done to determine whether it would be possible to find a pore size that would allow cell growth, but would not allow antigenic material to escape from the chamber and immunize the host. Will it be possible to obtain prolonged survival and growth of human cells in diffusion chambers by blocking the escape of antigenic material?

The studies will be extended to other cells of human origin, both normal and cancer.

PROJECT DESCRIPTION (Cont.)

No. 3

Problem: Mechanism of action of Chemotherapeutic Drugs on Tumor Cells.

Objectives: To determine the mechanism of action of various tumor-damaging drugs on tumors in terms of their circulatory effects, or direct cytotoxic properties.

Methods employed: Studies of the reaction of tumor cells to injected drugs were made under three conditions.

(1) Using diffusion chambers in which implanted cells are isolated from contact with host tissues by porous membranes;

(2) Solid vascularized tumors where circulatory effects may be involved;

(3) Solid vascularized tumors, as influenced by injected drugs, plus procedures to alter the circulatory response.

Major findings: Mouse sarcoma cells growing in diffusion chambers placed intraperitoneally are not affected by bacterial polysaccharide injected intraperitoneally. Subcutaneous injection of podophyllotoxin results in mitotic arrest without extensive necrosis of cells growing in diffusion chambers in the absence of blood supply. This result is in contrast to the hemorrhage and necrosis which occurs in solid vascularized tumors following injection of either of these drugs.

Preliminary evidence has been obtained that increased blood flow caused by local warming of tissue may prevent the tumor-damaging action of podophyllotoxin.

Significance to Cancer Research: Evaluation of therapeutic agents in cancer depends upon understanding their mode of action. Transparent chamber methods for microscopid observations in living mice, supplemented by diffusion chamber procedures, help to determine whether an agent acts directly upon the living cell or indirectly through its effects on the circulation of the host animal.

Proposed Course of Project:

1. To apply the diffusion chamber methods to studies of the effects of various chemotherapeutic drugs on cells isolated from host tissues with particular reference to leukemia and to human tumors.

2. To continue studies on the role of circulatory responses in the action of tumor-damaging agents.

PROJECT DESCRIPTION (Cont.)

No. 4

Problem: Mechanism of radiation protection by bone marrow.

Objectives: Lorenz and coworkers have shown that injections of isologous or homologous bone marrow will protect mice against amounts of X-radiation which are lethal to uninjected animals. The mechanism of protection is not clear. One hypothesis is that the injected bone marrow cells survive and proliferate to take over the function of the marrow of the irradiated mice; another, that protection results from some as yet unidentified humoral factor. The object of these experiments was to determine whether radiation protection can be brought about by bone marrow cells enclosed within diffusion chambers. A positive result would support the humoral theory; a negative result would be in accord with the cellular repopulation theory. This work was done in cooperation with Doctor C. C. Congdon.

Methods: Determination of the minimal effective number of marrow cells necessary for protection when given intraperitoneally; inclusion of this number of cells in diffusion chambers (and multiples of this dosage) for intraperitoneal implantation into irradiated mice and determination of the effect on survival as compared with controls.

Major findings: It has been found that marrow cells grow within diffusion chambers which are placed intraperitoneally. The dosage required for protection when marrow cells are injected directly into the peritoneal cavity has been found by Congdon to be approximately 5,000,000 cells. This number of cells and twice this population enclosed within diffusion chambers, were placed intraperitoneally in previously irradiated mice. The results were negative, that is, no protection was afforded by bone marrow cells enclosed within diffusion chambers and placed intraperitoneally. In following up this negative result, the volume of fluid in which the cells were suspended proved to be an important factor, since Congdon found that protection was not obtained after i.p. injection if the cells were suspended in the same volume of fluid (.02 cc) as used (necessarily) in diffusion chambers.

Significance to Cancer Research: Studies on radiation protection are of importance in enhancing the value of X-radiation as a therapeutic tool in cancer.

Proposed Course: This approach has been discontinued.

PROJECT DESCRIPTION (Cont.)

No. 5

Problem: Invasiveness of leukemia.

Objectives: Mouse leukemia cells differ greatly in their capacity to invade the tissues of the host. Studies were undertaken to determine whether this property is correlated with the ability of these cells to pass through filters of graded porosity. Parallel studies of the migratory behavior of leukemic cells are being carried out using time-lapse motion pictures.

Methods: Fragments of leukemia 1210 were placed in diffusion chambers formed from filters ranging from 4 millimicrons to 0.4 microns average pore diameter. Biological and histologic studies were made to determine the fate of the cells. Similar studies are in progress using Leukemia 5178, a less invasive neoplasm. These tumors are provided by Doctor Lloyd Law.

Major Findings: In the systems so far tested, it has not yet been possible to consistently prevent the development of leukemia in the hosts. Current studies are devoted to finding out whether leukemic cells pass through the pores of the filters or through the seal uniting the parts of the chamber. Alternative explanations for these results are possible and are being tested.

Significance for Cancer Research: These studies are concerned with factors which promote the spread of cancer cells in their invasion of normal tissues. Results of such experiments, using a series of filters of graded porosity and leukemias of diverse invasive properties, will contribute to an understanding of the mode of transmission of leukemia through membranes, and to a characterization of membranes in terms of their properties in a biological system.

Proposed Course: Studies will be continued as described above.

PROJECT DESCRIPTION (Cont.)

No. 6

Problem: Studies in carcinogenesis using diffusion chamber methods.

Objective: The diffusion chamber method for "tissue culture in vivo" provides a new approach to certain problems of chemical carcinogenesis. Earle and associates have found that normal adult fibroblasts grown in tissue culture in a completely heterologous media in the absence of deliberately added MCA give rise to sarcomas when inoculated into mice. One possible explanation for these results has been "growth in the heterologous media." This explanation would be ruled out if normal or embryonic mouse tissue underwent a malignant change when enclosed within diffusion chambers.

Goldblatt, following experiments and theories of Warburg (1927), has presented evidence that normal fibroblasts in tissue culture undergo a malignant change if subjected to conditions of intermittent anoxia. Through selecting filters of increasing thickness, and of consequent greater diffusion distance, one could study the effect of this variable on neoplastic change.

The immediate objective is to ascertain whether adult or embryonic subcutaneous tissue enclosed within diffusion chambers undergo neoplastic transformation.

Methods employed:

1. To determine whether the materials (plexiglas II, and Millipore filters) are carcinogenic for mice - (by intraperitoneal and subcutaneous implantation).

2. Fragments of muscle from young mice (strain C3H) were enclosed within diffusion chambers and placed intraperitoneally into adult hosts of the same strain. After 6 months these were reimplanted (open) into new hosts which had received 400-425 r total body irradiation. Another series will be done after 12 months. In other experiments, subcultures were made each month for 6 months before testing for neoplastic transformation.

Major findings: It has been found that fragments of muscle from young mice produce a vigorous outgrowth of connective tissue cells, with numerous dividing cells. Adult connective tissue cells also migrate out in diffusion chambers.

PROJECT REPORT FORM (Cont'd)

10. 410
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		TECHNICAL ASSISTANCE	

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT DESCRIPTION (Cont.)

Normal connective tissue cells have been carried in diffusion chambers in mice for 6 months. Reimplantation (open) into irradiated hosts has not led to the production of tumors.

Normal connective tissue cells have been carried in diffusion chambers with subculture at intervals of 1 month, and biological tests were made for the development of malignancy at the end of 6 months. The results have been negative.

No mice have developed tumors as a result of subcutaneous implantation of materials used in making diffusion chambers. These experiments are not yet completed.

Significance to Cancer Research: Increased understanding of factors involved in the transformation of normal to neoplastic cells is of fundamental importance in cancer research.

Proposed course of project: This experiment is still in progress. Various chemical carcinogens and related but inactive compounds will be used in studies of the effects on cells and organs using diffusion chambers.

14. 410
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Weaver, J. M., Algire, G. H., and Prehn, R. T.: The growth of cells in vivo in diffusion chambers. II. The role of cells in the destruction of homografts in mice. J. Nat. Cancer Inst. 15: 1737-1767 (1955).

Algire, G. H., and Merwin, R. M.: Vascular patterns in tissues and grafts within transparent chambers in mice. Angiology 6: 311-318 (1955).

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT

National Cancer Institute 2. Laboratory of Biology
INSTITUTE LABORATORY OR BRANCH

Cellular Biology Section 4. 5. 414
SECTION OR SERVICE LOCATION (IF OTHER THAN BETHESDA) SERIAL NO

Transparent Chamber Research
PROJECT TITLE

Ruth M. Merwin
PRINCIPAL INVESTIGATOR(S)

Dr. C. C. Congdon, Dr. K. K. Sanford and Dr. G. H. Algire
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Problem #1. Fate of homologous bone marrow cells injected intravenously into irradiated mice. Ruth Merwin and Charles C. Congdon

Objective; To study the mechanism by which marrow injected intravenously protects mice that have been given whole body irradiation. It has not yet been proven whether the introduced cells repopulate the tissues of the irradiated animals or whether the injected suspension provides some substance that enables the irradiated cells to recuperate. If cells from an animal not genetically like the irradiated one were injected and if one could identify these introduced cells and estimate their numbers it should be possible to find out whether the injected cells repopulate the marrow or whether the introduced cells only tide the animal over until its own cells can divide.

Method: Mice of the LAF strain were irradiated with 900 r and each was protected from this otherwise lethal dose by an intravenous injection of homologous marrow from a mouse of the C3H strain. At various intervals thereafter the marrows of the protected LAF mice were tested to see if homologous cells were present. The marrows were injected into LAF mice each of which carried a nonvascularized homograft of Harderian gland tissue from a C3H mouse. Nonvascularized homograft do not initiate immunity in the LAF host but if the injected marrow being tested contained homologous cells immunity would be initiated in the host and would cause the graft to disintegrate. The percentage of the grafts disintegrating and the interval before disintegration provided a means of determining whether the injected cells increased in number.

Major findings: All of the marrows taken from the protected LAF mice whether they were taken within 6 hours or not until 150 days after irradiation and protection showed the presence of homologous cells except about a third of those taken within 4 days.

An increase in the number of these homologous cells was indicated by 12 days after injection, and no decrease in number seemed to take place before 150 days, the longest interval after which the marrows were tested.

A few tests of the blood, spleen, lymph nodes, and thymus of the protected mice indicated that homologous cells were present in them also.

Significance: It is important to understand the mechanism by which injections of bone marrow protect animals that have been given an otherwise lethal dose of irradiation.

Proposed course: The data obtained will be prepared for publication.

Problem #2. The effect of local roentgen irradiation on the formation of new capillaries after injury. Ruth Merwin

Objective: In a previous study it was found that the capacity of vessels to form new sprouts was affected by doses of irradiation over 500 r. The interval before new vessels invaded the coating of exudate around wires placed in the skin became longer as the dose increased. At present a study is being made using a higher dose to find out what dose will completely prevent the formation of new capillaries. Also the growth of new capillaries around wires placed in the skin a year after irradiation is being studied to find out whether the endothlium will have regained its capacity to grow without delay into an injured area.

Methods; The growth of capillaries into the coating of exudate that forms around wire stitches placed in the skin of mice is being studied microscopically using transparent chambers.

Major findings: Preliminary findings indicated that a dose of 2,000 r completely prevents the growth of new vessels around some, but not all, wires. One year after local irradiation the delay before sprouts form in an injured area is approximately the same as when an injury is made immediately after irradiation.

Significance: An understanding of the changes in normal tissue irradiated locally is of importance because it is difficult to irradiate a tumor without including the surrounding normal tissues. The deleterious effect of irradiation on normal tissues sometimes limits the dose that can be given to a tumor.

Proposed course: To continue as stated above.

Problem #3. Tumor growth and vascularization. Initial vascularization of transplants of tumor and normal tissue. Ruth Merwin and Glenn Algire

Objective: The object of this study is to evaluate the significance of the activity of the graft and host vessels in the initial vascularization of grafts.

Methods employed: Microscopic observations were made on grafts placed in transparent chambers in living mice. With the usual methods of studying grafts one must focus through the graft in order to see the area in which the graft and host vessels establish connections. Therefore, grafts were placed so the area in which these changes take place would be beside rather than under the graft.

Major findings: The surviving vessels in grafts of normal tissues produce sprouts which may be active in establishing connections between the vessels of the graft and those of the host. On the other hand, the vessels in grafts

of tumor tissue survive only in some grafts and do not produce new vessels. Grafts of normal tissue of the size used did not cause host vessels to form new sprouts, whereas grafts of a similar size of a mammary adenocarcinoma caused a rich network of new sprouts to form.

Significance: The methods used here provide a means by which comparisons could be made between the effect of various kinds of stimuli and various kinds of tumors on blood vessels.

Proposed course: It is planned to compare various types of tumors in their capacity to stimulate host capillary proliferation.

Problem #4: Effect of irradiation on the capacity of mice to develop immunity to homografts. Ruth Merwin and Katherine Sanford

Objective: The objective of this study was to find out how long after irradiation mice are incapable of developing immunity to homografts.

Methods: A nonvascularized homograft will not initiate immunity but it will be destroyed if the host becomes immune. The interval after irradiation and after the injection of an immunizing dose of homologous cells before a homograft disintegrated was taken as the interval during which mice could not develop immunity.

Major findings: Preliminary results indicate that there is much variation between mice but that mice can acquire immunity as soon as 14 days after 900 r and protection by isologous marrow or as soon as 11 days after irradiation with 450 r.

Significance: To help identify the nature of the changes taking place in cells that become neoplastic (see the report of Dr. K. K. Sandford)

Proposed course: To continue as stated above.

10. 414
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	_____
REVIEW & APPROVAL	_____	TECHNICAL ASSISTANCE	_____

12. _____
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

Dr. C. C. Congdon is at Oak Ridge National Laboratory, Oak Ridge, Tenn.

13. _____
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 414
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Vascular patterns in tissues and grafts within transparent chambers in mice. Glenn H. Algire and Ruth M. Merwin. Angiology 6: 311-318, 1955

16 HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Cellular Biology Section — SECTION OR SERVICE
4. — LOCATION(IF OTHER THAN BETHESDA)
5. 416 — SER. NO.

6. The mechanism of destruction of homografts — PROJECT TITLE

7. J. M. Weaver — PRINCIPAL INVESTIGATOR(S)

8. G. E. Algire — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To elucidate the mechanism of homograft destruction. Work published in 1954 (J.N.C.I. 15: 493-507 and 509-517) suggested that such grafts are not destroyed by circulating antibodies and further data published in 1955 (J.N.C.I. 15: 1737-1767) suggested that they are destroyed by contact with host lymphocytes. There was some evidence in the latter work that these lymphocytes release a diffusible specific cytotoxin (antibody?) at the site of the graft. The purpose of further work in 1955 was to see if this was so.

Methods employed: Single and double diffusion chambers, tissue cultures, serologic methods, transparent chambers and conventional cytologic methods (see following section for further details).

Major findings: Modified intraperitoneal diffusion chambers were employed with two adjacent compartments separated by cell-impenetrable membranes with a pore size of about one micron. Lymphocytes from C57BL mice previously immunized with C3H tissue were combined in one compartment with C3H "target" cells (spleen or tumor). As expected from previous work, this resulted in the death of both the lymphocytes and the target cells. Similar "immunized" lymphocytes alone or target cells alone had been placed initially in the second compartments. Both of these two types of cells died in these latter compartments whereas control nonimmunized lymphocytes or control C57BL target cells survived here.

Furthermore, target cells combined with dead "immunized" lymphocytes (frozen and thawed) in intraperitoneal diffusion chambers with one compartment did not survive whereas such target cells did survive when combined with control dead nonimmunized lymphocytes. Whether, as might be expected, dead target cells will destroy "immunized" lymphocytes in diffusion chambers has not yet been studied.

These results can be interpreted most simply as signifying that homografts are destroyed by antibodies which are bound to host lymphocytes until these lymphocytes contact the grafted cells and, in addition, that this destruction of the graft is accompanied by the death of the host lymphocytes which is caused by antigens released from the dying grafted cells.

Further attempts to destroy target cells in vitro by combining them with living or dead "immunized" lymphocytes and efforts to visualize in vivo by means of modified transparent chambers the interaction of living graft and host lymphocytes have been unsuccessful to date -- quite possibly for various technical reasons.

Proposed Course: The above findings will be extended and methods will be sought to visualize in vivo the interaction between living grafted cells and host lymphocytes and also to cause this to occur in vitro where the mechanism involved can be more easily analyzed.

10. 416
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH X ADMINISTRATION

REVIEW & APPROVAL TECHNICAL ASSISTANCE

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 416
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Weaver, Algire, Prehn; J.N.C.I. 15: 1737-1767, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

SUPPLEMENTAL PROJECT DESCRIPTION OF SERIAL NO. 416 LABORATORY OF

BIOLOGY - DR. JAMES M. WEAVER

PROJECT DESCRIPTION (Addition)

Significance of work: "Cancer immunity" has long been a subject of considerable interest and of intensive investigation. Latency of cancer and occasional spontaneous regressions of cancer in man may well be manifestations of an immune reaction of the host to the tumor. If such reactions could be inteisified at will, the benefit to the host is obvious. However, the mechanism of rejection of homologous tumor grafts in animals and of homografts in general has long been disputed. This mechanism has certain features in common with better understood phenomena of immunity, such as reactions to bacteria or foreign proteins, but the search for antibodies to these tissues has usually been to no avail. Until the nature of this mechanism is clarified, it seems unlikely that attempts to utilize it to combat cancer in man will be successful. It is hoped that the research program described above will contribute toward an understanding of this mechanism.

PROJECT REPORT FORM

1. National Cancer Institute 2. Laboratory of Biology
INSTITUTE LABORATORY OR BRANCH

3. General Biology Section 4. 5. 417
SECTION OR SERVICE LOCATION (IF OTHER THAN BETHESDA SER.#

6. Investigation of the role of genes and their relationship to non-genetic
PROJECT TITLE
factors in the development of cancer.

7. W. E. Heston
PRINCIPAL INVESTIGATOR(S)

8. Margaret A. Deringer, Thelma B. Dunn, Delta Uphoff, George Vlahakis
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION.

Objectives: To determine the effects of genes on the occurrence of tumors, particularly of the lung and of the mammary gland, of the mouse. To obtain some indication of the number of genes involved and where possible to identify specific genes. To ascertain the manner in which the genes are related to non-genetic factors in the induction of tumors.

Methods employed: This report covers only certain facets of a long term program in which each year certain sub-projects are completed and certain new ones are initiated. Methods employed in various facets of the program may be much the same with new procedures interjected when needed.

In the study of pulmonary tumors a number of inbred strains of mice are available with specific incidences of pulmonary tumors ranging from 90 percent in strain A to less than 1 percent in strain C57L. Seven specific genes located on five specific chromosomes have been shown to be associated with the occurrence of pulmonary tumors - some through the effect of the gene itself and some through linkage. The occurrence of these tumors can be increased by extrinsic factors including the carcinogenic hydrocarbons, urethan, nitrogen and sulfur mustard and radiation, and the number of tumors occurring offers a valuable quantitative measure of response. Through proper matings specific desired genotypes can be produced and these can be studied in relationship to any of the extrinsic factors. Through transplantation of lung tissue various genotypic combinations between host and lung tissue can be created. The carcinogens can be introduced to shorten the duration of the project and also to study and compare their specific effects in combination with specific genotypes. Gold thioglucose has been injected to increase body weight comparable with effect of the lethal yellow gene.

In the study of mammary gland tumors a number of inbred strains are available with certain incidences of mammary tumors and differing in ability to transmit the mammary tumor agent. The line of strain C57BL here at the NCI is outstanding and valuable in our studies in that as shown by Andervont it will not transmit the agent for more than one generation. Through appropriate matings and foster-nursing of young the relationship of the genotype to the agent can be studied.

Major findings: - Lung Tumors.

In an attempt to determine whether or not the lethal yellow (A^y) gene of the mouse increases the occurrence of pulmonary through some general effect related to its increasing body weight, non-yellow a a sibs have been injected with gold thioglucose. Following this treatment the aa mice gained weight so that their average weight was approximately equal to that of the $A^y a$ mice without gold. Furthermore the average number of lung tumors observed in aa mice injected with gold and later with dibenzanthracene was about the same as that of the $A^y a$ mice without gold but with the dibenzanthracene. There are some unexplained sex differences in average number of tumors which may be clarified with further experimentation. Before publishing we want also to get data on effect of gold on occurrence of lung tumors in $A^y a$ mice and also the effect on occurrence of lung tumors of holding down the weight of $A^y a$ mice through dietary restriction. This study is underway.

In the study in which we were attempting to determine whether or not the effect of the A^y gene in increasing occurrence of pulmonary tumors was localized in the lung, the results were somewhat irregular. This was done by transplanting $A^y a$ lung into aa hosts as well as $A^y a$ hosts and transplanting aa lung into $A^y a$ hosts as well as aa hosts. The greatest number of tumors occurred in the transplanted lung when the genotype of the transplant and that of the host were the same. The highest incidence of tumors occurred in aa transplants in aa hosts and the next highest in $A^y a$ transplants in $A^y a$ hosts. The least incidence occurred in $A^y a$ transplants in aa hosts and next to the host occurred in aa transplants in $A^y a$ hosts. This experiment will be repeated before publication.

Reticulosis involving particularly the axillary and inguinal nodes has been found in a high percentage of these AYF_1 mice. Although scattered cases of this condition have been observed previously in a number of our stocks this is the first time we have noted it in a high incidence in any group. It has not been recorded in such an incidence previously in AYF_1 mice. The cause of the condition is not known.

In further dose-response studies on the induction of pulmonary tumors in strain A mice with dibenzanthracene the nature of the response curve has been determined in the region of very minute doses. A straight line response has been noted between dose .5 mg. and dose .01 mg. with the point for zero dose deviating upward from the extension of this straight line. The points for dose .005 mg. and dose .0025 mg. also deviated upward. Response was measured in terms of average number of nodules, and the lower points that deviated upward represented groups in which not 100 percent of the animals had one or more nodules. It may be that some theoretical response points can be established statistically assuming variation in the animals having no tumors and these points may not deviate from the straight line response.

Such dose-response studies have also been made on the induction of pulmonary tumors in strain C3Hf mice with urethan in doses ranging from 1.25 mg. to 40 mg. No increase in tumors was noted below the dose of 10 mg. and at doses 10, 20, and 40 mg. the response was so slight owing to the resistance of the strain that it was impossible to determine the dose-response relationship.

The outstanding observation in this group of C3Hf mice was definite evidence of increase in incidence and average number of hepatomas with increase in dosage of urethan. This was noted in the males only. This is the first observation, to our knowledge, of the induction of hepatomas in mice with urethan. An experiment has been set up to verify the observation before publication.

Mammary Gland Tumors:

Study of mammary gland tumors in strains without the mammary tumor agent.

In our C3Hf strain, mammary tumors are continuing to arise with an incidence of approximately 25 percent and an average tumor age of approximately 20 months. Although our colony is now in the 29th and 30th generation of inbreeding since the original foster-nursed litter from which this strain was started, we still have seen no evidence of high tumor lines segregating out as would be expected were the agent present.

In July 1953, a litter of Andervont's subline C3Hf/An, that in his laboratory had shown a considerably lower mammary tumor incidence than had our C3Hf, was obtained, and from that litter a colony has been bred. Of 23 C3Hf/An breeding females that have been autopsied, 6 or 26.6 have had mammary tumors at an average age of 13.3 months, the remainder died without mammary tumors at an average age of 15 months. It, therefore, appears that in our laboratory the incidence of tumors in his line is going to be comparable with that in our line and significantly higher than that in his line in his laboratory. Explanation for this difference may be one or more of the following. 1) In our laboratory the animals are fed Deerwood diet whereas in his the diet is Purina chow, 2) in our laboratory the females have been bred throughout the year whereas they are not bred during the winter months in his laboratory. 3) in our laboratory the females are housed in the same large room with animals with the milk agent, although on opposite sides of the room and on different racks, whereas in his laboratory agent free animals are not kept in the same room with animals with the agent. Since breeding has been shown to be an important factor in producing mammary tumors in our C3Hf line (the virgins having an incidence of less than 5 percent whereas the breeders have 25 percent or more) the second possible explanation is probably the most important. The third explanation would imply infection with the agent and as indicated above we have seen no evidence of this in our own C3Hf line.

The large mass of data on the relationship of the genotype to the milk agent has now been prepared for publication and should be submitted in the near future. The genetic control over the propagation and transmission of the agent is very pronounced, the agent being eliminated by the third backcross generation. Once eliminated the agent cannot be revived by again introducing a suitable genotype. The agent cannot be caused to arise de novo. The agent can be transmitted by the males and once introduced into the line in this way is immediately built up to create a high tumor line.

To test further for the presence of the agent these data were tabulated to show parent-offspring correlation. A positive correlation appeared only where it was certain from other observations that the agent was present. In other lines although quite a number of tumors were present there was no parent-offspring correlation which was in line with the previous conclusion that these tumors arose in the absence of the agent.

The data also were tabulated to determine whether or not more tumors occurred in females of later litters than in those of early litters. With the exception of the few females that were infected by the male and were infected after the first or second litter was born so tumors occurred more in later litters, there was no difference between early and late litters either in groups where the agent was known to be present or in groups where it was absent.

Cleft palate and harelip.

In Fraser's interpretation of his results on induction of cleft palate and harelip in strain A mice with cortisone he has contended that the cortisone did more than increase the occurrence of an anomaly that occurred spontaneously in the strain. His argument was that with cortisone he got some cleft palate without harelip, whereas cleft palate without harelip never occurred spontaneously. Since I had contested this interpretation and since we had the opportunity to observe a large number of newborn strain A mice we have gathered data on these anomalies. Of 1313 males born 8.3 percent had both harelip and cleft palate, .6 percent had harelip without cleft palate and .23 percent had cleft palate without harelip. Of 1351 females born 6.29 percent had harelip and cleft palate, .37. percent had harelip without cleft palate and .22 percent had cleft palate without harelip. Thus, without treatment of the mother the animal can have a cleft palate without evidence of the harelip.

Significance to program of the Institute: One of the approaches to the control of cancer in man is through the study of the factors in the development of cancer in mice, the manner in which these factors become effective and the way in which they are related to each other. Basic among these factors are the genes, but the observable effect of the genes is greatly influenced by nongenetic factors. The results reported help to round out our knowledge in this area and it is hoped that such knowledge will be of assistance in understanding the factors causing cancer in man and in directing investigations on cancer in man.

Proposed course of project.

The studies will be continued much along the same lines as in the present report. Linkage studies that were not at a stage suitable for reporting now will be continued and possibly concluded in the coming year. Data such as those on the transplanted foetal lungs will be written up for publication as soon as Dr. Steffee has finished going over the slides.

The experiment for verification of induction of hepatomas in mice with urethan will be carried on and possibly concluded. This will be extended to include repeated doses as well as single graded doses.

The dose-response studies on the induction of pulmonary tumors are being extended to urethan in strain A mice, the strain C3H having been too resistant to give analyzable results for the dosages used. It is necessary to get results from some carcinogen in solution since it is realized that the straight line relationship observed with the dibenzanthracene dispersion may have represented only distribution of DBA particles.

Studies on the effect of the $\underline{A}^{y}$ gene in increasing pulmonary tumors in mice are being extended to include data on $\underline{A}^{y}\underline{a}$ mice treated with gold and also on $\underline{A}^{y}\underline{a}$ mice whose weights are held comparable with that of their $\underline{aa}$ sibs through

The experiment attempting to localize the action of the A^y gene in increasing the occurrence of pulmonary tumors will be repeated before the results are published.

A new but related project on the effect of concentration of oxygen on the occurrence of pulmonary tumors in strain A mice is getting under way in collaboration with Dr. Arnold W. Pratt. This study is based upon the observation of increased rate of radiation induced mutations in Drosophila and other microorganisms with increased concentration of oxygen, increased melanotic tumors induced with radiation in Drosophila larvae with increased oxygen, and increased spontaneous melanotic tumors in the larvae with increased oxygen but without radiation. The studies will include 2 month old strain A mice injected with dibenzanthracene and exposed to various concentrations of oxygen, and also newborn strain A mice exposed to various concentrations of oxygen but not injected with dibenzanthracene.

10. 417
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	X	Administration	
REVIEW & APPROVAL		Technical Assistance	

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 417
SERIAL NO.

15.
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955.

Deringer, Margaret K., and W. E. Heston
Development of pulmonary tumors in mice segregated with respect to the three genes: dominant spotting, caracul, and fused. J. Nat. Cancer Inst. 16: 763-768, 1955.

16.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. 418 — SER. NO.
6. Host-tumor Relationships — PROJECT TITLE
7. M. K. Barrett; M. B. Melroy, M. K. Deringer — PRINCIPAL INVESTIGATOR(S)
8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

In multicellular organisms the morphology and physiology of each cell and each tissue is strongly influenced by other cells or tissues in its environment. There seems to be a cellular ecology which maintains organization and balance from earliest differentiation to death. Ample experimental evidence for such intercellular influences exists.

Cancer may be viewed as a disturbance of such an ecological balance and from such a view one may derive a definition of cancer. A definition in these terms is the only definition of which I am aware that does not require frequent important qualifications. For example, some normal tissues have enormous proliferative power and may even be invasive whereas some malignant cells multiply slowly and may remain localized indefinitely but in the end the normal usually responds to controlling influences and the malignant does not.

Such a definition is primitive but its constancy is a recommendation. The primitive quality is due to a lock of knowledge of the nature of the forces or influences that modify the intercellular balances. At present we call these influences "immunogenetic", for want of a better term. Our research is directed at learning more about the nature of these influences. The possibility of final

practical application of the knowledge is suggested by the occurrence of spontaneous remissions and even regressions in cancer of both man and animals - regressions that appear to be brought about by a resistance on the part of the host.

Methods

The basic procedure is transplantation of standard tumors into hosts of known genetic constitution and studying the effect of experimental variations on the host, the tumor, or both. The disciplinary techniques involved are those of genetics, immunology, and experimental surgery.

Major Findings

A review of this field, including our major findings and their place in the general perspective was reported in press last year. This paper entitled "The nature of tumor immunity" has now appeared as a chapter in a book; see publications.

We previously reported that a host can be made immune to the implantation of a tumor by prior inoculation of washed red cells or by stromata but not when the cells or stromata have been broken up by relatively gentle procedures. We also reported that the antigen was easily destroyed by heat or by formalin. Others have strongly suggested that the antigen concerned is a hemagglutinogen. Available data indicate that, genotypically, these antigens are related but we believe that phenotypical differences exist and may be important. Accordingly we have accumulated unpublished data that indicate such differences. Mild sonic vibration destroys the "resistance" antigen but not the hemagglutinogen for mice (dextran technique). This work has been hampered by "histocompatibility" limitations which prevented observation of the 2 types of phenomena in the same mouse. We have now succeeded in adapting a tumor so that future observations can be made in the same mouse. The "resistance" antigen (for mice) is destroyed by heating for 30 minutes at 42-44° C whereas the hemagglutinogen (for rabbits) remains potent after 30 minutes at 56° C. In one strain of mouse a high degree of resistance can be induced by certain red cells but such mice do not have hemagglutinens demonstrable by the new techniques. (Mr. Melroy has done most of the serologic work). Another aspect of the differential characterization of this antigen is found in a study of incubation periods. We reported a freakish incubation curve that seemed to characterize the development of

resistance following inoculation of red cells. This work is very laborious but we have made some additional progress. The first notch (decrease in relative immunity during the 3rd week) in the curve has been seen again and additional evidence for the second notch (5th week) has been obtained. This "gain-loss-regain" phenomenon has not been reported in immunology. We do not know what it means but it seems to have something to do with the particular antigen because it does not occur when tumor is used as the antigen. This might represent an obscure biologic difference between normal and malignant tissue but, because the cell type was different, this is uncertain. All these points permit us to continue with the hypothesis that there is something peculiar about this type of immunity and the antigens that generate it.

A paper reporting the effect of multiple inoculations of a tumor and the implications of the results in interpreting Lewis' "breaking down of resistance", Greene's "constitutional factors peculiar to a tumor bearing host" and Casey's "secondary XYZ" effects appeared in the December 1954 issue of J.N.C.I. which came out in January, 1955. The crux of the matter is that the cachectic host cannot muster such immunologic defenses as it previously possessed and this loss is nonspecific. As an outgrowth of that work we reinvestigated the effect of incision and partial excision on the progress of the disease. The work with tumors transplanted within strain and out of strain has been concluded. Neither simple cruciate incision nor partial excision increased the number of metastases and the latter prolonged the survival of the hosts. (Details given in previous report). We are now observing the effect in spontaneous mammary tumors. Accumulation of a properly matched series is very slow. So far the results are in agreement with those in transplanted tumors. These results are contrary to several reports in the literature, including one in the J.N.C.I. for 1949 and one in Krebsartz for 1952.

Following our discovery of a maternal influence on growth of a tumor and the specificity of "adaptation" we attempted to find a clear extrachromosomal (aside from milk agent (s)) or X-Y chromosomal influence on tumor growth. A special type of F_2 hybrid produced by Dr. Deringer furnished a very elegant substrate for this investigation but we were unsuccessful in demonstrating an effect. The experiments have been abandoned.

PROJECT DESCRIPTION (Cont.)

Several years ago Cloudman reported that mice of strain C3He, obtained by transferring fertilized C3H ova into C57BLK mothers, were tolerant to a tumor of C57BLK origin. If this were true it would furnish an interesting and instructive variation to the other known examples of "pseudohybridization" or "acquired tolerance." However, Cloudman reported tolerance in generations F_1 to F_{10}. Tolerance in the parental generation would be easy to believe but not tolerance in filial generations. The establishment of strain C3He by Dr. Deringer gave us an opportunity to reinvestigate this. A total of 174 C3He mice of generations F_2 to F_4 failed to yield any takes of a tumor of C57BLK origin and there also were none in 4 mice of the parental generation. Thus no confirmation of Cloudman's report was obtained. A manuscript reporting these negative findings is in preparation.

I participated in the Gordon Cancer Conference as an invited discussor but no manuscript for publication was involved.

A considerable amount of time has been spent in reviewing manuscripts, applications for fellowships, and grant-in-aid applications.

Significance

The field with which this work is concerned is so difficult and obscure as to make clear definitions and outlines almost impossible. Nevertheless there is a slowly growing body of evidence which indicates that there are interactions between tissues and between tissue and its host which involve critical factors in the phenomena of cancer, embryology, and transplantation of normal tissues. A defensible definition of cancer cannot be given today except in descriptive and biologic terms. The terms usually include things related to the experiments that we have been describing. The experiments may be significant in several ways both practical and theoretical.

The most important implication is that some of these results suggest progress toward understanding of what controls the growth of a cancer and how this differs from normal tissue. We have already seen that a tumor's power to overcome host resistance can be altered; its rate of growth, tendency to regress, and tendency to metastasize are all subject to experimental change. The host's reaction can be altered experimentally, not only between similar hosts but also at different times and sites in the same host. The changes in the tumor have eluded morphologic, chemical, or serologic definition. One of the antigens which changes the host would be missed by current chemical methods. However one might attain a biologic description of the factors that sometimes cause spontaneous tumors of man and animals to regress and that control metastasis. It has already been observed in this laboratory (and confirmed elsewhere) that such factors materially effect the outcome of many chemotherapeutic experiments.

PROJECT REPORT FORM (Cont'd)

10. 418
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH X ADMINISTRATION

REVIEW & APPROVAL TECHNICAL ASSISTANCE

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

The experiments on incomplete excision may lessen the fear of some surgeons in attempting palliation in certain selected cases, and thus lead to amelioration of a few.

Relation to others

There is a complex theoretical interlocking between this work and the work of Drs. Algire, Grobstein, Prehn, and Sanford.

14. 418
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Barrett, M. K. and Hansen, W. H.: Resistance of mice to multiple inoculations of a transplantable tumor. J. Nat. Cancer Inst. 15: 411-420, 1954 (Appeared in 1955).

Barrett, M. K.: The nature of tumor immunity. In Origins of resistance to toxic agents. M. G. Swag, R. D. Reid, and O. E. Reynolds, eds. Academic Press, New York, p. 308-333, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Laboratory of Biology (LABORATORY OR BRANCH)

3. General Biology (SECTION OR SERVICE) 4. (LOCATION(IF OTHER THAN BETHESDA)) 5. 419 (SER. NO.)

6. Gastric Cancer Investigations (PROJECT TITLE)

7. M. K. Barrett (PRINCIPAL INVESTIGATOR(S))

8. (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives

Efforts have been directed at finding methods for producing or studying some of the supposedly etiologic factors in gastric cancer. Many etiologic or predisposing factors have been suggested but laboratory investigation of them has been held back by a lack of adequate methods for producing them in animals. Our work has comprised a series of attempts to produce starting points from which gastric cancer studies could go forward. In addition, we have attempted to stimulate work in this field generally through the activities of the Gastrointestinal Cancer Committee.

Methods

New techniques of experimental surgery (some developed in this laboratory) and physiology have been used to study gastric polyps in dogs and peptic ulceration in rats.

Findings

It was reported last year that when artificial polyps are constructed in a dog's stomach the mucosa of the polyp undergoes degenerative changes ending in atrophic gastritis. A paper reporting

this appeared in Gastroenterology for March 1955.

In keeping with our general ideas regarding tissue specificity, we adopted an hypothesis that the stomach does not digest itself because some "recognition factor" prevents it. A recognition factor or specificity might exist between the enzymes and the tissue, or between some element of the mucus and the tissue, or among all three. If this were so the mucosa, which is relatively immune to the corrosive action of its own secretions, might be readily attached by foreign secretion. Under properly controlled conditions gastric juice was transferred between strains of rats. It was found that, although each strain was resistant to its own juice for a period of 4 hours (or more), the glandular mucosa of each was vulnerable to the juice of the other during a period of 3 hours (or less). A manuscript reporting these data has been submitted to Gastroenterology.

Significance

The experiments on gastric polyps should influence the clinical management of polyps, in the direction of radical treatment, generally. They also provide the long sought method for producing atrophic gastritis regularly, although better methods are still desirable.

The studies on peptic digestion may have far reaching implications, which at present are speculative: One might ask some questions. Is the pepsin specific in an immunogenetic sense? If so, do other enzymes have a biological specificity in the natural state and do they lose this specificity in vitro? (As pepsin may do). Is a different enzyme from pepsin involved in the initial attack upon the mucosa? (If so, the specificity remains). Does the mucus contain a mucopolysaccharide which is antibody-like in function? If so, is this where lysozyme enters the field in peptic ulcer and ulcerative colitis? Does gastric mucosa sometimes contain groups of cells which are immunogenetically aberrant? (Most clones of cells do contain atypical cells). Is the chronic ulcer case an example of this with an area of permanently lowered tolerance and the acute ulcer case merely a case of temporary imbalance of tolerance for transient causes? If so the first should always be excised for cure, the second may get well without surgery. Is this the reason that nearly all gastric cancers (at the surface) ulcerate? If so, one might be justified in deliberately augmenting ulceration in

PROJECT REPORT FORM (Cont'd)

10. 419
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH X ADMINISTRATION

REVIEW & APPROVAL TECHNICAL ASSISTANCE

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
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some small lesions - a long shot. At any rate, this appears to be a new and provocative idea in gastric digestion - and there has not been too many such during a century of investigation.

Gastrointestinal Cancer Committee work.

A large amount of time has gone into work with this Committee. A small Symposium was held in Stone House near the end of last year. At the request of the editor of Gastroenterology I prepared a short summary of the proceedings and this appeared in Gastroenterology for June 1955. There has been a demand from abroad for this reprint. I don't know why.

I carried the major burden in organizing the 6th National Gastrointestinal Cancer Conference which was held in New York in April. Editorial work and proof reading of the proceedings have been time consuming but are completed and the complete Proceedings appeared in Gastroenterology for October, 1955. This is much more rapid publication than was obtained previously.

Proposed Course

We are planning techniques whereby we may be able to determine whether the specificity of gastric juice for gastric mucosa also extends to unrelated tissues. If the specificity resides in the enzymes, such an extension could be very important. Other attempts will be made to see if stronger implication of the enzyme can be obtained.

14. 419
SERIAL NO.

15.
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Parrett, M. K., Lefco, T., and Hansen, W. H.: Degenerative changes in the mucosa of artificial gastric polyps in dogs. Gastroenterology 28: 393-401, 1955.

Barrett, M. K.: Summary of gastrointestinal cancer symposium, November 19, 1954. Gastroenterology 28: 969-971, 1955.

16.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Inst. 2. Laboratory of Biology
INSTITUTE LABORATORY OR BRANCH

3. General Biology Section 4. 5.420 (a)
SECTION OR SERVICE LOCATION(IF OTHER THAN BETHESDA) SER. NO.

6. Studies on the Rous sarcoma virus. I. Development of the virus-chicken test-system for general use in basic studies on carcinogenesis and other biological reactions.
PROJECT TITLE

7. W. Ray Bryan
PRINCIPAL INVESTIGATOR(S)

8. John B. Moloney, Dorothy Calnan
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To bring about adequate control of the biological and experimental factors which cause excessive variations and interference with precise systematic investigations involving the Rous sarcoma virus-chicken test system (a valuable system for crucial studies on carcinogenesis, particularly from the viewpoint of viral etiology).

Methods employed: Since satisfactory methods have now been worked out and the principal objectives noted above have been accomplished, this project is currently of minor importance, consisting only in the further refinement of existing procedures. The details of methods are therefore not reiterated here (See 1954 report under this heading).

Major findings: The major findings developing from this study, which had been in progress for about 11 years, have been detailed in a comprehensive review of the subject published during the past year)see references 1 and 2 below). The contributions of this laboratory to the problem are summarized as follows:

1. Control of the variations in initial potency of extracts was accomplished by showing a correlation between tumor initiating dose of virus, and the yield of virus in the tumor tissue extracts. By using only tumors initiated by strong doses of virus, the tumor source material can be kept highly potent, and predictable.

2. Control of the variable loss in biological activity of different virus preparations was accomplished by the use of citrate reagents, in which potency remains stable for at least 7 days at ordinary refrigerator temperatures.

Preservation for long periods of time (at least 2 years) was accomplished by freezing in citrate solutions and storage in a CO_2 ice chest. This finding permits the use of standard virus preparations for quantitative biological studies and investigations extending over long periods of time.

3. Control of variations in susceptibility among different test lots of chickens employed at different times was accomplished by the use of stable standard preparations of virus (see 2) and bioassay methods based upon reference to standard preparations.

4. The variations in susceptibility among individual chickens of a common lot was brought under statistical control by studies on the distribution of chicken sensitivities and the selection of appropriate statistical methods applicable thereto.

Future course of project: It is planned to initiate sometime next spring or summer, a study on different types of host chickens in their responses to the Rous sarcoma virus. The study will involve various inbred lines of chickens derived from the Regional Poultry Laboratory, U.S.D.A., at East Lansing, Michigan, and will be in collaboration with Dr. B.R. Burmester of that laboratory. The objective will be to find lines of high, intermediate and low genetic susceptibility to the virus, for further use in contemplated studies on virus-host interaction.

PROJECT REPORT FORM (Cont'd)

10. 420 (a)
SERIAL NO.

11. _____
BUDGET ACTIVITY

RESEARCH X ADMINISTRATION

REVIEW & APPROVAL TECHNICAL ASSISTANCE

12. _____
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

13. _____
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. 420 (a)
SERIAL NO.

15.
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

W. R. Bryan: Biological Studies on the Rous Sarcoma Virus. I. General Introduction. J. Nat. Cancer Inst. 16: 285-286, 1955.

W. R. Bryan: Ibidem. II. Review of Sources of Experimental Variation and of Methods for their Control. J. Nat. Cancer Inst. 16: 287-315, 1955.

16.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute — 2. Laboratory of Biology
INSTITUTE — LABORATORY OR BRANCH

3. General Biology Section — 4. — 5.420 (b)
SECTION OR SERVICE — LOCATION (IF OTHER THAN BETH.) — SER.NO.

6. Studies on the Rous Sarcoma virus. II. Purification of the virus.
PROJECT TITLE

7. John B. Moloney and W. Ray Bryan
PRINCIPAL INVESTIGATOR(S)

8. Dorothy Calnan
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To obtain the Rous sarcoma virus in sufficient quantity, and in a sufficiently high state of purity, to permit its physical and chemical characterization, as well as its general use in investigations on mechanisms of carcinogenesis.

Methods employed:

(a) Enzymatic degredation of impurities by the use of hyaluronidase and trypsin, (b) precipitation of the virus by protamine sulphate, and (c) differential ultracentrifugation, were combined to produce a purification technique which yields a product of 4 to 12 fold higher purity (on a nitrogen basis) than methods previously developed. A report of the procedures has been published (see reference, below).

It is apparent that these same procedures, carried through 2 or more additional cycles, might accomplish final purification of the virus if stronger source materials were available for their application. (See further discussion under "Proposed course of project", below).

PROJECT DESCRIPTION (Cont.)

Significance to cancer research: The rapidity of action of this tumor virus (microscopic tumors in 2 or 3 days, gross tumors in 5 or 6 days) indicates that it may enter directly into the intracellular reactions involved in malignant transformation. This virus may therefore be capable of guiding the investigator directly to the elements or reactions involved in the transformation from the normal to the neoplastic state. Studies on the chemical interactions are dependent upon the availability of practical quantities of essentially pure virus.

Proposed course of project: (a) Further refinement of present successful separative procedures with emphasis on increasing the final yield so that additional purification cycles can be undertaken, (b) the overwhelming amounts of non-virus material ("impurities") to virus in the present starting extracts of tumor tissue cause relatively great losses by any method of separation. Efforts are therefore being made to increase the virus content of the tumor tissue to be used as virus source material (See under project III).

PROJECT REPORT FORM (Cont'd)

420 (b)

10. SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		TECHNICAL ASSISTANCE	

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 and 16

PROJECT REPORT FORM

1. National Cancer Institute 2. Laboratory of Biology
INSTITUTE LABORATORY OR BRANCH

3. General Biology Section 4. 5. 420 (c)
SECTION OR SERVICE LOCATION(IF OTHER THAN BETHESDA) SER. NO.

6. Studies on the Rous sarcoma virus. III. The quantitative relationship between inducing doses of virus and the amount of virus extractable from experimental tumors.
PROJECT TITLE

7. W. Ray Bryan
PRINCIPAL INVESTIGATOR(S)

8. Dorothy Calnan and John B. Moloney
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The original objectives of this research were accomplished during the past year and a report of the findings was given in the publication listed below.

Major findings: The amount of virus extractable from experimental tumors was found to be related to the dose of virus used for initiating the tumor. Above certain initiating dose levels (250 or more ED50 units), the virus yields approached an upper limit asymptotically, but at lower virus initiating doses the yields were highly correlated with initiating dose. Doses of 1 ED50 unit, or less, produced tumors from which very little or no virus could be recovered on extraction.

Significance to cancer research: In addition to the practical usefulness of the information, which permitted methods for producing source tissue of constant high potency (see project I), the results of this study have important implications with respect to concepts in cancer research. For example, it has been generally assumed by workers in this field that tumors having a viral etiology should yield demonstrable virus on extraction, and that the failure to demonstrate virus in filtrates of tumors is justification for regarding them as being of nonviral etiology. The above findings show decisively that this is not a valid assumption, and that under certain reproducible experimental conditions even the most potent and most

PROJECT DESCRIPTION (Cont.)

rapidly acting tumor virus known is capable of initiating tumors from which little or no virus can be recovered on extraction.

Since the frequencies of experimental tumors are also related to initiating dose, the probability of detecting virus in tumor tissue extracts decreases as the frequency of the tumors decreases. At the extremely low frequencies with which "spontaneous" tumors occur in nature, therefore, one would not, on a basis of these findings, expect to demonstrate the presence of a viral agent even if the tumor should be of viral origin.

Proposed course of project: The developments in this project have opened up new areas of investigation. The most significant problem to be investigated is that of the reason for the observed correlation between initiating dose and virus yield. The final answer to this question will, of course, involve both the mechanisms of virus reproduction (in this particular case) and of neoplastic transformation. Many years would probably be required, therefore, for substantial progress toward this end.

The present efforts on this project are confined to: (a) The development of biological materials which will be necessary for the more basic studies, such as a transplantable tumor line of low dose origin which will provide ample amounts of tumor tissue yielding little or no virus on extraction; and similar stable tumor lines yielding extremely high, as well as intermediate potencies. (b) Exploratory experiments on possible mechanisms which could explain the correlation; such as the presence of inhibitor substances (suggested by certain results of earlier investigators), or differences in amounts of virus actually reproduced (electron-micrographic studies with Dr. Dalton - preliminary).

PROJECT FORM (Cont'd.)

10. 420 (c)
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		TECHNICAL ASSISTANCE	

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 420 (c)
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Bryan, W. R., Calnan, D. and Moloney, J. B.: Biological studies on the Rous sarcoma virus. III. The recovery of virus from experimental tumors in relation to initiating dose. J. Nat. Cancer Inst. 16: 317-335, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. General Biology Section
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA

5. 421
SERIAL NO

6. Genetic Studies in Mice A. Genetic Studies of Tumors B. Genetic Study of a Mutant. Urogenital Abnormalities in Strain A x C Line 9935 Rats.
PROJECT TITLE

7. Margaret K. Deringer
PRINCIPAL INVESTIGATOR(S)

8. Dr. W. E. Heston - Dr. M. K. Barrett
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives:

A. Genetic studies of tumors

1. To study the effect of the injection of the mammary tumor agent into agent-free strain C3Hf mice during the late stages of pregnancy at which time the agent has been reported to disappear from the blood of mice which carry the agent, and to see if the agent can be transferred through the uterine wall to the fetus (in cooperation with Dr. Heston).

2. To study the effect of the subcutaneous injection of o-aminoazotoluene on the production of hemangioendotheliomas in strain HR mice. It has been observed that hemangioendotheliomas developed spontaneously in 18% of a group of untreated strain HR mice. No other strain of mice has been described in which hemangioendotheliomas occur frequently but they have been produced by subcutaneous injection of o-aminoazotoluene in strains BALB/c, A, C3H, and C57BL.

3. To study the incidence of mammary gland tumors in virgin, in breeding, and in forced-bred females, and in stilbestrol injected males of strain C3HeC57BL (a substrain developed in this laboratory by the transfer of fertilized ova from a strain C3H mouse to a strain C57BL mouse).

4. To determine whether a mammary tumor agent is present in strain BL (Bagg L) and in strain SWR (Swiss) (in cooperation with Dr. Heston).

5. To study the relationship of certain known genes (W - dominant spotting, Ca - caracul, and Fu - fused) in mice to the production of pulmonary tumors induced by the injection of a hydrocarbon (in cooperation with Dr. Heston).

6. To produce special types of animals for collaborative experiments with Dr. M. K. Barrett (see Dr. Barrett's report for details).

B. Genetic study of a mutant

1. To test a mutant, phenotypically-like waltzer which arose in strain A mice maintained in this laboratory (in cooperation with Dr. Heston).

Urogenital abnormalities in strain A x C line 9935 rats.

At autopsy congenital absence of one kidney was observed in a rat of the strain A x C line 9935 colony. Subsequently, a number of rats of this strain were observed to have one kidney missing, and a number were observed to have other urogenital abnormalities. The extent and incidence of these abnormal conditions has been observed.

Methods employed:

A. 1. Groups of strain C3Hf mice have been injected intraperitoneally with 0.5 cc. of a 5% cell free extract of a mammary gland tumor from a strain C3H mouse. The groups of animals were injected 1-3, 4-6, and 7-9 days before the birth of the young; immediately following the birth of the young; and when the first litters were weaned. Two groups, maintained as virgins, have been injected at 45 and 65 days of age respectively. Uninjected female mice have been allowed to produce one litter each and then have been retired at the time at which these litters were weaned. Approximately 200 female offspring of mothers injected before the birth of the young have been kept as virgins. The animals in this experiment will be maintained as long as possible and a study of the occurrence of mammary gland tumors and of other types of tumors will be made.

2. One hundred strain HR mice (equally distributed as to sex and as to presence or absence of hair) were injected with 100 mg. of o-aminoazotoluene dissolved in olive oil. The injections were started when the animals were 2 to 2 ½ months of age and were given in monthly doses of 10 mg. each. One hundred uninjected animals served as controls. All of the animals were observed for the development of tumors and of any unusual lesions.

3. One hundred C3He females have been set aside as virgins in order to study the development of mammary gland tumors in them. One hundred C3He females are being forced-bred for the same purpose. These animals are isolated when pregnant and are returned to the breeding cage within 24 hours or less following the birth of the litter. One hundred males have been injected subcutaneously in the right axilla with 6-8 mg. pellets of 10% stilbestrol in cholestrol for the purpose of studying the development of mammary gland tumors. This will also be determined in the case of the breeding females which are used to maintain the colony of C3He mice.

4. Newborn strain C3Hf or C3He female mice are foster-nursed on strain BL and on strain SWR females. The foster-nursed animals are maintained as virgins and are observed for the development of mammary gland tumors.

5. The mice used in this experiment were offspring produced by mating F_1 hybrids of strain WCaFu and A with strain 1194. The animals were injected intraperitoneally with 0.5 mg. of 1,2,5,6-dibenzanthracene at two months of age. They were autopsied at 10 months of age and were observed for the development of pulmonary tumors and of any unusual lesions.

B. 1. Strain A males, showing the waltzing character were outcrossed to strain 1194, F_1, F_2, and backcross generations were produced and data were collected regarding the presence of the character in the different groups of animals.

A routine search for abnormalities of the urogenital system was made at autopsy in young animals (1 to 18 days of age) and in adult animals (2 to 17 months of age) of the strain A x C line 9935 rats.

Major findings:

A. 1. In the group injected 1-3 days before the birth of the young, 11 of a group of 57 females have developed mammary gland tumors at an average age of 20.9 months; the non-tumorous animals died at an average age of 21.1 months. In the group injected 4-6 days before the birth of the young, 13 of a group of 53 females have developed mammary gland tumors at an average age of 20.8 months; the non-tumorous animals died at an average age of 20.2 months. In the group injected 7-9 days before the birth of the young 4 of a group of 37 have developed mammary gland tumors at an average age of 16.0 months; the non-tumorous animals dying at an average age of 19.8 months. In the group of animals injected at the weaning of the first litter 5 of a group of 52 animals developed mammary gland tumors at an average age of 24.4 months; the non-tumorous animals died at an average age of 20.1 months. One female of a group of 16 injected following the birth of their young developed a mammary gland tumor at 25 months; the non-tumorous animals died at an average age of 19.9 months. Nine of 195 of the offspring of injected mothers developed mammary gland tumors at an average age of 20.1 months; the non-tumorous animals died at an average age of 20.6 months. Three of 57 of the females, retired after weaning of their first litter, developed mammary gland tumors at an average age of 18.3 months, the non-tumorous animals died at an average age of 21.1 months. In the groups injected at 45 and 65 days of age, 5 of 41 females in the former and 2 of 43 females of the latter group developed mammary gland tumors at average ages of 15.0 and 19.0 months respectively; the non-tumorous animals died at average ages of 20.0 and 20.3 months.

2. The incidence of hemangioendotheliomas and of hepatomas has been increased in the animals which have been injected with o-aminoazotoluene as compared with the untreated animals. Hemangioendotheliomas were observed in 13 of 23 injected haired and 12 of 22 injected hairless males as compared with 8 of 24 untreated haired and 2 of 16 untreated hairless males. They were observed in 13 of 22 injected haired and in 17 of 25 injected hairless females as compared with 6 of 23 untreated haired and 6 of 25 untreated hairless females. Hepatomas were observed in 8 of 23 injected haired and in 6 of 22 injected hairless males as compared with 3 of 24 untreated haired and 1 of 16 untreated hairless males. They were observed in 14 of 22 injected haired and 23 of 25 injected hairless females as compared with 1 of 23 untreated haired and none of 25 untreated hairless females.

Multiple tumors of both these types occurred more frequently in the injected animals.

3. No results to date.

4. Six of a total of 16 strain C3Hf or C3He females, foster-nursed by strain BL, have developed mammary gland tumors. The tumors have appeared at an average age of 13.0 months, the nontumorous animals have died at an average age of 15.8 months. All of the C3Hf or C3He females, foster-nursed on strain SWt, are still alive.

5. In the outcross resulting from crossing (A x WCaFu)F_1 and (WCaFu x A)F_1 animals with strain 1194, the segregants that were fused were less susceptible to induced pulmonary tumors than were the segregants that were nonfused. No significant difference in susceptibility was observed between the segregants with dominant spotting and those without spotting or between the caracul and noncaracul segregants. (Seventy-five of 110 animals exhibiting dominant spotting developed pulmonary tumors following intraperitoneal injection of dibenzanthracene. Sixty-nine of 107 animals without spotting developed pulmonary tumors. The difference between the

two is not significant; $X^2 = .33$, P is between 0.5 and 0.7. Thirty-one of 58 animals with caracul coat and 27 of 42 animals with normal coat developed pulmonary tumors. The difference between them is not significant; $X^2 = .05$, P is between 0.8 and 0.9. Twenty-seven of 50 animals with fused tail and 39 of 52 animals with normal tail developed pulmonary tumors. The difference between the two is significant; $X^2 = 4.9$, P is between 0.02 and 0.03.) Growth curves showed that the average weight of mice that were fused was less than that of mice that were nonfused and the mice that were caracul weighed less than those that were non-caracul. There was no difference between the average weights of the males with and without dominant spotting, but the females with spotting weighed more than those without.

B. 1. In the (1194 x A)F_2 animals, 75 of a total of 634 animals (11.8%) exhibited the waltzing character and in the (1194 x A)-ABC animals, 97 of a total of 251 animals (38.6%) exhibited it. These results indicate that possibly this character is due to a single recessive gene. No further results at present available.

Abnormalities of the urogenital system in strain A x C line 9935 rats were observed in 134 of a total of 748 females and in 137 of a total of 813 males autopsied at 1 to 18 days of age. In animals autopsied at 2 to 17 months of age, 45 of 189 females and 12 of 62 males were abnormal. The principal manifestations were the absence of one kidney or the presence of one cystic kidney. These were accompanied generally by other abnormal conditions. In females, they included a missing ureter, uterine horn, oviduct, ovarian capsule, or ovary; an incomplete ureter or uterine horn; a cystic ureter, uterine horn, or ovarian capsule; or hypertrophy of the remaining kidney, with variation in the degree of abnormality. In males, they included a missing ureter, epididymis, vas deferens, or vesicular gland; atrophy of the testis, vesicular gland, or epididymis; cystic ureter; undescended testis; and hypertrophy of the remaining kidney, all also varying in degree.

Significance to cancer research:

A. It is already well established that the genetic constitution influences the development of tumors. All facets of this project will add more information to that already existing concerning the mode of inheritance of various types of tumors.

B. The study of the mutant may be of significance in providing another known character which will be of use in the linkage studies of factors influencing susceptibility to various types of tumors.

Proposed course of the project:

A. 1. This study will continue through 1956.
2. This study will be completed early in 1956 (four experimental females remain to be autopsied) and the results will be prepared for publication.
3. This project will continue through 1956.
4. It is anticipated that this experiment will continue through 1956.
5. The results of this experiment will appear in the December 1955 issue of the Journal of the National Cancer Institute.
6. Special types of animals will continue to be produced for experiments with Dr. Barrett.

B. 1. Project will continue through 1956.

The results of this experiment have been written up and the manuscript has been submitted to the Editorial Board for approval for publication in the Proceedings of the Society for Experimental Biology and Medicine.

10. 421
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH X ADMINISTRATION

REVIEW & APPROVAL TECHNICAL ASSISTANCE

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 421
SERIAL NO.

15.
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

1. Deringer, M. K., and Lorenz, E.: Results of exposure of newborn HR mice to X radiation. J. Nat. Cancer Inst. 15: 923-929, 1955.

2. Deringer, M. K., Lorenz, E., and Uphoff, D. E.: Breeding behavior and tumor development in (C57L x A)F_1 hybrid mice receiving X radiation to ovaries shielded. J. Nat. Cancer Inst. 15: 931-941, 1955.

3. Deringer, M. K., and Heston, W. E.: Development of pulmonary tumors in mice segregated with respect to the three genes: dominant spotting, caracul, and fused. J. Nat. Cancer Inst. 16: 763-768, 1955.

4. Deringer, M. K., and Heston, W. E.: Abnormalities of the urogenital system in strain A x C line 9935 rats. (Submitted to the Editorial Board of the Journal of the National Cancer Institute).

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section — SECTION OR SERVICE
4. — LOCATION) IF OTHER THAN BETH.)
5. 423 — SER. NO.

6. Developmental Physiology — PROJECT TITLE

7. Clifford Grobstein — PRINCIPAL INVESTIGATOR(S)

8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Objectives: To increase understanding of the interaction of tissues and the components of rudiments in normal development as these relate to the differentiation of new cell types, and to apply information obtained to pathological problems, in particular to problems of tumorigenesis and malignancy.

B. Methods: Developing rudiments of mouse organs, e.g. kidney, salivary gland, and axial skeleton, are separated into their components and cultured in isolation and in various recombinations to determine the degree to which their development is interdependent and the nature of the interactive mechanisms involved. Development is observed in vitro and/or on reimplantation into the anterior chamber of the eye.

Major findings: Study continues of trans-filter induction between embryonic spinal cord and metanephrogenic mesenchyme. Methods have been developed, in collaboration with Dr. Dalton, for electron microscopy of the filter during induction. Culture methods have been further refined to permit elimination of the clot, thus making possible study of the induction in defined nutrient media as these become available. Preliminary studies on intact rudiments with the amino acid-vitamin mixture of Eagle indicate that it can be used if supplemented with horse serum or mouse ascitic fluid.

The nature of the trans-filter inductive influence cannot yet be specified. Filters in contact with inductively active tissues may show three types of penetration depending on tissue type, filter type, and duration of contact: 1) Cytoplasmic processes identifiable with the electron microscope; 2) Granular or filamentous material demonstrable by staining or phase contrast; 3) Diffuse material present after alcohol-formalin fixation and digestible by trypsin. Efforts are

PROJECT DESCRIPTION (Cont.)

continuing to determine possible correlations between the several kinds of penetration and biological activity, and to "trap" biological activity in the filter in the absence of living tissue.

Metanephrogenic mesenchyme, which never forms epithelial tubules by itself in vitro, does form tubules when implanted into the eye or brain of adults - despite the fact that tissues from these sites are negative as inductors in vitro.

Parotid epithelial rudiments undergo characteristic parotid-type branching in sub-mandibular mesenchyme - suggesting that the mesenchyme of the two salivary rudiments shares a common property not found previously in mesenchyme from non-salivary sources, and that the difference in type of branching is a function of epithelial rather than mesenchymal properties.

Preliminary experiments by Dr. Robert Auerbach, N.I.H. fellow, indicate that lens induction by the optic vesicle in the mouse can be obtained in vitro and that the process may be able to be analyzed by methods similar to those used for kidney, salivary gland and cartilage.

D. Significance to cancer research: As noted in last year's report these studies bear on the nature of factors which stabilize or disrupt tissue architecture.

E. Proposed course of project: Continued study is planned of the nature of the mechanisms of induction in the several in vitro systems available. Approaches in progress include: 1)Electron microscopy of the filter between interacting tissues; 2) Variation of filter porosity and other experimental conditions in an effort to separate the three types of filter penetration for correlation with biological activity; 3) Determination of the nature of the factors responsible for tubule formation in implanted kidney mesenchyme; 4) Culture of the interacting system in defined media so as to simplify identification of active materials.

PROJECT REPORT FORM (Cont'd)

10. 423
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH X ADMINISTRATION

REVIEW & APPROVAL TECHNICAL ASSISTANCE

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957:

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 423
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

1. In vitro studies of cartilage induction in mouse somite mesoderm (with H. Holtzer) J. Exp. Zool. 128: 333-358 1955

2. Tissue disaggregation in relation to determination and stability of cell type. Ann. N. Y. Acad. Sci. 60: 1095-1106 1955

3. Tissue interaction in the morphogenesis of mouse embryonic rudiments in vitro. In "Aspects of synthesis and Order in Growth", Princeton Univ. Press 1955

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section - SECTION OR SERVICE
4. ____ LOCATION (IF OTHER THAN BETH.
5. 424 SERIAL NO.
6. Tissue Compatibility Studies — PROJECT TITLE
7. Richmond T. Prehn — PRINCIPAL INVESTIGATOR(S)
8. Joan M. Main — OTHER INVESTIGATORS
9. Project Description

Object: The long range objective is, of coarse, the understanding of the physiology of cancer, which in turn will some day lead to control of the disease.

It is axiomatic that the well-being of the cancer cell is dependent upon its social acceptability to the normal cells of its host - i.e. upon its recognized failure to arouse a significant immune response or foreign body reaction. The cancer cell, in order to survive, produces little or no detectable response of an antigenic nature, but is apparently accepted by the host organism as a legitimate, if somewhat undisciplined part of itself.

This fact stimulates the question: why are some tissues or cells antigenic and others not? The facile answer that, "only foreign cells are antigenic," not only begs the question - it is not correct. The discovery of a variety of autoantibodies demonstrates the cogency of the question. It is not irrational to believe that the understanding of growth in general and of cancer in particular may await a better understanding of why cells are antigenic in some cases and not in others. Such knowledge might make it possible to produce autoantibodies, i.e. to induce immunological growth inhibitors artificially against cellular types such as cancer which probably have little or no natural antigenicity.

The immediate object of the project is therefore to investigate the causes or sources of cellular antigenicity or its lack - to investigate the origin of the ability of the organism to "recognize" some cells as endogenous and others as foreign and why it sometimes makes mistakes.

Methods and Results: The immediate work of the project can be conveniently divided into three related categories:

I. The phenomenon of specific acquired tolerance: -- The original observation was that when strain DBA mice were exposed to an X-ray dosage of 800 r and then protected from the lethal effects of the radiation by the intravenous injection of (BALB/c x DBA), F_1 bone marrow, the DBA mice, throughout their subsequent life were tolerant to skin grafts from BALB/c donors. Subsequently, it was found that bone marrow from

BALB/c rather than F_1 hybrid mice would accomplish the same result. The effect was profound, almost 100% of the homografts surviving as though they were autografts. The tolerance produced was apparently specific for the antigens contained in the bone marrow, since resistance to other antigens such as those of a C57BL skin graft, returned as soon as the mice recovered from the acute effects of the radiation. Further studies suggested that second set homografts are accepted as well as first set by "tolerant" mice. To-date, attempts to reverse the tolerance by injections of immune splenic tissue have failed. Preliminary results suggest that the tolerance producing factor may be subcellular and perhaps capable of serial transmission in irradiated hosts. The phenomenon is not limited to strain BALB/c grafts to strain DBA recipients, but has been demonstrated with (CxDBA) F_1 grafts to (LxA) F_1 mice and with BALB/c grafts to C3H. It is apparent however, that certain strain peculiarities do exist although these have not yet been fully explored. A qualitatively similar though quantitatively much weaker effect has been obtained by substituting L.D. 50 doses of nitrogen mustard for the radiation.

II. Antigenicity of methylcholenthrene induced sarcomas: ---- A number of investigators have reported the apparent production of isologous immunity to methylcholanthrene induced sarcomas. Since it is the prevailing opinion that tumors are non-antigenic in the animal or strain of origin, the results of these investigators have been attributed to an unrealized genetic heterogeneity of materials. However, it has now been found in this laboratory that such immunity can be regularly produced by MCA induced sarcomas but not by spontaneous sarcomas or mammary carcinomas. It is, therefore, apparent that if the immunity produced is due to the small residual genetic heterogeneity, then MCA induced sarcomas are for some reason peculiarly susceptible. The only other possible explanation would seem to be that, due to some peculiarity of the MCA, those tumors may differ from the others by being antigenic in the animal of origin - i.e. they may be capable of producing true autoimmunization. Fortunately, experimental means are available to distinguish between these alternative hypotheses.

III. Miscellaneous: A number of miscellaneous but related experiments have been done during the past year: a. The growth of strain C skin grafts in (CxDBA) DBA backcross mice has been studied. Results indicated that 5 or more histocompatibility genes were segregating. b. The claim of Hardin and Verder that multiple successive skin homo-grafts could produce a state of acquired tolerance was investigated. The results did not substantiate this claim. c. Red cell agglutination within diffusion chambers was studied. Red cell agglutinins could not be demonstrated in the chambers in vivo even in immune mice. d. The immunizing power of red cells in diffusion chambers was investigated. It was found that the diffusion chamber prevented immunization even though the red cells themselves remained potentially antigenic. e. It was found in a preliminary experiment that, contrary to the results obtained at other sites, intra-diffusion chamber homologous cells were killed when diffusion chambers were implanted intra-splenically in immunized mice. f. An attempt with the aid of X-radiation and bone marrow to produce an effect in mice by the Rous chicken tumor agent

was an apparent failure. g. An attempt to "adapt" tumor cells to a foreign strain by prolonged growth of the tumor cells within diffusion chambers in the foreign strain gave possibly slight but generally inconclusive results. This despite the exposure of the cells to the foreign medium for a period of 9 months. h. A preliminary study of the efficacy of various routes of administration in producing immunity to homologous rod cells has suggested that the subcutaneous is significantly poorer than the intravenous, intraperitoneal or intracutaneous routes. i. A statistical study of the influence of the occurrence of a mammary tumor upon the probability of the occurrence of a second in the same mouse was undertaken in high tumor strain C3H mice. The results are not yet apparent. j. A study was made of the effects of trypan blue on MCA carcinogenesis. This was inconclusive. k. Preliminary results suggest that the "immunization" of adult BALB/c mice to the mammary tumor agent interferes with the growth of transplantable BALB/c mammary tumors.

Major Findings and Significance: Two findings are possibly of major importance:

a. The production of specific acquired tolerance by X-radiation and bone marrow administration. This is a significant advance in our understanding of the mechanism by which an animal is capable of distinguishing foreign from endogenous cells. It is too early to say whether this work will in addition, have any direct practical application though it is apparent that a useful investigative tool has been discovered.

b. The antigenicity of isologous MCA induced sarcomas. This work demonstrates an apparently unique characteristic of such tumors. It is, however, too early to foresee where this observation will lead.

Proposed Course of project:

A. With regard to the specific acquired tolerance produced by radiation and bone marrow administration, it is planned to investigate the following. Some of these studies are already under way.

a. Farther studies of specificity and possible strain limitations.
b. Effects of varied dosages of radiation and of marrow on the phenomena.
c. Effect of varying the period between radiation and antigen administration.
d. Can tolerance be produced in immunized mice?
e. Effect of different routes of foreign antigen administration.
f. Influence of tissues other than marrow as a source of foreign tolerance producing factor.
g. Study of possible changes in transplantation specificity of the skins of tolerant mice or of grafts in tolerant animals.
h. Further study on the cell free nature of the tolerance factor and its serial transmissibility.
i. Can tolerance be reversed by marrow and X radiation treatment.

It is doubtful if time will permit all of these studies to be initiated, let alone completed during the following year.

B. With regard to the antigenicity of MCA induced sarcomas, a initial experiment is now in progress to determine whether autologous MCA tumors will show the same antigenicity as isologous. The future of this

investigation must await the results of that experiment.

10. 424
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		TECHNICAL ASSISTANCE	

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 424
SERIAL NO.

15.
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955.

1. Main, J. M., and Prehn, R. T.: Successfull skin homografts after administration of high dosage x-ray and homologous bone.marrow. J.N.C.I. 15: 1023 - 1028 (1955).

2. Weaver, J. M., Algire, G. H., and Prehn, R. T.: Growth of cells in diffusion chambers II. J.N.C.I. 15: 1737-1767 (1955).

3. Prehn, R. T., Algire G. H., and Weaver, J. M.: Diffusion chamber in homograft research Trans. Bull. 2: 147 (1955).

4. Prehn, R. T., and Main J. M.: Lack of immunizing capacity of homologous cells within diffusion chambers J.N.C.I. - In press.

16.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute — institute
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. 426 — SERIAL NO.
6. "Effects of the A^y and ob Genes on Mouse Metabolism and Physiology" — PROJECT TITLE
7. George L. Wolff — PRINCIPAL INVESTIGATOR(S)
8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objective: The objective of this project is to determine the primary and pleiotropic effects of the A^y (lethal yellow) and ob (obese) genes on mouse metabolism and physiology, especially as related to the increased lung cancer incidence in $A^y a$ mice and to the hormone balance in mice carrying the A^y and/or the ob genes.

Methods Employed: An inbred strain of mice carrying the A^y and ob genes is being developed in order that the effects of these genes, both singly and in combination, may be studied against the identical genetic background.

The anaerobic glycolysis of Harding-Passey melanomas (in the presence or absence of exogenous insulin and/or testosterone) after transplantation to normal and gonadectomized, male and female, Ob- and obob, $A^y a$ and aa mice is determined by means of a Warburg respirometer apparatus.

The anaerobic glycolysis of kidney minces (in the presence or absence of added insulin and/or testosterone) of male and female Ob- and obob mice at various ages, after growth of the Harding-Passey melanoma, or after exposure to high temperature (35°C) has been studied by the same method.

Acid and alkaline phosphatase activities of $A^y a$ and aa mouse lung and Harding-Passey melanoma homogenates are being determined.

The possibly differential resistance of Ob- (non-obese) and obob (obese) mice to high temperature stress is being studied by exposing the animals to 35°C for 24 hours.

Major Findings: The yellow obese mouse phenotype (presumably A^yaobob) has been produced and found to be viable.

The rate of establishment and growth of the Harding-Passey melanoma, six weeks after inoculation, has been found to be higher in obob mice than in Ob- mice.

The in vitro rate of anaerobic glycolysis of the Harding-Passey melanoma grown in obob females was found to be higher than that of melanomas grown in any other sex-genotype group, including ovariectomized obob females. In the presence

of exogenous testosterone this difference has a probability of less than .01 of being due to chance.

Stimulatory effects of exogenous insulin on melanoma glycolysis were observed in the presence of added testosterone.

In vitro kidney mince glycolysis of obob mice has, in general, been found to be higher than that of Ob- mice at 30 and 63 days of age and after 24 hours' exposure to 35°C. Female kidney glycolysis in all of these categories tended to be higher than the corresponding male kidney glycolysis.

Growth of the Harding-Passey melanoma depresses the in vitro kidney mince glycolysis of obob mice, but seems to have no effect on that of Ob- mice.

Testosterone inhibits in vitro kidney mince glycolysis of Ob- and obob mice, while insulin has been found to stimulate kidney mince glycolysis, especially after the mice had been exposed to 35°C.

The partial control of the potential glycolytic capacity of mouse kidney and Harding-Passey melanoma by an insulin: anti-insulin system and a pleiotropic effect of the obob genotype on this system is suggested by the data obtained.

Significance to Cancer Research: In order to obtain an understanding of cancer, it is necessary to learn how genes control the balance of hormones regulating normal and tumor cells so that any difference between the latter may be exploited in the treatment of neoplasms.

To explain the relatively high incidence of lung tumors in mice carrying the AY gene as opposed to a relatively lower incidence in aa littermates, it is necessary to know which specific metabolic reaction is under the control of this gene. The solution of this problem might ultimately provide a method of preventing lung tumors and of treating lung tumors chemotherapeutically.

The use of the Harding-Passey melanoma as a bio-indicator of the hormonal balance and the study of the combined and separate effects of the AY and ob genes on this hormonal balance are designed to provide clues to possible relationships between altered metabolism and lung cancer.

Proposed Course of Project: The determinations of alkaline and acid phosphatase activities in A/a and aa mouse lung homogenates and in Harding-Passey melanomas grown in A/a and aa mice will be continued and extended to determine specific phosphatase activities.

As soon as the new strain carrying both the AY and ob genes is sufficiently inbred, determinations of the in vitro rates of glycolysis of Harding-Passey melanomas grown in normal and gonadectomized, male and female mice of the four different phenotypes (AYaOb-, AYaobob, aaOb-, aaobob) will be begun. Similar determinations will be made on non-malignant tissues, e.g. kidneys, of these mice.

The role of adrenal steroids in the insulin: anti-insulin system in relation to the AY and ob genes will be investigated.

10. 426
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		TECHNICAL ASSISTANCE	

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 and 16.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Leukemia Studies Section — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. 427 — SERIAL NO.

6. Studies on the etiology and chemotherapy of experimental lymphomas.

7. Lloyd W. Law — PRINCIPAL INVESTIGATOR(S)

8. Michael Potter in certain collaborative projects. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Our investigations in leukemia and other lymphomas in mice come under two major headings: 1) Studies of the etiology and pathogenesis of the disease, and 2) Studies of mechanisms involved in inhibition of leukemic cell growth through the use of certain selected compounds, particularly such antimetabolites as antifolics, antipurines, and antipyrimidines, e.g., methotrexate, 6-mercaptopurine, 8-azaguanine, azaserine, 6-azauracil, and the pyrazolo pyrimidines.

Heretofore major emphasis has been placed upon lymphocytic neoplasms. As may be seen from this report, our interest is now extended to include etiology and therapy of other morphologic forms, such as Type A and Type B neoplasms (Dunn), plasma cell neoplasms, etc.

1) Studies of the etiology and pathogenesis of experimental leukemias.

A. The rôle of the thymus in spontaneous cases.

Additional data are now at hand concerning the role of thymic tissue in the production of lymphocytic neoplasms in (C3H x AKR)F_1 mice. AKR thymic fragments significantly increase the incidence and time at death from leukemia and, in the majority of cases (57 of 77), the transplanted thymic tissue is neoplastic. C3Hf thymuses have no such influence and none of the recovered thymic fragments were found to be neoplastic. AKR spleen likewise is found to be ineffective. This study has been extended (with Dr. M. Potter) to include the rôle of AKR thymic tissue in thymectomized (AKR x C3H) mice. It is known (from a small series) that the incidence of lymphomas in thymectomized (AKR x C3H) mice is 56% (compared to 53% in intact controls), but the average age at death following thymectomy is increased from 14.8 to 19.0 months. Furthermore, lymphocytic neoplasms have not been found in the thymectomized mice.

(Project description continued)

B. The influence of grafted thymic tissue following exposure of recipient mice to x-radiation.

Certain data are now available concerning the influence of C57BL thymic tissue grafted into thymectomized, irradiated 4 x 225 r (C57BL x A)F_1 mice:

1. Thymectomectomy completely inhibits the induction of lymphocytic neoplasms (but not type A reticulum cell neoplasms).

2. Grafted thymic tissue, from 1 to 10 day old C57BL mice, becomes leukemic in 80% of the cases if transfer is made either on day 1 or day 7 after x-radiation.

3. Transplantation studies of these induced neoplasms indicate that those arising early, at 5 and 6 months (4 cases), transplant to C57BL and the F_1, whereas those arising later, 8 to 10 months (9 cases), grow progressively only in the F_1 mouse (not in C57BL, origin of thymic tissue).

These results provide the information suggested by Kaplan of an "indirect mechanism of x-ray induction" dependent on the post-irradiation state, but also provide further data on the thymic "sphere of influence", indicating that this tissue is capable of inducing lymphocytic neoplasms following repopulation with F_1 recipient round cells.

The following additional experiments using this model are now underway:

a) Influence of strain A thymic grafts of various ages 1, 10, 30 days on the induction of lymphocytic neoplasms.
b) Study of the persistence of the post-irradiation state. Thymuses from 1-7 day C57BL donors have been grafted at 1, 7, 14, and 30 days.
c) Influence of C57BL and A bone marrow on the induction of leukemia in grafted C57BL thymuses in thymectomized, irradiated (C57BL x A) mice.

C. Influence of thymectomy in C3H/Fg subline.

Nearly 80% lymphomas have been observed in a milk-agent free subline of the C3H strain obtained from Dr. Figge. Approximately one-third of these neoplasms are lymphocytic but very few involve thymic tissue. On the other hand, fractionated x-radiation (4 x 90 r, weekly), produces a preponderance of lymphocytic neoplasms. Thymectomy has been accomplished in 2 groups: those observed for the spontaneous disease and a group receiving x-radiation.

D. The role of cell-free materials in the induction of leukemia and parotid gland neoplasms.

In a study conducted jointly with Dr. Thelma Dunn, Pathology, results have been published concerning the incidence of leukemia, parotid gland neoplasms and other neoplasms in C3Hf/Lw, (C3H x AKR)F_1 and (C3H x C3H/Fg)F_1 mice following introduction of leukemic materials (extracts, centrifugates, and filtrates) from the high-leukemic lines AKR, C58, and C3Hf/Fg. The incidence of

(Project description continued)

leukemia and the age at death were found not to be influenced by the introduction into young (< 24 hour old) mice.

Certain other observations, however, were of interest:

1. Parotid gland neoplasms were found in all 3 groups of test mice, especially in the (C3H x C3H/Fg)F_1 cross.

2. In association with the parotid neoplasms in this latter group (25%) nearly all the test mice showed highly invasive subcutaneous tumors.

3. Many other uncommon forms of neoplasms were found, e.g., adrenal medullary tumors, early mammary tumors, but these occurred among the control as well as the experimental mice.

Since Gross has suggested that subline differences may play a part in the response to a leukemogenic agent, this work has now been extended to include 105 C3H/Bittner subline mice inoculated < 24 hours with centrifugates (9500 RPM) and filtrates (Berkfeld and Selas) from 12 different spontaneous AKR leukemias. These same preparations have been given simultaneously to 85 C3Hf/Lw subline mice.

We had observed, at the last annual report, that a transplantable adrenal medullary neoplasm gave nearly 40% parotid gland neoplasms in C3H and (C3H x AKR) mice when tested at the G2, G3, and G8 transfers. Consequently, a series of studies were commenced relating to:

1. Influence in different inbred mice;
2. Effects of different types of filtration;
3. Tests for activity after various periods at -60°C.;
4. Titration of stored material.

More than 250 mice were set-up and, to date, after 11 months, no parotid tumors have been observed and the ability of L5665 (the adrenal medullary neoplasm) seems to have disappeared with the later transfer generations.

Crosses between the 2 sublines discussed above, C3Hf/Lw and C3H/Fg, have now been made reciprocally, and inoculations of cell-free materials from AKR and C3H/Fg leukemias are being accomplished into infant mice to study the unusual occurrence of a high incidence of parotid gland neoplasms associated with subcutaneous neoplasms. The subcutaneous growths appear to differ morphologically and biologically from those seen spontaneously; for example, in old C3H strain mice.

E. Study of certain protective factors in induced and spontaneous lymphomas.

1. Bone marrow repressive factor.

a) Data are now nearly completed on the influence of high-leukemic (AKR) bone marrow and low-leukemic (C3Hf) bone marrow on

(Project description continued)

spontaneous and x-rayed induced leukemia in (C3Hf x AKR)F_1 mice. It is clear that C3Hf bone marrow represses lymphomas in these hybrids (36%) whereas AKR bone marrow influences the incidence and mean age of leukemic death in a positive direction (75%). It is clear also that 4 monthly IV inoculations is most effective in repressing lymphomas in the F_1 hybrid (7% at 12 months compared with 27% in controls).

b) This work is being extended (by Miss Delta Uphoff) to study the influence of C3Hf bone marrow in the AKR strain. Periodic inoculations of bone marrow into AKR mice of various ages from 2 weeks to 3 months have been commenced.

c) It has been found (Uphoff) that intact bone marrow cells from certain H-2 lines will protect against other H-2 irradiated mice (mortality), e.g., C58 bone marrow gives maximum protection to (C3Hf x AKR)F_1 mice. This would appear to be a good experimental model to study the relationship of protection against 1) mortality; 2) thymic degeneration;and 3) induction of lymphomas by radiation. Such studies are now in progress.

d) The role of bone marrow in protection against x-ray induction of Type A and B reticulum neoplasms is being investigated in DBA/2 thymectomized, irradiated mice, and in BAF_1 thymectomized, irradiated mice. In both these groups there appears to be an induction by x-rays of Type A and B neoplasms.

2. Maternal resistance influence (MRF)

An attempt to further characterize an influence of resistance to leukemia in AKR and C58 mice by foster-nursing upon old (> 32 week) STOLI mothers.

Although AKR mice in most instances show less leukemia, which appears late in life (beyond 1 year), there are found litters which develop the disease as expected even though fostered by old STOLI mothers.

F_2 and F_3 mice obtained from AKR mothers,fostered on old STOLI mice,are now under observation to determine if MRF can be passed on in successive generations.

Following reciprocal crosses are now being accomplished in the hope of studying the influence of MRF on radiation-induced lymphomas:

(Old) STOLI x C57BL/Ka

(Young) STOLI x C57BL/Ka

Fractionated irradiation at 90 r x 4, of 7 days, and at 225 r x 4, of 7 days.

(Project description continued)

2) Studies of mechanisms involved in inhibition of leukemic cell growth.

A. Studies are now underway to develop resistant and dependent variants of other lymphocytic neoplasms and, in addition, of type A and B reticular neoplasms. Our interest here is to determine if cross-resistance and collateral sensitivities are the result of specific drugs used, or are dependentin part on the neoplastic cell population. Dr. H. Potter has established, in ascitic form, a battery of 15 such neoplasms from which selection will be made. From this group an Azaserine-resistant plasma cell neoplasm 70429, which exhibits a new pattern of resistance, has been established. This resistant population of cells will be of interest since specific sites in biochemical pathways at which Azaserine acts are known (in pigeon lines and E. coli systems). The response to various antileukemic agents is being studied of neoplasms which do not show exponential growth in the ascitic form.

B. In collaboration with Dr. Arnold Welch, Yale University, studies are in progress of several uracil and orotic acid analogs which have shown great promise as inhibitors of bacterial growth. Three of these compounds: 6-azauracil and the methyl and benzyl derivatives of 6-uracil sulfone have been shown to give striking inhibition of the neoplasms L1210, L4946, and L5178 only if given at least 3 times daily. It has been found also that if given in the drinking H_2O (5 mg/ml) optimal inhibition is attained, thus simplifying the projected work of 1) developing resistance to these compounds and 2) studying reversal mechanisms.

C. In collaborative work with Dr. Charles Nichol of Yale University studies are being made on 1) content of PGA; 2) capacity to alter PGA to compounds measurable as CF; and 3) differences in sensitivity to PGA antagonists in in vitro systems employing ascitic forms of our L1210 resistant and dependent variants.

Certain findings to date are of interest:

1. An extremely high activity of ascitic cells in contrast to lymphomatous cells has been found, making possible the development of cell-free systems to study such changes as cell permeability and differences in enzyme systems as they relate to resistance.

2. Antipurine variants have a far greater capacity to convert PGA to CF-like compounds in comparison with the sensitive ascitic cells, and also show a striking sensitivity to inhibition of this conversion by A-methopterin. These results fit in well with studies on formate incorporation, and suggest a shift in the metabolic pattern from utilization of exogenous metabolites to one involving de novo synthesis from precursors in the cell.

10. 427
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH [x] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

12. Laboratory of Pathology*
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

* Provided personnel for collaborative work in certain projects.

13. None.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 427
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Mandel, H. George and Lloyd W. Law; "The Effect of 4-Amino-5-imidazole-carboxamide on the Carcinostatic Action of 8-Azaguanine." Cancer Research 14: 808-11, December 1954.

Law, L. W.:"Studies on Transformations to Resistance and Dependence in Leukemic Cells," Origins of Resistance to Toxic Agents, pp. 268-286, 1955, published by Academic Press, Inc., New York.

Law, L. W., Thelma B. Dunn, and Peter J. Boyle: "Neoplasms in the C3H Strain and in F_1 Hybrid Mice of Two Crosses Following Introduction of Extracts and Filtrates of Leukemic Tissues," J. Nat. Cancer Inst. 16(2): 495-539, October 1955.

16. None.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Leukemia Studies Section
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. 427(a)
SERIAL NO.

6. Studies on the etiology and chemotherapy of experimental lymphomas.
PROJECT TITLE

7. Michael Potter
PRINCIPAL INVESTIGATOR(S)

8. -
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

1) Leukemogenesis: chemical

DBA^{F}/2 mice have been painted with 0.2% methycholanthrene in ether 3 x /wk in various experiments. Many of the experiments in progress here have revealed that this mouse develops many different morphologic forms of reticular neoplasms, mainly Type A and B reticulum cell sarcomata. Some of the information obtained, it is hoped, will reveal a method for inducing such types of reticular disease with greater regularity.

A. Path of action

Within cages, mice have been selected and painted, while others in the same cages have not been painted. This study has been designed to determine the role of absorption through the skin as the path of introduction of the carcinogenic material. If the non-painted cage mates develop leukemia to the same degree as the painted mice, greater emphasis will be placed on studying the oral route of this material in leukemogenesis. Ten cages have completed the painting schedule; in these cages there are 32 painted and 28 non-paintedmice.

B. Crude tobacco smoke condensate

Crude tobacco smoke condensate has been obtained and is being painted on the skin of DBAF/2 mice. The carcinogenic activity of this crude material, if based on the presence of hydrocarbons, may increase the incidence of lymphocytic neoplasms in these animals prior to one year of age. The mode of action of known carcinogenic hydrocarbons in this mouse, i.e., a cumulative action on a distant system, administered during a susceptible physiologic age, suggests this type of biologic test may indicate the presence of materials leukemogenic to the mouse. Through the cooperation and kindness of a commercial firm a large supply of crude tobacco smoke condensate is being made available, and with the supervision and cooperation of Dr. J. Hartwell of this Institute, concentration of hydrocarbons by vacuum distillation will be carried out and this material will also be tested. In experiments thus far, the presence of nicotine has limited the use of this material, and probably obscures the action of substances which may be present. The complexity of any

(Project description continued)

biologic test of this material is taken into consideration.

C. The influence of bone marrow on methylcholanthrene-induced lymphocytic neoplasms.

DBA F/2 mice painted in the routine manner for 10 or 20 treatments have received 2 intravenous bone marrow injections following the last treatment. In each case the bone marrow is of DBA F/2 origin. The dose of bone marrow consisted of that amount of bone marrow obtained from 2 femora, 2 humeri, and 2 tibia from one mouse. The injections were given on the 2nd and 16th day following the last painting. Ninety-six mice are involved in the experiment: 48, 10 painting; 48, 20 painting; each group further subdivided in half and half treated and not treated with bone marrow.

D. The comparative action of x-radiation and methylcholanthrene in the induction of reticular neoplasms in the DBA F/2 mouse.

The DBA F/2 mouse develops lymphocytic neoplasms in the thymus and in other organs in the reticular system. This mouse also has a moderate incidence of spontaneous reticulum cell sarcomata, of the A and B type. Gonadectomy in this mouse has been reported by Kirschbaum to have two effects on methylcholanthrene-induced lymphocytic neoplasms in the DBA/2 mouse: (1) increase the incidence of lymphocytic neoplasms; (2) to overcome the physiologic barrier of age.

In order to test in the same strain mouse whether the mechanisms of leukemogenesis are the same, DBA F/2 mice have been irradiated with 125 r every 7 days, for 4 exposures; subgroups of thymectomized mice, and gonadectomized mice, have been included. Other pertinent groups of mice are included. More animals are being added to the series.

Group		At 10 - 12 months		
Intact	X-ray	63	13	20.3%
Gonadectomized	X-ray	21	5	24 %
Gonadectomized	None	42	0	0 %
Thymectomized	X-ray	19	0	0 %
Intact	MCA	30	12	40 %

It has been found by examination of the autopsy data, and leukemia tissue sections, that the character of methylcholanthrene/is quite different from that of x-ray/leuk. X-radiation appears to produce a monotonous type of lymphocytic neoplasm in the thymus, whereas in the methylcholanthrene group lymphocytic neoplasms, in combination with type B reticulum cell sarcomata, have been found with great frequency. Gonadectomy thus far has not greatly augmented x-radiation-induced leukemia in the DBA F/2 mouse. Further studies are in progress.

(Project description continued)

2) Genetic Studies

In 1946 Furth described multiple osteomata in thymectomized AKR mice. Those tumors appeared in animals of 12 months of age or over. Normally this type of growth would not be seen in these mice because the mice would be dead of leukemia before the osteomata could appear. Employing papain digestion of mouse carcasses, skeletons of (C3H x AKR)F_1 mice 12 months and older were found to contain those neoplasms. They are multiple; commonly appear on the skull, pelvis and lower extremity; almost never in the upper extremity; more common in females (over 80% of females have one or more of these lesions after 14 months). An interesting finding has been that these lesions have not been seen in only 2 of 37 (AKR x STOLI)F_1 and (STOLI x AKR)F_1 mice of similar age. This problem is of real interest since the incidence of leukemia in the 2 AKR hybrids is also different. STOLI is known to contribute a nursing influence which delays and inhibits leukemia. Further studies employing other AKR hybrids, and genetic studies of backcross animals, are underway.

Osteomata

Strain	Sex	No.	No. of animals bearing tumors	Total number tumors
C3H x AKR	♀♀	27	21	95
	♂♂	28	15	32
STOLI x AKR	♀♀	17	2	3
	♂♂	20	0	0

3) Hormonal Studies

A. Gardner has reported that estrogen treatment of C3H mice, of the Strong subline, developed thymic neoplasms following estrogen treatment. Weekly doses of 20 mgm of estradiol benzoate are being administered to 2 sublines of C3H. The C3H F/Lw and the Zf line from Bittner. Gross has reported that subline differences exist, when he attempts to induce leukemia with extracts of AKR leukemic tissue. It is of interest to determine if a similar difference exists with estrogenic induction of thymic tumors.

B. A study of leukemogenesis in (DBA x CE)F_1 and (CE x DBA)F_1 mice. Virgin females of this hybrid type develop hyperestrogenism, characterized by a proliferation of the endometrium. Reciprocal hybrids of these strains have been produced, though it has taken some time to get enough CE mice, and the first group has been painted with methylcholanthrene. Lymphocytic neoplasms in the virgin females will be transplanted to determine if these neoplasms are dependent on this abnormal hormonal state. The role

(Project description continued)

of genetic factors, gonadectomy, etc. will be evaluated. To date, no lymphocytic neoplasms have been found in the DBA x CE hybrid mice.

4) The development of transplantable reticular neoplasms of the mouse, in ascites form, for the study of chemotherapeutic mechanisms.

A spectrum of transplantable tumors, principally in DBA/2 mice, has been developed in the last year in order to:

a. increase the range of morphologic forms which can be studied with chemotherapeutic agents;

b. to test differences within morphologic classes of response to the antimetabolites, azaserine, methotrexate, 6-mercaptopurine.

For this purpose the following tumors have been established, and some of their characteristics are listed.

A. Lymphocytic Neoplasms

A series of 10 ascitic lymphocytic neoplasms, originating in the DBA/2 mouse, has been developed. With these ascitic tumors studies on drug sensitivity were carried out; further, using cell doses varying from 10^7 to 10^2 cells, titrations of these tumors were undertaken. In order to establish true sensitivity or resistance to a given antimetabolite, with survival time as the end point, the cell population must be uniform. Studies of titration reveal that/Semi-uniformity is acquired in most cases gradually./ straight line re- log-lationships between cell dose in log units and days of survival arith-are the criteria for uniformity. Such relationships have been mic found to hold for L1210, and for 3 of the tumors in this series, P288, P312, P335. These straight lines have been found to extend from cell doses of 10^7 to 10^2. Some tumors have never shown this character or have developed it very slowly. L5178 and P330, and the plasma cell tumor, 70429, are examples.

In many experiments employing antimetabolites it has been difficult to obtain good results with early transplant generations, principally because the tumors take too long to kill, thus allowing the animals to live after the maximal optimal dose of the antimetabolite can be given.

From this work facts regarding the administration of antimetabolites to animals bearing tumors of a logarithmic and a non-logarithmic character are being learned.

(Project description continued)

RETICULAR NEOPLASMS

I. Lymphocytic

No.	Induction or origin	Strain	Time of death from doses of 1-3 x 10^6 ascites cells: At generations	Time of death from doses of 1-3 x 10^6 ascites cells: Days survival	Transplant generation at which converted to ascitic tumor
288	MCA*	DBA/2	7 - 11	13.5 - 11.8	3
312	MCA	"	6 - 17	14.2 - 13.1	4
335	MCA	"	7 - 11	14.3 - 11.7	1
389	MCA	"	8 - 9	17.2 - 16.7	1
330	MCA	"	5	42.5	2
433	X-ray	"	5	25.8	1
5178	MCA	"	8 - 15	24.6 - 16.7	?
553	Spont.	"	4	34.2	1
421	X-ray	"	8	-	2
413	MCA	"	8	-	2

II. Reticulum Cell Sarcomata (DBA/2)

228	MCA	"	8	25.2	3
329	MCA	"	6	About 30	3

III. Hodgkins-like Lesion

195	MCA	"	G4	Not ascitic	
L7235	Filtrate ?	C3H	G3	" "	

IV. Plasma Cell Tumor

70429	Spont.	C3H	G21-25	30-40 days	20

V. Granulocytic Leukemia

H5530	Spont.	C58	G2	14-20 days	2

*Methylcholanthrene

(Project description continued)

B. The Reticulum Cell Sarcomata (Type A and B)

Dunn in her review described a classification of reticular neoplasms of the mouse. An important category of neoplams in the mouse has not been evaluated as to the relationship to human disease, cell type involved, and response to chemotherapy. The relationship of these neoplasms to the more chronic lymphomata of man has been repeatedly cited. Type A and B neoplasms have been transplanted in DBA/2 mice. Ten neoplasms are again available and have been transplanted. These neoplasms are being converted to the ascitic form where the cells are studied with phase microscopy. Two Type A neoplasms have been converted to ascites tumors. Two Type B neoplasms have been carried for 3 generations. These neoplasms are appearing sooner and it is hoped this most interesting cell type will be available in the ascitic form. Studies thus far reveal these Type A tumors to be sensitive to triethylene melamine. The problems of administering this drug effectively again have important bearing on human, chemotherapeutic problems.

Two Type B neoplasms, L7235 in C3H, and P195 in the DBA/2 mouse, are unique in their transplant character. These tumors require many months to develop. When they do appear, it is first in the spleen and then generalizes in the lymph nodes. Studies of the morphologic changes during transplantation are being carried out with Dr. Clyde Dawe and Dr. Thelma Dunn of the Pathology Section. These tumors have not yet been tested with chemotherapeutic agents.

C. Plasma Cell Tumor

The plasma cell tumor #70429 arose in the ileocecal region in a C3H/He mouse, and originally was a plasma cell neoplasm. Following 20 transplant generations as a solid tumor, it was converted in one transplant generation to an ascitic tumor. The ascitic tumor cell no longer resembles a differentiated plasma cell. At ascitic generations 3, 9, and 17 this tumor was titered by inoculating doses of cells which varied from 10^7 to 10^3. Cell doses of 10^7, 10^6 cells do not kill anymore rapidly than doses of 10^4. In 5 experiments utilizing 165 animals, employing doses of .35 - 2.25 x 10^6 cells, 87 percent of control animals were dead by the 40th day, whereas only 15 percent animals treated with 20 or more daily doses of 5 mgm/kg b.w. azaserine were dead at the 40th day. It has been found by these and other experiments that:

1. Azaserine is a powerful inhibitor of this tumor.
2. Azaserine is relatively non-toxic and can be given for extended periods, thus providing a chemotherapeutic model for the study of a) the development of resistance, and b) the reasons for eventual curative failure; c) the mechanism of action of azaserine; d) some crude idea of the rate of resistant lines developing in a group of animals can be explored.
3. Two resistant and probably dependent lines of this tumor have been isolated. Others are being tested.
4. Azaleucine, another analog, has been obtained and has been found to also be inhibitory to this tumor. Studies on dosage, cross resistance, and other properties, are underway.

(Project description continued)

D. Granulocytic Leukemia

This tumor was at first a chloroma and has only recently developed. It has developed virulence rapidly. It is in ascitic form and consists mostly of myeloblasts with some myelocytes and more differentiated forms. No studies have as yet been undertaken.

5) Radiation Induced Leukemia

A. The pathophysiologic mechanism by which thymic lymphocytic neoplasms are induced in C57BL/Ka mice are not clearly defined. The most striking influence thus far depends upon fractionation of the x-ray exposures. This suggests that an injury-regeneration phenomenon may be involved. The fractionation procedure has been carried out by Kaplan at 4, 8, and 16 day intervals with little difference in the incidence of leukemia. An experiment is underway in which groups of C57BL/Ka mice are receiving 225 r four times at intervals of 16, 30, and 45 days. This prolongation of the interval is designed to break up the injury-regeneration time-relationship, if such a relationship is the determining factor.

B. The development of the Rf strain (with Dr. L. W. Law)

The Rf strain of mice has been reported to have a moderate incidence of granulocytic leukemia, following the exposure to single doses of 128 r. Rf mice have been bred in this laboratory for the last year, and good breeding colony has been established. Mice from this colony have been used for preliminary, confirmatory experiments regarding the incidence of granulocytic leukemia. The mice have been placed in the following groups:

	Leukemogenic Treatment
1. Intact	128 r single dose
2. Intact	128 r, 4 doses, 7 day intervals
3. Thymectomized	128 r single dose
4. Thymectomized	128 r, 4 doses, 7 day intervals
5. Intact	Painting with methycholanthrene
6. Intact	Weekly injections of 25 y estradiol
7. Intact breeding colony.	

Leukemias are beginning to appear. Several chloromas and non-thymic leukemias have appeared in various groups. This mouse may be the instrument whereby further information on the etiology of granulocytic leukemia will be forthcoming.

10. 427(a)
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH [x] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

12. Doctors Clyde Dawe and Thelma Dunn, Lab. of Pathology, *
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

* All aspects regarding the pathology of reticular disease.

13. None.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHER IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14, 15 & 16: NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Leukemia Studies Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETH.)
5. 427(b) — SERIAL NO.

6. Studies on the etiology and chemotherapy of experimental lymphomas.
PROJECT TITLE

7. Bernard Shacter
PRINCIPAL INVESTIGATOR(S)

8. -
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Biochemical mechanisms of resistance to metabolic antagonists in chemotherapy of experimental lymphomas.

Objectives:

The purpose of this project is to discover the mechanisms involved in the development of resistance to the growth inhibitory action of agents initially active against experimental lymphomas.

Methods employed:

The effects of known chemotherapeutic agents on various metabolic activities of lymphoma cells are determined, in order to establish possible differences in the action of the agents on sensitive as opposed to resistant cells. Metabolic activities studied include uptake of radioactive precursors into proteins and nucleic acids, effects on significant enzyme systems, and effects on concentration of important cellular metabolites.

Major findings:

In the course of determining the effect of amethopterin on glutathione and ascorbic acid levels of sensitive and resistant leukemic cells, it was found that there was a decrease in liver glutathione levels of animals bearing the rapidly growing lymphocytic leukemia L4946 in ascitic form. Administration of 3 mg/kg amethopterin to animals bearing rapidly growing tumors was followed by a cessation of tumor growth, as measured by change in total volume of ascitic cells, and by a return of liver glutathione levels to considerably above normal. The techniques of measuring changes in total volume of ascites cells and changes in liver glutathione levels are being applied as a simple yet precise means for determining action of other possible chemotherapeutic agents on the growth of experimental lymphomas.

(Project description con'td.)

Significance to cancer research:

One of the major problems in the use of chemotherapeutic agents for the clinical control of leukemia is the eventual development of resistance to the action of the drug, such that the leukemic process can no longer be controlled. If it were possible to establish the mechanisms involved in development of resistance, it might be possible to institute measures to either avoid this undesirable effect, or else to circumvent it, perhaps by combined therapy.

Proposed course of project:

During the next calendar year it is proposed to investigate some of the mechanisms which have been suggested as leading toward resistance. These include a study of deamination of 8-azaguanine by lymphomas sensitive to, resistant to, and dependent on the agent, since increased deamination of azaguanine has been proposed as a mechanism for resistance to the agent. The primary effort will be directed toward a search for alternative pathways of synthesis of essential products by resistant cells, as the possible mechanism for development of resistance.

10. 427(b)
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH [x] ADMINISTRATION

REVIEW & APPROVAL TECHNICAL ASSISTANCE

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. No entries for Items 14 & 16.

15. _____
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955.

Shacter, B., Interrelations in Respiratory, Phosphorylative and Mitotic Activities of Ehrlich Ascites Tumor Cells: Influence of Dinitrophenol. Arch. Biochem. and Biophys. 57, 387-400 (1955).

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Leukemia Studies Section — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETH.)
5. 427(c) — SERIAL NO.

6. Studies on the etiology and chemotherapy of experimental lymphomas.
PROJECT TITLE

7. S.E. Reaume
PRINCIPAL INVESTIGATOR(S)

8. None.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Studies on the etiology and chemotherapy of experimental lymphomas.

Objectives: Investigate genetic and biochemical mechanisms involved in the development of drug-resistance and -dependence in mouse leukemias.

Methods employed: 1) Model bacterial systems have been employed for genetic studies of the frequency and mode of development of resistance to purine analogs which are effective antileukemic drugs. 2) In vitro studies have been made of specific biochemical reactions considered likely to be involved in the development of resistance to amethopterin. 3) Patterns of activity of a number of antipurine drugs have been studied with respect to their effect on a number of drug-sensitive, -resistant and -dependent leukemias.

Major findings: 1) No organisms tested have proven to be satisfactory for the genetic investigations.

2) The reaction of most interest in this phase of the work has been the cleavage of aminopterin at C-9 to yield pterdine and p-aminobenzoylglutamate (measured as diazotizable amine). Although such cleavage has been reported in a number of microorganisms, no such activity has been detectable in aminopterin (or amethopterin-)-resistant leukemic lines. Leukemias sensitive to this drug, as well as those dependent upon it, appear also to lack ability to cleave it. Liver, kidney, spleen, and skeletal muscle, from both normal and leukemic mice, showed no such activity.

3) The adenine analog, 4-aminopyrazolo-(3,4-D)-pyrimidine (APP), and guanine analog, 4-hydroxy-6-aminopyrazolo-(3,4_)-pyrimidine (HAPP), have been investigated for their ability to inhibit various leukemias or to satisfy the drug requirement of anti-purine-dependent lines. APP inhibits all sensitive, resistant and dependent leukemic lines tested to the extent of 40 to 60 percent, including one (L-4946) which is naturally resistant to other antipurines. APP does not substitute for 8-azaguanine or thioguanine in lines dependent upon these drugs. Purified HAPP does not inhibit any of the tumors inhibited by APP; an unpurified sample had shown some such activity. An interesting point is that HAPP appears to satisfy the antipurine requirement of an 8-azaguanine-dependent leukemia. APP inhibits the growth of an amethopterin-dependent line. Other,

(Project description continued)

variously substituted, members of this series of compounds, as well as the (4,3-d) congener of APP, have shown little or no antileukemic activity. APP is quite toxic to mice $(C/DBA)F_1$, producing detectable weight-loss at doses of 7.5 mg/kg X 3. At levels of 15-20 mg/kg X 5, severe extraorbital hemorrhages may occur.

All data obtained so far apply to activity in terms of weight of tumors grown as subcutaneous lymphomas. Survival times of mice carrying the same leukemias as ascitic tumors are not prolonged by APP.

Significance to cancer research: 1) If one or more bacterial strains can be found which imitate the responses of our leukemias to the pyrazoles and other antipurines, it is felt that much can be learned about the genetic aspects of resistance and dependence which, thus far, has not been amenable to a direct analysis in the leukemic material. Of particular interest in this respect are data on the frequency of mutation to dependence and resistance; allelic relationships among genes affected; genic interactions such as suppression which may occur. At present, available technics are inadequate to permit a direct attack on such problems involving mutation in leukemic cells.
2) Detailed knowledge of biochemical events involved in the expression of dependence or resistance offer expanded hope of effective chemotherapeutic attack against fulminating or refractory leukemia encountered in the clinic. Negative information, while rarely conclusive, can help narrow the area in which fruitful exploration is likely to be made. It is tentatively concluded that, in our leukemic material, cleavage of aminopterin does not play a role in the expression of resistance to this drug.
3) The activity of 4-aminopyrazolo-(3,4-d)-pyrimidine against antipurine-resistant mouse leukemia suggests that it might be of interest clinically. Little appears to be known concerning its pharmacology or toxicology. Given such information, it might prove satisfactory for trial against antipurine-refractory leukemias, or used in combination with antifolics or other antipurines.

The fact that APP inhibits leukemias resistant to other antipurines, whereas other antipurines show cross-resistance among one another against these same leukemias, suggests that APP is active at a biochemical locus different from that of other purine analogs. The failure of APP to substitute for 8-azaguanine for the 8-azaguanine-dependent tumor is consistent with this view. These and other data suggest several different points of action of purine analogs in the sensitive, resistant and dependent leukemias. Further analysis of the interactions and cross-resistance patterns should yield insights into the biochemical mechanisms underlying them, as well, perhaps, as specific points where rational chemotherapeutic attacks might be made.

Proposed course of project: 1) Further attempts will be made to obtain suitable bacterial strains to serve as genetic models as discussed above. Emphasis will be placed on obtaining antipurine-sensitive, -resistant and -dependent lines of organisms which provide opportunity for relatively extensive genetic analysis (e.g., Escherichia coli, Salmonella spp.).
2) In vitro studies will be concentrated on the problem of pinpointing the loci of action of APP and HAPP in the leukemic lines in which they are active metabolically. An attempt will be made to follow, qualitatively, purine synthesis by tumor homogenates and ascitic cell suspensions and the effects produced by the pyrazoles in these systems. If these surveys are successful, it may be possible to extend and quantitate them by means

(Project description continued)

of radioisotope studies in collaboration with someone competent in such technics. If such brei and cell suspensions prove satisfactory, they could be used in place of the intact animals now being used in studies of reversal of activity of APP and HAPP.

3) Many difficulties have been encountered in attempts to study the effects of the pyrazoles combined with normal purine bases or other purine analogs. These problems arise as a result of the low solubilities of the compounds, toxicity of many combinations, etc., so that activity measurement in terms of tumor weight often become unreliable at best and impossible at worst. Shacter has shown that, under certain conditions, ascitic tumor packed cell volume can be correlated with antileukemic activity. Host liver glutathione levels can also be used as a measure of such activity. It is planned to investigate the extent to which these criteria can be used to replace tumor weight as a measure of antileukemic action.

10. 427(c)
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

12. None.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16.

General Project 428
Serial No.

Study of chemically defined medium and cell nutrition.

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology Laboratory or Branch
3. Tissue Culture Section or Service
4. Bethesda, Md. Location
5. 428(a) Serial No.

6. Project Title: Development of a defined medium for the cultivation of strain L cells in vitro.

7. Principal Investigator(s): V. J. Evans

8. Other Investigators: J. C. Bryant, W. R. Earle, K. K. Sanford, B. B. Westfall, M.C. Fioramonti, and W. T. McQuilkin.

9. Project description:

Objective: To develop a satisfactory defined medium adequate for producing proliferation of strain L mouse cells in order to study their nutritional and metabolic behavior.

Methods employed: Replicate cultures are prepared and enumeration of nuclei are made after exposure to defined media for varying intervals. Such media were devised from the collected information on the nutrition of bacteria, mammalian and avian cells in vitro and the data from physical and chemical fractionations of naturally occurring medium on which the strain L cells are maintained.

Major findings: Several media have been devised and tested. Media have been tested containing amino acids, amides glutathione, vitamins, coenzymes, unsaturated fatty acids, desoxyribosides of nucleic acids, carbohydrate sources and inorganic salts. No antibiotics, fungicides or protein sources are included. A medium has been devised which allows a continued (11 months to date) reasonably rapid rate of proliferation for clone L cells. More recently the unsaturated fatty acids have been omitted from these media with no deleterious effect upon proliferation. Vitamin B 12 has been added with a resulting small increment of additional growth. In a continuation of this work the essential amino acid requirements of strain L have been determined as well as the essentiality of the coenzymes group and the nucleic acid derivatives group.

Significance: A chemically defined medium is essential for determing differences in the physiological between normal and malignant cells and for research in the nature and origin of malignant transformation. The present medium, essential in all respects, serves as a prototype medium for a prototype cell in tissue culture. Such a medium also makes tissue cultured cells a most valuable tool for chemotherapeutic and virological studies. Actually the best chemically defined medium described for strain L cells so far, from these studies has already been found to be an exceptionally excellent medium for virus studies with human cell strains, when it is supplemented with heterologous serum in small volumes.

Projected studies: Continued research will be done to determine the essentiality of each of the groups of components as well as the individual members of the group and to fortify the medium with additional known growth promoting material. At the same time metabolic studies are in progress using this medium.(See report B. B. Westfall)

10. 428 (a)
Serial No.

11.
Budget Activity:

Research	x	Administration	
Review & Approval		Technical Assistance	

12.
Cooperating units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

Medium has been made available to cooperating units of the Public Health Service such as the Microbiological Institute.

13.
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

The organization of Dr. Harry Eagle in the Microbiological Institute is also working on cell nutrition in tissue culture.

14. 428 (a)
Serial No.

15. Publications other than abstracts from this project during calendar year 1955.

Studies of Nutrient Media for Tissue Cells in Vitro. I. A protein-free chemically defined medium for cultivation of strain L cells. Cancer Research 16:00-00 Jan 1956. V.J. Evans, J. C. Bryant, M.C. Fioramenti, W. T. McQuilkin, K.K. Sanford, and W. R. Earle.

Studies of Nutrient Media for Tissue Cells in Vitro. II. An improved protein-free chemically defined medium for long term cultivation of strain L 929 cells. V. J. Evans, J. C. Bryant, W. T. McQuilkin, M.C. Fioramenti, K. K. Sanford, B. B. Westfall, and W. R. Earle, Cancer Res, 16: 00-00, Jan. 1956.

16. Honors and awards to Personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer — INSTITUTE
2. Biology — LABORATORY OR BRANCH
3. Tissue Culture — Section or Service
4. Bethesda, Md. — Location
5. 428 (b) — Serial No.

6. A study of the stability of protein-free chemically defined medium. — Project Title

7. V. J. Evans, — Principal Investigator(s)

8. W. T. McQuilkin — Other investigator (s)

9. Project description:

Objective: To determine whether the chemically defined medium is stable biologically after being maintained in solution at 5° C for a prolonged period of time.

Methods employed: Cultures of strain L-929, mouse fibroblast cells were maintained for a period of six weeks in protein-free chemically defined medium NCTC 109 the component solutions of which had been stored at 5°C as long as 3 to 4 months. The cultures were fluid changed and serially transferred in the manner of routine cultures.

Major findings: The morphology and cell proliferation of the cultures appeared excellent and equal to stock cultures maintained in chemically defined medium that was made up fresh every two weeks.

Significance: Reports in the literature of the development of chemically defined culture media and accepted data regarding the stability of certain of the component solutions indicate rather extreme instability of certain of the components of the medium. According to this information such constituent elements as glutathione, coenzymes, glutamine and vitamin B-vitamin B-12 solutions must be kept fresh or in a frozen state until immediately before use. To know that the chemically defined medium is not so biologically unstable makes possible substantial saving of labor in the repetitious preparation of the medium. It also makes possible a wider range of use of the medium in experimentation.

Proposed course of project: To study other aspects of the stability of the medium with the view toward further simplification of its preparation.

10. 428 (b)
Serial No.

11.
Budget activity:

Research	x	Administration	
Review & approval		Technical assistance	

12. None
Cooperating units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 428 (b)
Serial No.

15. None
Publications other than abstracts from this project during calendar year 1955.

16. None
Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service — 4. Bethesda, Md. Location — 5. 428(c) Serial No.

6. Project Title: The effect of horse serum residue and certain chemically defined supplements on prolieration of strain L clone 929 cells from the mouse.

7. Principal Investigator(s): V. J. Evans

8. Other investigators: M. C. Fioramonti, K. K. Sanford, W. R. Earle, J. C. Bryant, W. T. McQuilkin.

9. Project description:

Objective To develop a partially defined medium incorporating washed serum residue and free amino acids as well as other growth factors that is capable of supporting proliferation of strain L cells as the whole serum.

Methods employed: The replicate culture techniques accompanied by nuclei enumeration methods were used to obtain data on the action of horse serum residue obtained by ultrafiltration supplemented with certain chemically defined media. The horse serum residue was obtained by ultrafiltration procedure as described by Sanford et al. The supplements were those of Morgan, Morton and Parker's mixture 199.

Major findings: Quantitative experiments were used to test the effect of supplements to a basic medium of the residue fraction remaining after ultrafiltration of horse serum. This study was carried out on washed cell suspensions of clone 929 strain L cells, originally obtained from a strain C3H mouse. These cultures were planted in T-15 flasks on glass substrate and changes in population levels were determined by enumeration of the nuclei at seven, ten, thirteen, fourteen and twenty-one day intervals. The unsupplemented fraction of the horse serum was incapable of maintaining the inoculum level beyond seven days. This residue medium, supplemented with the amino acids, amides and amine of horse serum was less effective in maintaining population levels. The further addition of niacin, p-aminobenzoic acid, niacinamide, pyridoxine HCl, thiamin HCl, d-Ca pantothenate; i-inositol, choline chloride; riboflavin, ascorbic acid, glutathione, cysteine HCl, biotin, folic acid, vitamin A, vitamin D(calciferol), tween 80, menadione, vitamin E and ATP as contained in mixture 199 of Morgan, Morton, and Parker gave population levels superior to those obtained by use of unfractionated horse serum. Under the experimental conditions used, addition of the other components of mixture 199 to this medium gave no added increase in proliferation and, in fact, indicated a possibly inhibitory action.

Significance: Development of a chemically defined medium is of major importance for ultimate comparison of normal and malignant cells and this was one step in the development of such a medium.

Proposed course of project: Continued study to develop a chemically defined medium of general use.

10. 428(c)
Serial No.

11.
Budget activity:

Research x	Administration
Review & Approval	Technical Assistance

12. None
Cooperating units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

The organization of Dr. Harry Eagle in the Microbiological Institute is also working on cell nutrition in tissue culture.

14. 428 (c)
Serial No.

15.
Publications other than abstracts from this project during calendar year 1955.

The effect of horse serum residue and chemically defined supplements on proliferation of strain L clone 929 cells from the mouse. Cancer Research v. 15: 00-00, 1955. M.C. Ficramenti, J.C. Bryant, W. T. Mc Quilkin, V. J. Evans, K. K. Sanford, and W.R. Earle.

16.
Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section Section or Service — 4. Bethesda, Md. Location — 5. 428 (d) Serial No.

6. Simplification of a protein-free chemically defined medium NCTC 109
Project Title

7. V. J. Evans
Principal Investigator(s)

8. J. C. Bryant, N.M. Hawkins and M.C. Ficramenti, K. K. Sanford and W.R.Earle
Other Investigators,

9. Project Description:

Objective: To determine the essentiality of individual members of component groups of coenzymes mixtures glucuronic acid mixtures and fatty acid now in the complex chemically defined protein-free medium NCTC 109 used to grow strain L-929 cells.

Methods employed: Replicate cultures of strain L cells are prepared and enumeration of nuclei are made after exposure to the test medium for varying intervals. From changes in the number of nuclei on test medium in relation to the complex but complete medium containing at least all the essentials and perhaps some non-essentials for strain L it is possible to determine the essentiality of each individual component.

Major findings. Exploratory data indicate that it may be possible to omit. Glucuronolactone, methyl linolenate, methyl arachidonate, diphosphopyridine nucleotide, cocarboxylase, flavin adenine dinucleotide, uridine triphopyridine nucleotide.

Significance: The data indicate that it is possible to prepare for practical purposes a simpler medium. Further, it is learned that the substances which can be omitted are not essential nutritional requirements for this strain of cells because their absence does not result in lessening of growth or cause death of the cultures.

Projected studies: Continued simplification of the medium will be pursued and the data will serve to act as a basis for determining the nutritional essentials for other normal and malignant cell strains.

10. 428 (d)
Serial No.

11.
Budget activity:

Research	x	Administration	
Review & Approval		Technical Assistance	

12. None
Cooperating units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957

13.
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

The organization of Dr. Harry Eagle in the Microbiological Institute is also working on cell nutrition in tissue culture.

14. 428 (d)
Serial No.

15. None
Publications other than abstracts from this project during calendar year 1955.

16. None
Honors and awards to personnel relating to this project during calendar

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology Laboratory or Branch
3. Tissue Culture Section or Service
4. Bethesda, Md. Location
5. 428(e) Serial No.

6. Project title: Supplemental studies of the effect of ultrafiltrate of whole chicken-egg extract on the cultivation of cells in vitro.

7. Principal investigator(s): V. J. Evans

8. Other investigators: W.T.McQuilkin, J. C. Bryant, W. R. Earle, K. K. Sanford, M.C. Fioramonti.

9. Project description:

Objective: To supplement the study of ultrafiltrate of whole chicken-egg extract that was carried out and reported in 1954, by studying certain aspects of the stability of the ultrafiltrate.

Methods employed: Replicate cultures of strain L cells were cultured on egg ultrafiltrates that had been 1) heated to 60°C for 1 hour, 2) dried and reconstituted, 3) frozen for over two months and 4) a comparison was made in long term cultures between the ultrafiltrate made from fertile eggs and that made from non-fertile eggs.

Major findings: The proliferative ability of the ultrafiltrate was not diminished by heating or by drying and reconstitution. The two-month old frozen ultrafiltrate had a proliferative ability comparable with that of fresh embryo extract medium. The fertility or non-fertility of the eggs used as a source of the ultrafiltrate appeared to make no difference in its proliferative ability.

Significance: These findings emphasize the biological stability of egg extract ultrafiltrate and its potential usefulness as a substitute for embryo extract in tissue culture media.

Proposed course of project: Stock and experimental cultures of various cell strains have been and will increasingly be carried on this medium.

10. 428 (e)
Serial No.

11. _____
Budget Activity:

Research	x	Administration	
Review & Approval		Technical Assistance	

12. None
Cooperating units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 428 (a)
Serial No.

15. In Preparation
Publications other than abstracts from this project during calendar year 1955.

16. _____
Honors and awards to personnel relating to this project during calendar year 1955.

W. T. McQuilkin used this material as a basis for a thesis submitted to George Washington University in partial satisfaction of the requirements for the degree of Master of Arts in Zoology, which was awarded in October, 1955.

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology Laboratory or Branch
3. Tissue Culture Section or Service
4. Bethesda, Md. Location
5. 428 (f) Serial No.

6. Development of a defined medium for the cultivation of human skin cells. Project Title

7. V. J. Evans and W. R. Earle Principal Investigator(s)

8. See Item 12. Other Investigators

9. Project description:

Objective: To develop a satisfactory defined medium adequate for producing proliferation of the human skin strain, #1764. This is essential for use of this cell strain in problems of cancer research, homografting and viral research.

Major Findings: Exploratory data recently obtained indicates that the human epidermal cell strain #1769, will grow in the chemically defined medium NCTC 109 as already worked out in this laboratory provided there is human or horse serum supplementation at low concentration.

Significance: On these media, NCTC 109 previous exploratory virological studies have been successful. For cancer research and human transplantation work such a defined medium is imperative. Information resulting from studies of such a human strain may also serve as exploratory data for preparing a tissue preservation medium for the living cells. The implication of usefulness in nutritional and metabolic studies in relation to skin cancer are manifold and obvious.

Proposed course of project: a) With this strain as with all the others, the essentiality of all the individual components and component groups of medium NCTC 109 are being investigated to improve the chemically defined portion of the medium; b) Similarly the action of the horse serum is investigated to determine its biochemical and biophysical function; c) The chemically defined media will be used to study long term preservation of normal tissue at refrigeration temperatures; d) To use NCTC 109 as medium for studying (the biochemical products and the rejection-acceptance phenomenon. (See separate project)

10. 428 (f) Serial No.

11. Budget activity:

Research	x	Administration	
Review & Approval		Technical Assistance	

12. Cooperating units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 156 or 1957.

Tissue Bank, Naval Medical School, National Naval Medical Center. Much of this work was actually done by Tissue Bank Personnel under supervision of N.C.I., senior investigators.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 428 (f)
Serial No.

15. None
Publications other than abstracts from this project during calendar year 1955.

16. None
Honors and Awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service — 4. Bethesda, Md. Location — 5. 428 (g) Serial No.

6. The effect of serum fractions on the proliferation of strain L cells in vitro. Project Title

7. K.K. Sanford and B. B. Westfall. Principal Investigator(s)

8. W. R. Earle, J. C. Bryant, V.J. Evans, E. V. Peppers, M. C. Ficramenti, and W.T. McQuilkin. Other Investigators

9. Project Description:

Objective: To examine those components of the large molecular portion of serum that appear to be essential for the rapid proliferation of strain L mouse cells in vitro, with the ultimate objective of isolating components that could be added to a chemically defined medium for the purpose of increasing the rate of cell proliferation.

Methods employed: Horse serum was fractionated by a modification of Cohn's low-temperature procedure, Method No. 10. The effect of four serum fractions on the rate of proliferation of a clone of mouse fibroblasts was tested in quantitative experiments.

Major findings: All protein fractions isolated from horse serum were found to promote growth of strain L cells. When tested in a protein-free basal medium, the gross-globulin fraction could be substituted for the large molecular portion of horse serum to yield comparable rates of increase in cell numbers. After removal of the gamma globulins from the gross globulin fraction, the residual globulins could also be substituted for the proteins of whole serum with only slight decrease in numbers of cells. A lower rate of increase in cell numbers was obtained with the albumin and gamma globulin fractions tested at a concentration of 1.3 percent; when tested at a higher concentration, however, (2.6%) the rate of increase in numbers of cells grown in gamma globulins was the same and in the albumins was less than that of cells grown in the same concentration of the unfractionated serum proteins.

Significance to cancer research: Although a protein-free chemically defined medium has been developed for strain L cells, at least four other cell strains in this laboratory grow in the defined medium only when a small amount (0.05 to 1 percent) of serum protein is added. To pursue carcinogenic, metabolic and nutritional studies on these strains, defined serum fractions appear to be essential at the present time.

Proposed course of this project: Experiments will be continued testing the relative value of certain commercial serum fractions prepared as nearly as possible according to the methods developed in the present study.

10. 428 (g)
Serial No.

11.
Budget activity:

Research	x	Administration	
Review & Approval		Technical Assistance	

12. None
Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

13.
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

The organization of Dr. Harry Eagle in the Microbiological Institute is also working on cell nutrition in tissue culture.

14. 428 (g)
Serial No.

15.
Publications other than abstracts from this project during calendar year 1955.

The effect of serum fractions on the proliferation of strain L mouse cells in vitro. K. K. Sanford, B. B. Westfall, M.C. Fioramenti, W.T. McQuilkin, J. C. Bryant, E.V. Peppers, V. J. Evans and W.R. Earle, J. National Cancer Inst. 16:789-802, 1955.

16. None
Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service — 4. Bethesda, Md. Location — 5. 428 (h) Serial No.

6. Study of chemically defined medium and cell nutrition for clone 929 cells. Project Title

7. K. K. Sanford Principal Investigator(s)

8. M.C. Ficramenti, W.T. McQuilkin, J.C. Bryant, V.J. Evans and W.R.Earle. Other Investigators

9. Project description: Attempts to simplify and improve a protein-free chemically defined culture medium, NCTC-108 for the proliferation of strain L mouse cells.

Objective: To determine which components of the chemically defined mix are essential for cell proliferation and to establish the optimal concentrations of these components.

Methods employed: The effects of varying concentrations and depletions of components on the proliferation of strain L cells have been determined by quantitative replicate culture procedures.

Major findings: Of the 26 amino acids, amines, and amides of the protein-free medium NCTC 108, those essential for proliferation of strain L cells have been determined. From dose-response curves on these essentials, new mixtures have been devised and are now being tested.

An analysis is being made of the effects of the deoxyribosides on cell proliferation.

Proposed course of project: This project will be continued until the effect of the deoxyribosides and other nucleic acid derivatives on cell proliferation have been established. The study of the amino acid mixtures will be completed soon.

10. Significance to cancer research: A simplified chemically defined medium is of primary significance in order to determine differences that may exist in the nutritional requirements of malignant cells in vitro as compared with the normal cells from which they arise.

10. 428 (h)
Serial No.

11.
Budget activity:

Research	x	Administration	
Review & Approval		Technical Assistance	

12. None
Cooperating units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

13.
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

The organization of Dr. Harry Eagle in the Microbiological Institute is also working on cell nutrition in tissue culture.

14. 428 (h)
Serial No.

15. None
Publications other than abstracts from this project during calendar year 1955.

16. None
Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology Laboratory or Branch
3. Tissue Culture Section or Service
4. Bethesda, Md. Location
5. 428 (i) Serial No.

6. A study of the preservation and storage of strain L cells. Project Title

7. V. J. Evans Principal Investigator(s)

8. W.T. McQuilkin Other Investigators

9. Project description:

Objective: To determine how long strain L cells could be preserved at 5°C with no, or infrequent, fluid changes.

Methods employed: Flasks of stock cultures were placed in the cold room for periods ranging from three to eight weeks during which times the medium was either not changed at all or was changed at infrequent intervals. When cultures were withdrawn from the cold storage, they were fluid changed immediately and then maintained in the usual manner to determine whether the cells would revive.

Major findings: All cultures in cold storage for 3 weeks survived and recovered satisfactorily -generally within one week after removal from cold room and return to routine procedures. Cultures could survive and recover from an additional three-week cold storage period if returned to normal treatment for about a week beforehand. No cultures survived when stored at 5°C for 5 weeks or longer even with fluid changes at the end of the third or fourth week.

Significance: The usefulness of such preservation methods was under consideration for the maintenance of strains at minimal expense. This work partially corroborates the work done during the same period by Swim and Parker and reported in Proc. Soc. Exp. Biol. and Med., vol. 89; Aug.-Sept. 1955.

10. 428 (i) Serial No.

11. Budget Activity:

Research	x	Administration	
Review & Approval		Technical Assistance	

12. None
Cooperating units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 428 (i)
Serial No.

15.
Publications other than abstracts from this project during calendar year 1955.

16. None
Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology Laboratory or Branch
3. Tissue Culture Section or Service
4. Bethesda, Md. Location
5. 428 (j) Serial No.

6. Project title: Development of a defined medium for the cultivation of mouse liver epithelial cells.

7. V. J. Evans
Principal Investigator(s)

8. W. R. Earle and N. M. Hawkins
Other Investigators

9. Project description:

Objective: To design and test a defined medium which will support proliferation and allow determination of effect of individual components of the medium on substrains of mouse liver cells of low and high tumor incidence, (designated #1469 and #1795 respectively).

Methods employed: Replicate cultures are prepared and enumeration of nuclei are made after exposure of various intervals to the defined media. Such media are devised from collected data on the nutrition of bacteria and mammalian and avian cells in vitro and also from the data on physical and chemical fractionation of naturally occurring media.

Major findings: The prototype media NCTC 107, 108 ; 109 for strain L cellshave been found unable to support continued proliferation in the normal clone liver strain 1469. However, the incorporation of serum fraction such as gross globulin as well as, in as little as 0.05 gm. percent of amino acid free horse serum residue produces minor proliferation of the cells. The essential amino acids and amides for this strain have been determined. Exploratory data indicate to be identical with those required for strain L cells. With this medium, glucose and amino acid utilization, keto acid formation, and formation of lactic acid on both the high and low incidence tumor cell have been determined in replicate cultures. (See report of B. B. Westfall)

Exploratory data from cultures of the high tumor producing strain of liver cells when subjected to medium NCTC 108 in which the amount of glutamine has been decreased show almost twice the proliferation that the low tumor producing strain shows.

Significance: A partially defined medium for studying the behavior of liver epithelial cells has been devised. A slightly modified medium will support proliferation also of a strain of liver cells of high tumor incidence. This suggests a difference between a normal and malignant cells in tissue culture. It must be learned if this exploratory data can be confirmed and whether additional differences can be demonstrated *in vitro* from a nutritional point of view.

Proposed course of project: Efforts will be made to substitute for and augment the effect of the 0.05 gm % of amino acid-free protein in this medium. It is proposed to do this by introducing further purified fractions of serum proteins and to supplement the medium with single materials which can be closely attached to the protein residue. It is further proposed to continue to demonstrate nutritional differences between the two cell lines, and to constantly attempt to devise media free of any protein for these strains also.

10. 428 (j)
Serial No.

11.
Budget activity:

Research	x	Administration	
Review & Approval		Technical Assistance	

12.
Cooperation units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

Laboratory of Clinical Investigation, National Microbiological Institute.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:.

14. 428 (j)
Serial No.

15. None
Publications other than abstracts from this project during calendar year 1955.

16. None
Honors and awards to personnel relating to this project during calendar year 1955.

General Project 429
Serial No.

Studies of the influence of cell population on proliferation.

Project Report Form

1. National Cancer Institute
2. Biology Laboratory or Branch
3. Tissue Culture Section or Service
4. Bethesda, Md. Location
5. 429(a) Serial No.
6. Studies of the influence of cell population on proliferation. Project Title
7. K. K. Sanford Principal Investigator(s)
8. G. L. Hobbs and W.R. Earle Other investigators
9. Project description: Single cell studies and the development of cell clones.

Objective: To develop clones of human skin, mouse mammary carcinoma, and four strains of mouse fibroblasts. Each of these clones is desired for specific research projects which will be considered under other headings. The development of a clone of mouse mammary carcinoma is for use in a collaborative study with Dr. H. B. Andervont on the relation of the milk factor to the malignant cell in tissue culture.

Methods employed: A sieved cell suspension is prepared and drawn into capillary pipettes. Capillary segments are cut, each segment containing one isolated cell. The segments are embedded in plasma clot in a Carrel flask, and the culture fluid is renewed thrice weekly.

Major findings: Attempts have been made to define more accurately the conditions allowing the proliferation of single isolated cells. Some progress has been made.

Significance to Cancer Research: The development of clones of cells is of fundamental importance for future studies in carcinogenic cell transformations among populations of cells in vitro, virus studies, and in studies of the differences in the physiology and nutrition of malignant cells and the normal cells from which they arise in culture.

Proposed course of project: Renewed efforts will be made to develop clones of the several cell strains listed above and to define more accurately the conditions that allow the proliferation of single isolated cells.

10. 429 (a)
Serial No.

11.
Budget activity:

Research x Administration

Review & Approval Technical Assistance

12. None
Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

13. None
If this Project Resembles, Complements, or Parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds) identify such research.

14. 429 (a)
Serial No.

15. None
Publications other than abstracts from this project during calendar year 1955.

16. None
Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute 2. Biology Laboratory or Branch

3. Tissue Culture Section Section or Service 4. Bethesda, Md. Location (if other than B 5. 429 (b)

6. Growth of massive fluid suspension cultures of animal tissue cells. Project Title

7. W. R. Earle Principle Investigator(s)

8. Jay C. Bryant, E. L. Schilling, B. B. Westfall, Ellison Peppers and V.J.Evans. Other Investigators

9. Project Description: Growth of massive fluid suspension cultures of both normal and malignant animal tissue cells. Application of these methods in defining factors responsible for proliferation of representative tissue cell types in such cultures.

Objectives: The general objective of this project is to develop methods and equipment for growing massive fluid suspensions cultures of animal tissue cells. Factors receiving attention in the attainment of this objective include particularly various types of fluid media, control of air inflow, control of pH, and regulation of volume and compositions of culture fluid in relation to numbers of cells and rate of proliferation.

Methods Employed: The standard Brunswick type platform shaker enclosed in an incubator box, which had been developed previously, was used for continuing studies with massive cultures. The shaker was operated at about 12,000 revolutions per hour, and the size of culture flask used was 1½ liter. Continuous aseptic flow of a gas mixture of 5% CO_2, 20% oxygen and 75% nitrogen, saturated with water vapor, was maintained through each culture flask. The rate of gas flow through each flask was held constant at either 140 or 280 ml. per hour. Phenol red at a concentration of .002 percent was used in all of the culture fluid, as a pH indicator. The fluid was either renewed periodically without appreciable loss of cells or was increased by increments of fresh fluid. The rate of proliferation of cells in suspension was determined periodically by nuclei counts of carefully sampled aliquots.

Major Findings: Considerable progress has been made during the year in attaining better control of the major factors governing the growth of cell suspension cultures in shaker flasks. With all cells used the concentration of glucose in the medium has been increased up to several times the concentration in Earle's normal saline in order to maintain cell growth during intervals between fluid changes unlimited by exhaustion of glucose.

General Project 430
Serial No.

Studies on the cultivation of epithelial cells.

PROJECT FORM REPORT

1. National Cancer Institute — 2. Biology, Laboratory or Branch

3. Tissue Culture, Section or Service — 4. Bethesda, Md., Location — 5. 430(a), Serial No.

6. Study of a strain of Human Liver Cells.
Project Title:

7. V. J. Evans
Principal Investigator(s)

8. N. M. Hawkins, W. R. Earle, and B. B. Westfall
Other Investigators

9. Project Description:

Objective: To cultivate a strain of human liver cells for long term studies for ultimate comparison of normal human cells and malignant cells derived from it.

Method: Minced human liver prepared by trypsin treatment together with stirring and subsequent tissue culture cultivation in human serum and chick embryo extract, has yielded a strain of cells.

Major findings: For the first time a human liver strain gives some promise of continuing to grow in vitro. This is contrary to data from 5 or 6 other endeavors to cultivate human liver for any prolonged interval. To date this strain of cells subcultures readily in fluid suspension and exploratory data indicate that it may grow in shaker cultures thus facilitating metabolic studies.

Significance to Cancer Research: In addition to significance to cancer research the strain of cells should be an invaluable tool to virologists. Techniques developed in establishing this strain are of usefulness in establishing human liver strains for comparative studies in the physiology of the normal and malignant cell.

Proposed course of project: Since adequate amounts of tissue and fluids are obtained from the large culture these will continue to be used for glucose and glycogen analysis and for comparison with similar studies on the other strains of mouse and human epithelial cells in the laboratory. Attempts to produce a malignant transformation of this cell in vitro will be made. Since the HeLa strain of cells from a human cervical carcinoma has given rise to small identifiable growths in the anterior chamber of the mouse eye similar techaniques may be worthy of consideration for other strains of cells developed and in particular for this human liver epithelium. [illegible] strain of cells will be cloned as soon as feasible.

10. 430 (a)
Serial No.

11.
Budget Activity:

Research	x	Administration	
Review & Approval		Technical Assistance	

12. None

Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 430 (a)
Serial No.

15. None
Publications other than abstracts from this project during calendar year 1955

16. None
Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer INSTITUTE
2. Biology, LABORATORY OR BRANCH
3. Tissue Culture Section or Service.
4. Bethesda, Md. Location
5. 430 (b) Serial No.
6. Preservation of culture cells. Project Title
7. V. J. Evans and W. R. Earle, Principal Investigators.
8. See item 12 Other Investigators.

9. Project description: Now that cells can be cultures in substantial amounts is it desirable to be able to preserve these cells in a latent state, for extended periods of time. The most promising method reported to date, has been reported by Polge, C. (Nature 164; 666; 1949) who used glycerine impregnation for preservation of bull spermatazoa. The Tissue Bank, Naval Medical School has utilized this method for the preservation of skin and cornea. It is desirable to learn the length of preservation; whether this inexpensive material (dry ice and glycerine) is satisfactory; and, whether this material is less toxic to cells than other materials. Tissue culture viability of preserved tissue will serve as the index of the physiological conditions of the cells.

Methods employed; Rabbit cornea cultures were planted in a thin plasma clot under perforated cellophane in Carrel 3.5 flasks. A nutrient medium of horse serum and which embryo extract LA Earle's saline was used. One half of a rabbit cornea was used in each culture flask. Initially fresh medium and were planted within 30 minutes from the time of removal from the eye. Soaked corneas were placed in test tubes containing 15% USP glycerine by volume in Earle's saline, or, in Ringer's saline for 1 hour. Division, explantation and incubation of unfrozen corneas took place within an hour. Frozen cornea were frozen either with (1) no soaking, (2) after soaking in glycerine in Earle's balanced saline for 1 hour and (3) after soaking in 15% glycerine in Ringer's saline for 1 hour. The excess fluid was decanted and the tubes of corneas were first immersed in a container of carbon dioxide alcohol slush at -76°C for 3 minutes. At the end of 1 hour at -76°C the tubes containing corneas were removed, thawed by immersing in a water bath at 38°C and corneas were divided, explanted and incubated.

In the exploratory skin study to date, the procedures were approximately similar to that used to the cornea study, except that human serum was substituted for horse serum, and the skin used, was of human origin.

Major findings: In the study on rabbit cornea the fresh cornea showed excellent migration of epithelial and fibroblastic cells in all instances within 48 hours. Corneas which were soaked in dilute glycerine or soaked and then frozen showed a slight lag in migration but were soon indistinguishable in migration and cell appearance from fresh corneas. Corneas frozen without glycerine protection showed no migration in 28% of the cultures. The remainder showed retarded migration and severe cell injury. Corneas preserved by the 15% glycerine, in 85% Earle's saline by volume, and frozen and preserved at -76°C. have been found to be viable in tissue culture up to 6 months. Exploratory data, obtained to date on skin preserved by these methods does not appear to be viable in tissue culture.

Significance: Before beginning the study the feasibility of using human corneas was considered. Since insufficient numbers could be obtained, the rabbit cornea was selected since most experimental work to date has used this source. The tissue viability test was used to test the physiological condition of corneas preserved by the above methods because it was considered of greater critical usefulness than either the clarity of the graft after transplantation or tests of the respiratory enzyme systems. The results of the study demonstrated for the first time that rapidly frozen glycerine impregnated corneas were consistently viable. Four factors rather than any independent one appears to be responsible for this: (1) glycerine soaking, (2) fast freezing, (3) fast thawing and (4) the use of a highly buffered balanced physiological saline, such as Earle's saline rather than Ringer's Saline.

Corneal endothelial cells (which are required in a viable state for successful keratoplasty) may assist in establishing criteria for the preservation of cells cultured in large masses and stored in glycerine. If it can also be demonstrated that corneal endothelial cells, which are preserved by glycerine impregnation and stored at dry ice temperature, exhibit viability in vitro, possibly, cells of less specific function may be preserved in a like manner. The human skin which has been preserved by a modified glycerine method serves well as a clinical homograft dressing. In spite of the failure to date to establish viability in vitro, it still appears important to learn whether or not improvements in methods of preservation of skin, so that viability in vitro is demonstrable, will yield a superior dressing.

11. Budget Activity

Research	x	Administration	
Review & Approval		Technical Assistance	

12. Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

a) Tissue Bank, b) Dept. of Ophthalmology, c) Naval Medical Research Institute, National Naval Medical Center.

13. None
If this Project Resembles, Complements, or Parallels Research Done Elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research.

14. 430 (b)
Serial No..

15. Publications other than abstracts from this project during calendar year 1955.

The viability of fresh and frozen corneas as determined in tissue culture. McPherson, S. D., Draheim, J. W., Evans, V. J. and W. R. Earle, American J. of Ophthalmology - In press.

16. None
Honors and Awards to Personnel relating to this Project During Calendar Year 1955.

PROJECT FORM REPORT

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service — 4. Bethesda, Md. Location — 5. 430 (c) Serial No.

6. Studies on the cultivation of human endocrine secreting tissue in vivo.
Project Title

7. V. J. Evans
Principal Investigator (s)

8. N. M. Hawkins, W. R. Earle
Other Investigators

9. Project Description:

Objective: To cultivate cells of endocrine tissue as made available by the Endocrinology Branch of the National Cancer Institute in order to learn the extent and duration of hormone secretion of isolated cells in vitro.

Methods employed: Additional islet cell tumor tissue from the pancreas of a clinically diagnosed case of carcinoma of the pancreas has been obtained from the Endocrinology Branch, of the National Cancer Institute. Dissociation of the tissue to small but multiple colonies for explantation of cultures has been done. This method has been successful for cultivation of other human epithelial cell strains.

Major findings: Presently this new strain of cells is still in an early stage of cultivation in vitro.

Significance to cancer research: The development of techniques for growing human endocrine tissues in vitro so that they continue to function should have considerable practical value for therapy. From the point of view of cancer research any endocrine assays or data from histochemical assays should serve as valuable indices of the changes in cells in vivo and in vitro both in clinical and experimental conditions.

Proposed course of project: Experiments will be continued to culture various tissues of endocrine origin as they are made available from services in the Clinical Center and other sources. Methods must be developed for culturing such highly specialized cells. The duration and amount of hormone secretion and study of the morphology of the tissue growth with photographic recordings will be made. Histochemistry, biochemical analyses and morphology and mouse chamber techniques will be

employed and correlated with function and therapy. Attempts will be made to devise new methods of assay of function by grafting as suggested by mouse chamber techniques and as justified by the growth of cells in vitro.

10. 430 (c)
Serial No.

11. _____
Budget Activity:

Research	x	Administration	
Review and Approval		Technical Assistance	

12. _____
Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

Endocrinology Branch, National Cancer Institute, and Transparent Chamber Unit, Laboratory of Biology, National Cancer Institute.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 430 (c)
Serial No.

15. None
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955.

16. None
Honors and Awards to Personnel Relating to this Project during Calendar Year 1955.

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service — 4. Bethesda, Md. Location — 5. 430(d) Serial No.

6. A study of long term human skin strains. Project Title

7. V. J. Evans and W. R. Earle Principal Investigators

8. See item 12. Other investigators

9. Project Description: Objective: a) To produce and maintain long term human epthelial cell tissue culture. This will tend to an understanding of tissue culture cell metabolic needs as a preliminary to transplantation studies. b) To determine by continuous in vivo transplantation of cells, the possibility of spontaneous in vitro malignant transformation of human skin cells.

Methods employed : a) Human skin from a 65 year old man obtained by the Tissue Bank, Naval Medical School, was separated into dermal and epidermal elements. A cell suspension of epidermal elements was planted in human serum and has been carried in vitro now for over 2 years. A subline was established in horse serum for experimental work where human serum is undersirable. b) Shaker studies were established according to the procedures of Earle, Bryant and Schilling.(c) The strain 1769, human skin has been implanted in the anterior chamber of the C3H mouse eye, and the pouches of golden hampster. Mice were irradiated and cortisine treated, as described by Toolan.

Major findings: Human skin has been successfully cultivated over two years in human serum. The subline on horse serum has been carried now for over 6 months. The details for preparation of the strain, description of the cells and related use of the cells have been reported in a manuscript. In exploratory studies the strain on human serum when in the presence of viable homogenous tissue will elicite what appears to be a rejection-acceptance phenomenon (see report on rejection-acceptance phenomen in vitro). b) The complete detail of methods employed and size of cultures attained in the report of Earle, Bryant, and Schilling, "Growth of massive fluid suspension cultures of animal tissue cells". Fluid and cells from these were made available to Dr. Westfall (see report of Westfall and Peppers) for studies on utilization of glucose, storage of glycogen and production of keto acids, etc. c) No overt

tumors were found in the anterior chamber of the mouse eye or in the hampster mouth pouch. This was contrary to the findings of tumor tissue when HeLa (Human cervical carcinoma) was implanted.

Significance: For the first time a strain of human skin cells has been cultured in vitro for over two years and continues to grow luxuriantly, resembling morphologically epithelial cells. Numerous types of studies have been made with it. It is known to support some 15 viruses. The strain can be grown in amounts adequate for metabolic studies. It has been possible to initiate problems related to homotransplantation. The cells have possible application in clinical use, particularly in reparative surgery. They may have additional significance in investigations on cancer therapy, both because it supports viruses, and as a stable strain they may be of value in screening chemotherapeutic agents. Comparative studies on their metabolism with that of epithelial cancers might be significant.

Proposed course of study: To continue studies on the nutrition and metabolism of the cells for ultimate use in clinical work. To confirm studies on homograft response and to continue studies on large shaker flasks to facilitate the above.

10. 430 (d)
Serial No.

11.
Budget activity:

Research	x	Administrative	x
Review & Approval		Technical Assistance	

12.
Cooperating units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957

Much of the work on this problem has been done by personnel of the Tissue Bank, Naval Medical School, National Naval Medical Center.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds) identify such research:

14. 430 (d)
Serial No.

15.
Publications other than abstracts from this project during calendar year 1955.

Perry, V. P., Evans, V. J., Earle, W. R., Hyatt, G. W. and Bedell, W.C.- Long Term Tissue Culture of Human Skin, Am. J. of Hygiene, Jan. 1956.

Bassett, C.A.L., Evans, V.J., Campbell, D. and Earle, W. R. -Characteristics and Potentials of Long-term Culture of Human Skin. N.M.R. I. Project Report 007 081.10.11.

16. Secretary of Navy Commendation Medal to Hospitalman Vernon P. Perry 1C for for his participation in this work as carried on under our supervision in the Tissue Culture Section, Laboratory of Biology.

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service — 4. Bethesda, Md. Location — 5. 430 (e) Serial No.

6. To adapt strains of cells to media of heterologous composition. Project Title

7. V. J. Evans, Principal Investigator(s)

8. W. R. Earle and see item 12 Other investigators

9. Project Description:

Method: a) Present experimental studies include: subjecting human malignant cells and human cells from non-malignant skin to media of various combined mixtures of human and horse serum until horse serum alone could be used. The cells were maintained in Carrel D-3.5 flasks and Earle T-30 and T-60 flasks. After intervals of 120 hours or more, the less foreign and it is presumed less toxic serum concentrations permit subculturing of substrains of cells for study.

Major Findings: It has been found possible by the method described above, that cultures of human malignant tissue (HeLa 1881) initially propagated in human serum, can human serum, can be adapted to heterologous horse serum medium to produce HeLa strain 1985. By a similar procedure a strain of normal human skin epithelium grown in human serum and designated strain 1769 has been adapted to horse serum. This adapted strain was designated strain 2198. Both horse serum adapted strains have been found of usefulness in homografting problems in mouse chamber work (see report Algire et al.), and for cultivation of human viruses (see report Habel and McBride, National Microbiological Institute).

Significance: Both these strains have already been found of significance in studies of cultivation of human viruses. (Strain 1985 has already been used in studies on mechanisms of homografting. The mechanism of adaptation should be an interesting situation for study by the immunologist interested in the problem of homografting as well as by the protein chemist. Habel, Gregg and McBride of the Microbiological Inst. and Dental Institute reported results on large numbers of horse serum adapted cultures submitted to them from this laboratory. In one experiment cells adapted to horse serum were less susceptible than those grown in human serum and less susceptible than fresh kidney to poliomyelitis virus. However, a second experiment indicated an equal susceptibility of all three types of tissue culture to the three types of poliomyselitis. HeLa cells grown in horse

serum were just as responsive to polio virus isolated from 23 separate stock emulsions of polio virus as those grown in human serum.

HeLa cells grown in horse serum in suspended cell shaker type of culture and then carried in a maintenance medium for 24 hours and tested for poliomyeletis as efficiently as those grown in human serum. This indicates that viruses are able to propagate in cells growing in the shaker type culture and an advantage is gained where horse serum is used both because of its availability and because the specific antibodies of human serum may be eliminated.

Projected plan: Further study of problems of homografting in mouse diffusion chambers with both strains of cells and their respective substrains to determine likenesses and differences in behavior will be made.

10. 430 (e)
Serial No.

11. _____
Budget Activity:

Research	x	Administration	
Review & Approval		Technical Assistance	

12. _____
Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957

Laboratory of Infectious Diseases, National Microbiological Institute; Tissue Bank, Naval Medical School, National Naval Medical Center; Transparent Chamber Unit, Laboratory of Biology, NCI

13. None
If this Project Resembles, Complements, or Parallels Research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 430 (e)
Serial No.

15. _____
Publications other than abstracts from this project during calendar year 1955.

Perry, V.P., Evans, V. J. and Earle, W. R. Cultivation of large cultures of HeLa Cells in horse serum. Science 121:805, 1955.

16. _____
Honors and awards to personnel relating to this project during calendar years 1955.

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology Laboratory or Branch
3. Tissue Culture Section or Service
4. Bethesda, Md.. Location
5. 430(f) Serial No.

6. Studies on the utilization of a long term strain of skin epithelium for propagation of virus. Project Title

7. V. J. Evans, Principal Investigator(s)

8. W. R. Earle, - Also see item 12 Other Investigators

9. Project description: To test whether human skin epithelium will support cultivation of certain human viruses.

Method - More than 2000 roller tube culture of a long term strain of human skin cells from a 65 year old man were studied for cytopathogenic effects and production of complement fixing antigens when exposed to numerous significant viruses.

Major Findings: To date these viruses apparently multiplying in this cell strain are: adenoidal pharyngeal conjunctival viruses types 1, 2 and 3; Coxsacki virus type B-3; herpes simplex; B virus; vaccinia; mumps; encephalomyocardities virus; lymphocytic choriomeningetis virus; St. Louis encephalitis virus; and yellow fever virus.

Significance: This strain of cells for virus studies has significance because of its being an epithelial cell of human origin. Many viruses have not been propagated *in vitro* since this cell strain grows luxuriantly in large amounts, and maintains many viruses in rapid proliferation, it offers substantial potentialities both as a basic research tool and as a practical basis in large scale production of viruses. Already, this cell strain, can be maintained in a defined medium supplemented with only limited amounts of serum for these specific studies on viral propagation. In other cases, where desired, an entirely heterologous serum may be used.

This tissue may be assumed to be non-malignant until exhaustive studies prove otherwise.

Proposed course of project: Exploratory data on the cultivation of this strain of cells in mass amounts in the shaker type culture will be confirmed for other studies but the obvious value of this strain of cells from a normal tissue source useful for vaccine production is suggested for consideration by virologists, and will not be studied by our group. Continuation of efforts to demonstrate any potential malignancy must be pursued. Methods presently available for doing so will be tested and new ones will be sought. Methods of controlling possible development of malignacy in tissue culture must be tried and their validity tested.

10. 430 (f)
Serial No.

11. None
Budget Activity:

Research	x	Administration	
Review & Approval		Technical Assistance	

12.

Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

Laboratory of Infectious Diseases, National Morphological Institute; Clinical Investigation, National Institute of Dental Research; Tissue Bank Naval Medical School, National Naval Medical Center. Over 2000 cultures have been furnished the first two of these for virological research.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research.

14. 430 (f)
Serial No.

15.

Publications other than abstracts from this project during calendar year 1955.

Long Term Tissue Culture of Human Skin. Perry, V. P., Evans, V. J., Earle, W. R., Hyatt, G. W., Bedell, W. C. Am. J. of Hygiene, (Jan) 1956.

16.

Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service — 4. Bethesda, Md. Location — 5. 430 (g) Serial No.

6. Rejection-Acceptance phenomenon in vitro.
Project Title

7. V. J. Evans and W. R. Earle
Principal Investigator(s)

8. See item 12.
Other Investigators

9. Project description: To determine if viable and non-viable tissue fragments in vitro will elicite an in vivo homograft-like reaction.

Method: Fresh and freeze dried tissue have been, procured from the Tissue Bank, Naval Medical School and planted in Carrel D-3.5 flasks with plasma and a fluid medium phase containing strain 1769 (long term human epithelial) and allowed to clot. Observations were made at 40,96 and 120 hours to determine if rejection of strain 1769 has taken place.

Major Findings: In an exploratory series, observations suggest that there is a recognizable homograft-like reaction in vitro. The 24-72 hour rejection phase of strain 1769 is evidenced by the formation of a definite zone of partial or complete growth inhibition at the periphery of the fresh tissue explant. This inhibition zone has not been observed with freeze dried tissue explants.

Significance to Cancer Research: If these preliminary observations can be confirmed they might well be most sighificant in further elucidation of the homograft response that occurs in vivo.

Projected Plans: To repeat and expand these findings with different types of tissue, both viable and non-viable, of homogenous and heterogenous origins.

10. 430 (g)
Serial No.

11. Budget Activity:

Research	x	Administration	x
Review and Approval		Technical Assistance	

12. Cooperating Units of the Public Health Service, or other organizations providing funds, facilities, or personnel for this project in either 1956 or 1957.

Tissue Bank, Naval Medical School, National Naval Medical Center. Much of this work was actually done by Tissue Bank Personnel under supervisio of N.C.I., senior investigators.

13. None

If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 430 (g)
Serial No.

15. None

Publications other than abstracts from this project during calendar year 1955.

16. None

Honors and awards to personnel relating to this project during calendar year 1955.

General Project 431
Serial No.

Studies of cell transformation *in vitro*.

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology Laboratory or Branch
3. Tissue Culture Section or Service
4. Bethesda, Md. Location
5. 431 (a) Serial No.

6. Studies of cell transformation in vitro.
Project Title

7. K. K. Sanford
Principal Investigator(s)

8. G. L. Hobbs, M. Fioramenti, and W. R. Earle
Other Investigators

9. Project description: Characteristics of two lines of cells originating from a single adult mouse cell.

Objective: 1) To define quantitatively the sarcoma-producing capacity of cells of these two lines as dependent on the numbers of cells injected into mice and as influenced by the period of cell growth in vitro. 2) To determine whether the difference in sarcoma-producing capacity of these two cell lines is related to a difference in immunologic properties affecting their transplantability to the mouse strain of origin and to define the autonomy and immunologic specificity of the cell lines through homologous transplantation studies. 3) To compare the proliferation rates in vitro of the two cell lines. 4) To compare the growth in vivo of these two cell lines. 5) To establish and analyze the change in sarcoma-producing capacity induced in these cell lines by mouse passage. 6) To determine differences in oxidative metabolism of these two lines of cells. 7) To determine differences in the nutritional requirements of these two lines of cells.

Methods employed: Quantitative procedures in which known numbers of cells were injected into both X-irradiated and non-irradiated, immunized and non-immunized mice of the inbred strain of origin have been used. Injections of cells were also made into foreign inbred strains of mice. Proliferation rates in a stock horse serum-chick embryo extract culture medium have been determined and nutritional requirements are being studied by the use of quantitative replicate culture procedures. In collaboration with Dr. Mark Weeds the oxidative metabolism of the two cell lines is being investigated. In collaboration with Dr. Ruth Merwin an attempt is being made to determine the latent period for sarcoma production from cells of these two lines injected into mice treated so as to be unable temporarily to develop a homograft reaction. Cell growth in vitro has also been studied by implanting known numbers of cells into diffusion chambers, each placed in the peritoneal cavity of a mouse.

Major findings: 1) Marked differences in the sarcoma-producing capacity of these two cell lines were demonstrated. 2) The sarcoma-producing capacity of cells of these two lines was found to be dependent on the numbers of cells injected into mice and was influenced by the period of cell growth in vitro. 3) Both lines of cells were found to be antigenic to the strain C3H mouse. Immunologic cross reactions occurred between these two lines and also between these lines and a different clone of cells, clone 929 of strain L. 4) The cells of these two lines grew specifically in the strain C3H mouse and not to any extent in other strains of mice tested. 5) In mice treated so as to be unable temporarily to develop a homograft reaction (see report by Dr. Ruth Merwin) marked differences were observed in the latent periods for tumor development from injections of equivalent numbers of cells of the two lines. These data indicated that the main difference in sarcoma-producing capacity of these two cell lines was not a difference in their transplantability to the mouse but was a difference in their ability to survive and grow in a non-resistant host. 6) The malignancy of cells of the low sarcoma-producing line could be markedly increased by animal passage. After one generation in a mouse, the cells were changed. This cell transformation lasted for approximately 6 months of growth in vitro for the one tissue culture line studied. 7) The proliferation rate of cells of these two lines when cultured in a horse serum-chick embryo extract medium was not significantly different. 8) Cells of these two cell lines could not proliferate on chemically defined mixture NCTC 107-108 unless 0.5 to 1 percent serum proteins were added. The amino acid requirements appeared to be the same as those of strain L cells. 9) Initial studies by Dr. Woods indicated a marked difference between these two cell lines in their rates of aerobic and anaerobic glycolysis and in their rates of respiration.

Significance to cancer research: This analysis of the behavior of cell lines that differ markedly in their ability to produce sarcomas in the strain C3H mouse and that have transformed from normal to tumor-producing cells should provide some information as to the type of metabolic change that these cells have undergone in their transformation.

Proposed course of project: To complete the studies outlined above and to pursue particularly the biochemical studies in an effort to detect the mechanisms underlying the changes in malignancy of these cells.

10. 431 (a)
Serial No.

11.

Budget Activity:

Research x Administration

Review and approval Technical Assistance

12. Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

Cooperation with Dr. Dean Burk, Dr. Mark Woods, Laboratory of Biochemistry and with Dr. Ruth Merwin, Laboratory of Biology, N.C.I.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 431(a)
Serial No.

15. None
Publications other than abstracts from this project during calendar year 1955.

16. None
Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute | 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service | 4. Bethesda, Md Location | 5. 431 (b) Serial No

6. Studies of cell transformations in vitro .
Project Title

7. K.K. Sanford,
Principal Investigator(s)

8. G. L. Hobbs and W.R. Earle.
Other investigators.

9. Project Description: A study of the tumor-producing capacity of clone 929 of strain L.

Objective: To test the sarcoma-producing capacity of a clone of cells, clone 929, derived from a single cell of strain L. Strain L was originated in 1940 from an explant of subcutaneous connective tissue taken from a strain C3H mouse. After two years of growth in vitro, cultures of strain L gave rise to sarcomas when injected into strain C3H mice. The incidence of mice developing sarcomas in 1943 from the injected cells was 68 percent. Three years later in 1946, this incidence had dropped to 1 percent. The object of the present study was to explain this change in sarcoma-producing capacity of the cells.

Methods employed: The sarcoma-producing capacity of the cells has been tested by injecting the cells intramuscularly into X-irradiated and non-irradiated strain C3H mice as well as into foreign strains of mice and strain C3H mice immunized with clone 929 cells.

Major findings: When clone 929 cells were injected into X-irradiated strain C3H mice, the percentage of mice developing sarcomas was comparable to that obtained in 1943 when strain L cells were first tested for sarcoma-producing capacity. An injection of clone 929 cells was found to induce an immune reaction in strain C3H mice that completely prevented the growth of clone 929 cells subsequently injected. It was thus demonstrated that the apparent decrease in sarcoma-producing capacity of these cells resulted from the development of an incompatibility between the tissue culture cells and the strain C3H mouse rather than from any demonstrable change in the malignancy of the cells. Two sarcomas derived from clone 929 cells were found to grow in all strain C3H mice injected after serial transfers in vivo. A specificity of the sarcoma tissue for the strain C3H mouse was also demonstrated.

Significance to cancer research: At the present the only method for determining the malignancy of tissue culture cells is to inject the cells into animals of the inbred strain from which the tissue originated. The significance of the present study is to point out that negative results of such a test do not necessarily indicate a lack of malignancy of the cells tested. It has been demonstrated that an incompatibility may develop between the cultured cells and the mouse strain of origin such that the cells become antigenic to the host. The change observed earlier in the sarcoma-producing

capacity of this tissue culture strain has thus been explained as a change in the transplantability of the cells and not as a change in their malignancy. It has also been demonstrated that cells grown for 10 years in a heterologous culture medium have not lost their specificty for the animal strain of origin.

Proposed course of sub-project: This sub-project will not be continued.

10. 431 (b)
Serial No.

11.
Budget Activity:

x Research — Administration

Review and approval — Technical Assistance

12. None
Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957

13. No
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 431 (b)
Serial No.

15.
Publications other than abstracts from this project during calendar year 1955.

The tumor-producing capacity of strain L mouse cells after 10 years in vitro. K. K. Sanford, G.L. Hobbs, and W.R. Earle, Cancer Research. In press.

16. None.
Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer | 2. Biology

3. Tissue Culture (Section or Service) | 4. Bethesda Md. (Location) | 5. 431 (c) (Serial No.)

6. Study of two strains of mouse liver epithelium from the same single cell. (Project title.)

7. V. J. Evans, (Principal Investigator(s))

8. N.M. Hawkins and W.R.Earle (Other Investigators)

9. project Description:

Objective: To study two substrains, arising from a common clone of mouse liver epithelium. One of these strains (1795) is of high tumor producing ability the other is low in tumor production. The primary objective is to have available two such strains for comparing their metabolic and physiologic characteristics.

Methods employed: Cell cultures have been studied in vitro and have been continuously injected in both x-irradiated and non-irradiated C3H mice. The behavior of these cells in replicate culture has been studied for their response metabolically.

Major findings: Mouse liver clone (1469) was originated from a single cell of strain 721 in April 1948. Metabolic studies will be dealt with in detail by Dr. B.B. Westfall. This clone cell strain has shown a low capacity for developing tumors on injection, only 1 tumor has been found in non-irradiated mice while 2 have been found in x-irradiated mice in over 200 animals. Practically 100% tumor incidence was obtained from one of these lots on trocar injection into both non-irradiated and x-irradiated mice. Tissue culture from one of these tumors were grown to produce strain 1795. This strain developed a much enhanced capacity in a much shorter interval for its cells to give rise to tumors on reinjection into C3H mice. The incidence of takes with this strain is 81.0% on injection in x-irradiated C3H mice and 69.8% by injection in non-irradiated C3H mice. Tumor tissue on subinoculation into irradiated mice was 100% while those in non-irradiated mice was only 10%.

A manuscript is in preparation describing these two cell strains.

Significance to Cancer Research: A comparative study of the metabolic and physiologic behavior of these two strains in tissue culture appears to be of significance in understanding the characteristics of transition from a cell strain able to give rise to practically no tumors to one able to give rise to a high percentage of tumors.

Projected work: Clone cells of this high incidence tumor strain will be used for experimental work to compare and to determine if tumors can be obtained from the second pure cell strain as well as the mixed cell strain (1795). Studies are still in progress to determine if the mixed cell strain (1795) continues to give the same high incidence of tumors or whether this incidence is lessened by continued proliferation in vitro.

10. 431 (c)
Serial number

11.
Budget activity:

x Research | Administration

Review and approval | Technical Assistance

12. None
Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in epither 1956 or 1957.

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research.

14. 431 (c)
Serial No.

15. None
Publications other than abstracts from this project during calendar year 1955.

16. None
Honors and awards to personnel relating to this project during calendar year 1955.

General Project 432
Serial No.

Comparison microcinematography.

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology Laboratory or Branch
3. Tissue Culture
4. Bethesda Location
5. 432 (a) Serial No.

6. Studies with the Comparison Micrccinematograph.
Project Title.

7. W. R. Earle
Principal Investigator (s)

8. W. T. McQuilkin (part time)
Other Investigators

9. Project Description:

A three optical system time lapse cinematograph has been developed and constructed in the NIH Instrument Shops. This is to be used in comparative study of normal and malignant cultures, and comparison of cultures under different experimental conditions. Particular attention will be paid to factors which influence population changes in cultures.

One person was given preliminary training in use of this instrument. Final adjustments were begun on the instrument after the long delay reported last year. Several experimental film strips were run on behavior of various cell types. Person being trained recently resigned to take outside position at a much higher salary. No replacement has been authorized but Mr. McQuilkin (Biologist GS-7) is much interested in the equipment and is carrying out further part time work on it. This equipment needs full time work on it to make it of practical usefulness however.

Significance to Cancer Research: This camera is a powerful instrument in study and comparison of various normal and cancer cell types under controlled experimental conditions.

Proposed course of project. As technical assistance is available the studies projected are as follows: 1) Comparison of cell strains with various nutrients missing from medium. 2) Transition of epithelial cells to more spindle shaped forms, allowing possible clearer understanding of transition of carcinomas to "sarcoma" and transition of epithelial cells to "fibroblasts" like forms. 3) Influence of cell population density on manner of migration and the morphology of epithelial cells. 4) Action of specific reagents on cells.

10. 432 (a)
Serial No.

11. Budget Activity:

Research	x	Administration	
Review and approval		Technical Assistance	

12. None
Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957

13. None
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 432 (a)
Serial No.

15. None
Publications other than abstracts from this project during calendar year 1955

16. None
Honors and Awards to Personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Leukemia Studies Section
4. ____
5. 433 — SERIAL

6. Studies on the etiology of mouse leukemias and neoplasms of the parotid and adrenal glands. — PROJECT TITLE

7. Sarah E. Stewart — PRINCIPAL INVESTIGATOR(S)

8. None — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Studies on the etiology of mouse leukemias and neoplasms of the parotid and adrenal glands.

Objectives: To find good sources for the parotid gland tumor agent and the leukemia agent in order to recover them and determine if they are identical.

Methods employed:

I. Newborn mice were inoculated with cell-free extracts prepared from leukemic mouse tissues, from parotid gland tumors, and from other tumors arising in mice having received extracts of leukemic mouse tissues.

For controls, similar mice were inoculated with the following:

a) Extracts prepared from tissues of normal strain C3H mice.
b) Extracts from C3H mammary tumors carrying the milk agent.
c) Extracts from human lymphomas.
d) An active mumps virus.
e) Methylcholanthrene applied to the skin of newborn mice.

II. By using a method previously described (Stewart, Proceedings of American Assoc. Cancer Research, April 1955), attempt to recover a virus from transplanted leukemias, parotid gland tumors and adrenal gland tumors and test these for carcinogenesis.

III. Study the histopathology observed in the organs of mice (irradiated* and non-irradiated) carrying transplanted mouse tumors, and of mice inoculated with the virus recovered in II to note if there are similarities.

* 250 r total body radiation.

(Project description continued)

Major findings:

I. Two good sources for the parotid gland tumor agent have been found; one is AKR leukemia #60, extracts of which, on repeated tests over a 2-year period, have yielded an incidence of 50 to 80 percent parotid gland tumors, some of the mice have had both leukemia and parotid tumors and others only leukemia. The other, C3H leukemia #19, has yielded about a 30 percent incidence of parotid gland tumors and adrenal tumors over a period of 3 years.

Other sources for the parotid gland tumor agent have been an AKR leukemia #RIL6, C3H leukemia #1124, a paraganglioma, and a mammary tumor. The last two arose in (C3Hf x AKR) hybrid mice as a result of inoculating with AKR leukemia extracts.

None of the mice inoculated with extracts prepared from parotid gland or adrenal gland neoplasms from normal tissues, from human lymphomas, or from milk agent mammary tumors, have developed parotid gland tumors or leukemias.

Mice receiving mumps virus, and those receiving methylcholanthrene, have remained free of leukemia and parotid gland tumors.

II. A virus has been recovered from the following leukemias: AKR leukemias #60 and #RIL6, and from C3H leukemias #1124 and #19. These 4 leukemias have repeatedly been good sources for the parotid gland tumor agent. It has not been possible to recover a virus from the parotid gland tumors, or from the adrenal tumors. Extracts prepared from these tumors have not yielded parotid gland tumors.

This virus is highly lethal for newborn mice, especially for strain C3H. A few (C3Hf x AKR)F_1 hybrids have survived the inoculations, and 4 out of 15 which received virus after serial passage developed parotid gland tumors in from 5 to 12 months.

III. Five C3H parotid gland tumors transplanted into irradiated mice grew in from 50 to 80 percent of the first passage transplants and retained their original histology even after repeated transplanting. One tumor has been carried for 3 years and 2 for 2 years. Mice carrying these tumors lived for from 3 to 6 months and developed marked hepatomegaly, cardiomegaly, hemorrhagic adrenal glands, enlarged spleens, and edematous peripheral nodes. The microscopic findings were as follows: The sinusoids of the adrenal cortex and of the liver were so greatly dilated with blood that in many there was almost complete atrophy of the parenchymal cells. Marked myeloid metaplasia and erythropoiesis were observed in the spleen and liver. Congestion of the kidney glomeruli and other organs was also observed. From the histologic findings a diagnosis of hypervolemia has been made (Dr. Thelma Dunn). Atrophy of lymphoid tissue was generally found. Metastasis of the parotid gland tumor to the lungs has been a common finding.

(Project description continued)

These same tumors, when inoculated into non-irradiated mice, failed to grow in a high percent of transplants, and when they did grow they were rapidly transformed to sarcomas. Mice carrying the sarcomas did not develop hypervolemia. This was also true in those instances where the tumor growth was slow and the mice remained alive 4 to 5 months (tumors principally sarcomatous). The myeloid metaplasia and lymphoid atrophy observed in the irradiated mice was also found here.

All mice with the transplanted tumors, whether sarcomas or parotid gland tumors, developed a granulocytosis; the white blood counts were frequently over 100,000/cubic mm. Also, all developed a severe anemia, the hemoglobin frequently dropping to 3 or 4 grams/100 cubic ml.

The histologic changes observed in mice that developed an acute infection, after inoculation with the filterable agent (Proceedings Am. Assoc. Cancer Research, April 1958), were similar to those observed in mice with the parotid gland tumor in that myeloid metaplasia, lymphoid atrophy, and hyperemia of the different organs were also noted. Severe anemia with a granulocytosis was also present.

Significance to cancer research:

It is felt that this study has contributed to the study on the etiology of the parotid gland tumor and leukemias.

Proposed course of project:

To be continued with more emphasis on demonstrating leukemogenic activity of the agent.

Major findings:

I. Two good sources for the parotid gland tumor agent have been found; one is AKR leukemia #60, extracts of which, on repeated tests over a 2-year period, have yielded an incidence of 50 to 80 percent parotid gland tumors, some of the mice have had both leukemia and parotid tumors and others only leukemia. The other, C3H leukemia #19, has yielded about a 30 percent incidence of parotid gland tumors and adrenal tumors over a period of 3 years.

Other sources for the parotid gland tumor agent have been an AKR leukemia #RIL6, C3H leukemia #1124, a paraganglioma, and a mammary tumor. The last two arose in (C3Hf x AKR) hybrid mice as a result of inoculating with AKR leukemia extracts.

None of the mice inoculated with extracts prepared from parotid gland or adrenal gland neoplasms from normal tissues, from human lymphomas, or from milk agent mammary tumors, have developed parotid gland tumors or leukemias.

Mice receiving mumps virus, and those receiving methylcholanthrene, have remained free of leukemia and parotid gland tumors.

II. A virus has been recovered from the following leukemias: AKR leukemias #60 and #RIL6, and from C3H leukemias #1124 and #19. These 4 leukemias have repeatedly been good sources for the parotid gland tumor agent. It has not been possible to recover a virus from the parotid gland tumors, or from the adrenal tumors. Extracts prepared from these tumors have not yielded parotid gland tumors.

This virus is highly lethal for newborn mice, especially for strain C3H. A few (C3Hf x AKR)F_1 hybrids have survived the inoculations, and 4 out of 15 which received virus after serial passage developed parotid gland tumors in from 5 to 12 months.

III. Five C3H parotid gland tumors transplanted into irradiated mice grew in from 50 to 80 percent of the first passage transplants and retained their original histology even after repeated transplanting. One tumor has been carried for 3 years and 2 for 2 years. Mice carrying these tumors lived for from 3 to 6 months and developed marked hepatomegaly, cardiomegaly, hemorrhagic adrenal glands, enlarged spleens, and edematous peripheral nodes. The microscopic findings were as follows: The sinusoids of the adrenal cortex and of the liver were so greatly dilated with blood that in many there was almost complete atrophy of the parenchymal cells. Marked myeloid metaplasia and erythropoiesis were observed in the spleen and liver. Congestion of the kidney glomeruli and other organs was also observed. From the histologic findings a diagnosis of hypervolemia has been made (Dr. Thelma Dunn). Atrophy of lymphoid tissue was generally found. Metastasis of the parotid gland tumor to the lungs has been a common finding.

(Project description continued)

These same tumors, when inoculated into non-irradiated mice, failed to grow in a high percent of transplants, and when they did grow they were rapidly transformed to sarcomas. Mice carrying the sarcomas did not develop hypervolemia. This was also true in those instances where the tumor growth was slow and the mice remained alive 4 to 5 months (tumors principally sarcomatous). The myeloid metaplasia and lymphoid atrophy observed in the irradiated mice was also found here.

All mice with the transplanted tumors, whether sarcomas or parotid gland tumors, developed a granulocytosis; the white blood counts were frequently over 100,000/cubic mm. Also, all developed a severe anemia, the hemoglobin frequently dropping to 3 or 4 grams/100 cubic ml.

The histologic changes observed in mice that developed an acute infection, after inoculation with the filterable agent (Proceedings Am. Assoc. Cancer Research, April 1955), were similar to those observed in mice with the parotid gland tumor in that myeloid metaplasia, lymphoid atrophy, and hyperemia of the different organs were also noted. Severe anemia with a granulocytosis was also present.

Significance to cancer research:

It is felt that this study has contributed to the study on the etiology of the parotid gland tumor and leukemias.

Proposed course of project:

To be continued with more emphasis on demonstrating leukemogenic activity of the agent.

10. 433
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH [x] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

12. None.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

13. None.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

14. 433
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Stewart, Sarah E.: "Neoplasms in mice inoculated with cell-free extracts or filtrates of leukemic mouse tissues. I. Neoplasms of the parotid and adrenal glands," J. Nat. Cancer Inst. 15: 1391-1415, April 1955.

Stewart, Sarah E.: "Neoplasms in mice inoculated with cell-free extracts or filtrates of leukemic mouse tissues. II. Leukemia in hybrid mice produced by cell-free filtrates," J. Nat. Cancer Inst. 16: 41-53, August 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

General Project 434
Serial No.

Metabolic studies on tissue cells *in vitro*.

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service — 4. Bethesda, Md. Location — 5. 434 (a) Serial No.

6. Metabolic Studies on Tissue Cells in vitro
Project Title

7. B. B. Westfall
Principal Investigator(s)

8. E. V. Peppers, V.J. Evans, K.K. Sanford, N. M. Hawkins, J.C. Bryant, E. L. Schilling, M.C. Fioramonti, G. L. Hobbs, and W.R. Earle.
Other Investigators.

9. Project description: Alpha-keto acids in tissue cultures.

Objective: No information was available on the behavior of alpha-keto acids in the medium during growth of the cells in tissue culture, and since these are important substances in the metabolic cycle it was deemed desirable to study certain key ones both to ascertain any changes that might be taking place during growth of the cells and to see if they might substitute for the comparable amino acids in culture as certain ones have been found to do in mammalian growth.

Methods: Existing methods for the estimation of the alpha-keto acids were modified and adapted so as to give good separation of the alpha-ketoglutaric acid, oxal-acetic acid and the two geometric isomers of pyruvic acid dinitrophenylhydrazones. The methods were then applied to the ultrafiltrates of the horse serum and chick embryo extract and to the protein-free supernatants from the media after growth of the cells in the media.

Major findings: The medium supplied to the cells was found to be low in keto acids, but in those media showing good growth of the cells the cells were quickly able to restore the level to a more nearly plasma-like concentration.

Significance: This study was partly an orientation work aimed at getting methods well in hand and determining concentrations of the keto acids supplied to the cells. Further work is planned with different strains and various media to see what effect these combinations have. This is part of the program concerned with the general metabolism of the cell. It is therefore proposed to extend the study as indicated.

10. 434 (a)
Serial No.

11.
Budget Activity:

Research x Administration

Review & Approval Technical Assistance

12. None
Cooperating units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

13. No resemblance or complementation.
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 434 (a)
Serial No.

15.
Publications other than abstracts from this project during calendar year 1955.

"a-Keto acids in tissue culture. I. The concentration of pyruvic and a-ketoglutaric acids in the ultrafiltrates from horse serum and chick embryo extract. B.B. Westfall, E.V. Peppers and W.R. Earle. J. National Cancer Inst. 16:1: August 1955.

16. None
Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology Laboratory or Branch
3. Tissue Culture Section or Service
4. Bethesda, Md. Location
5. 434 (b) Serial No.

6. Metabolic study on tissue cells in vitro.

7. B. B. Westfall
Principal Investigator(s)

8. E.V. Peppers, K.K. Sanford, V. J. Evans, N. M. Hawkins, and W.R.Earle.
Other investigators

9. Project description: Fraction of horse serum proteins for use in cultivation of cells in vito.

Objective: It had been shown horse serum freed of its readily diffusible substances by ultrafiltration when properly supplemented gave excellent growth of the tissue cells in culture. As a first attempt to see if the growth was perhaps associated with some one of the more or less well recognized discrete fractions, separation by means of a modified Cohn low-temperature technique was carried out and the fractions tested for growth by Dr. K. K. Sanford. If one of these contained all the growth stimulating factors it would have been a distinct forward toward getting a well-characterized medium for growth studies.

Methods: As noted above.

Major findings: Growth was reasonably good with all fractions, but better with some than others; the gross globulin fraction was that one giving as good proliferation as the original serum residue. The study indicated that growth was not necessarily associated with any particular moiety, or if such should be the case that it was effective. If time and suitable facilities are available further work on fractionation is contemplated. It is hoped that outside sources may be found that might undertake such fractionation.

10. 434 (b)
Serial No.

11. Budget activity:

Research x Administration

Review & Approval Technical Assistance

12. None

Cooperating units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

13.

If this project resembles, complements, or parallels research done elsewhere in the Public Health Service(without interchange of personnel, facilities or funds), identify such research:

It has been rumored that Dr. H. Eagle's group and Dr. H. Steinman have been working along similar lines, but we have no direct information.

14. [illegible]
Serial No.

15.

Publications other than abstracts from this project during calendar year 1955.

Effect of serum fractions on the growth of strain L cells. K.K.Sanford, B.B.Westfall, M.C. Fioramenti, W.T. McQuilkin, J.C.Bryant, E.V. Peppers, V.J. Evans and W.R.Earle. J. National Cancer Inst. 16:3: Dec. 1955.

16. None

Honors and awards to personnel relating to this project during calendar year 1955

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory of Branch

3. Tissue Culture Section or Service — 4. Bethesda, Md. Location — 5. 434 (c) Serial No.

6. Metabolic studies on tissue cells in vitro.

Project Title.

7. B.B. Westfall
Principal Investigator(s)

8. E.V. Peppers, V.J. Evans, K. K. Sanford, N. M. Hawkins, J. C. Bryant, E. L. Schilling, M. C. Fioramonti, G.L. Hobbs, and W.R. Earle.
Other investigators.

9. Project description: Chemical studies on the changes in the nutrient medium when cells are grown in large quantity and volume of the shaker flask.

Objective: No information was available as to change in nutrient in the medium during growth of cells on the large shaker apparatus. Also since growth was very good and it was known that the quantity of certain of the amino acids were supplied in low amount it was deemed desirable to see if any of these were limiting in concentration since Fischer had demonstrated in 1953 that the free amino acids lysine, histidine, arginine, valine, leucine, isoleucine, threonine, phenylalanine, tryptophane, methionine, cystine and the amine glutamine were needed for good growth of cells.

Major Findings: In one study with the HeLa strain of cells it was found that by using the medium made up of human placental fluid and whole egg extract ultrafiltrate with their attendant low concentration of amino acids, certain of the amino acids appeared to be exhausted from the medium in a 3-day interval with a heavy inoculum and good growth.

Significance: This study was primarily part of a program for the study of cellular behavior under different conditions, cancer being thought of as mainly a phenomenon in the growth of cells. The strain of cells under study were human malignant cells of epithelial origin.

Methods: The cells were grown on the shaker flasks with continuous gas exchange and continuous shaking. The medium was examined for free amino acids by paper chromatography; for alpha-keto acids by extraction and paper chromatography, for lactic acid and glucose by adaptation of existing spectrophotometric methods.

It is proposed to continue and extend similar studies to other cells and to various conditions of maintenance.

10. 434 (c)
Serial No.

11.
Budget activity:

Research x Administration

Review & Approval Technical Assistance

12. None
Cooperating Units of the Public Health Service, or other organizations, providing funds, facilities, or personnel for this project in either 1956 or 1957.

13. No resemblance or complementation known.
If this project resembles, complements, or parallels research done elsewhere in the Public Health Service (without interchange of personnel, facilities or funds), identify such research:

14. 434 (c)
Serial No.

15.
Publications other than abstracts from this project during calendar year 1955.

"The change in concentration of certain constituents of the medium during growth of the strain HeLa Cells." B. B. Westfall, E.V.Peppers and W.R. Earle. The American J. of Hygiene, 16;3: April, 1955.

16. None
Honors and awards to personnel relating to this project during calendar year 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Pathology Section — SECTION OR SERVICE
4. — LOCATION(IF OTHER THAN BETHESDA)
5. NCI ~~509~~ 500 — SERIAL NO.

6. Histogenesis and pathology of induced and spontaneous tumors of laboratory animals
PROJECT TITLE

7. Harold L. Stewart
PRINCIPAL INVESTIGATOR(S)

8. Katharine C. Snell
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Object: To gain knowledge of the etiologic and pathogenic factors involved in the production of neoplasms in experimental animals, and to make information obtained available to other investigators.

Methods: 1. A fascicle on transplantable and transmissible tumors of animals has been prepared for publication in the Atlas of Tumor Pathology under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council. This fascicle includes the history, transplantation behavior, detailed pathological description, and microphotographs of 50 of the most widely used animal tumors. (Dr. Lucia Dunham has collaborated on this work.)

2. A study is being made of the pathology of spontaneous and induced tumors of the liver of animals. This study will form the basis of papers to be presented at the Symposium on Liver Cancer at Kampala, Uganda, and Leopoldville, Belgian Congo, in August 1956 under the auspices of the Unio Internationalis Contra Cancrum. It will also be published as a chapter in the revised edition of The Physiopathology of Cancer (F. Homburger and W. H. Fishman, editors).

3. A series of untreated rats of seven strains, males and females, breeders and non-breeders, is being necropsied at various age intervals and subjected to detailed pathological examination in order to enumerate and classify spontaneous tumors, to study tissue changes with increasing age, and to furnish controls for present and future experiments.

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

4. An attempt is being made to produce adenomatosis of the lung by feeding 1,2,5,6-dibenzanthracene to DBA mice.

5. Monkeys that received injections of carcinogenic substances into the wall of the stomach several years previously are being necropsied and studied to ascertain whether these substances may have produced gastric neoplasms.

6. A study is being made of the tumors occurring in the NHO strain of mice. These mice are of interest because of the fact that their ancestors received a single dose of methylcholanthrene in an effort to produce an hereditary gastric carcinoma.

Major Findings: 1. Fascicle on Transplantable and Transmissible Tumors of Animals. The manuscripts on 50 tumors, together with 300 microphotographs, have been completed and these have been approved for publication by the N.I.H. Editorial Board.

2. The study of liver tumors is in progress and microphotographs and lantern slides are being prepared.

3. A total of 404 untreated rats have been necropsied. The series of necropsies is complete on the Marshall 520, the AXC, and the Osborne-Mendel strains. Microscopic evaluation of the material is in progress. Unusual tumors noted have been carcinomas of the adrenal cortex, a carcinosarcoma of the jejunum, and tumors of the lung and testis.

4. Necropsies and evaluation of sections of the DBA mice fed 1,2,5,6-dibenzanthracene are being made, but it is too early in the experiment to report any significant findings.

5. No monkeys necropsied to date have shown gastric carcinoma nor any lesions that can be attributed to the injection of carcinogenic substances into the wall of the stomach.

6. Various tumors of the NHO strain of mice have been observed, and these are being studied histologically and evaluated. There have been no carcinomas of the glandular stomach, but tumors of the nervous system, lung, uterus, and reticulo-endothelial system have been found.

Significance to Cancer Research: The knowledge gained from a study of neoplasms in laboratory animals should provide a basis for further work on the mechanism of carcinogenesis in the human being.

. PROJECT DESCRIPTION (Cont'd)

Proposed Course of Project: It is expected that the fascicle on Transplantable and Transmissible Tumors of Animals will be published in 1956.

The study of the pathology of liver tumors will be completed and ready for publication by the summer of 1956.

Experiments dealing with carcinogenesis (enumerated as parts 3-6) will be continued.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~509~~ 500
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None to my knowledge
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI 5~~09~~ 500
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Stewart, H. L. and Hare, W. V. Chronic gastritis of the glandular stomach, adenomatous polyps of the duodenum and calcareous pericarditis in strain D.B.A. mice. J. Nat. Cancer Inst. In press.

16. None
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Pathology Section — SECTION OR SERVICE
4. New York, Israel, Washington, D.C. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~519~~ 5-01(a) — SERIAL NO.

6. Geographic pathology field studies on uterine cancer in New York City, Israel, and Washington, D. C.
PROJECT TITLE

7. Lucia J. Dunham, Harold F. Dorn, Harold L. Stewart
PRINCIPAL INVESTIGATOR(S)

8. John H. Edgcomb, Louis B. Thomas
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Field studies of uterine cancer (cervix and corpus) in New York City, Israel, and Washington, D. C.

Objectives: The objective of this project is to complete an extensive questionnaire study of women with uterine cancers in different geographic areas and racial groups, and to analyze the data obtained with a view to identifying factors suspected of predisposing to or causing female genital cancers. Also, basic data on the incidence and pathologic (microscopic) diagnoses of the cancers are obtained.

Methods: For the area under study and the period of study all patients possible with the diagnosis of uterine cancer are interviewed, and an equal number of control patients as well. The confidential data recorded include social, medical, and surgical history, and menstrual, marital, and pregnancy data. Records are kept of the small number of patients not interviewed, their diagnoses, and the reasons for failure to interview. Pathologic material is studied in all cases.

A coding statistician and two medical social worker interviewers are currently employed in the project.

Major findings: 1. Data from about 2,000 interviews with Jewish women in Israel have been reviewed and coded.

2. Data from about 2,000 interviews with Jewish and non-Jewish white women in New York City have been reviewed and coded.

3. Data from about 400 interviews of non-white women in New York City have been reviewed and coded.

4. Data from about 20 interviews of non-white women in Washington, D. C., have been reviewed and coded. This study was started late in 1955.

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

5. Pathologic material has so far been examined in about 90 percent of the above cases.

6. The data from about 2,500 cases have been transferred to punch cards for IBM analysis.

7. The medical literature on geographic and environmental factors in uterine cancer has been reviewed.

Significance to cancer research: The significance of the study lies in the hope of supplying a sound scientific basis for the understanding of factors of custom, habit, or environment as they may be related to relatively high frequencies of uterine cancers in some groups of women as contrasted to intermediate or relatively low frequencies in other groups.

Proposed course: The proposed course is to tabulate, analyze, and appraise the material referred to above, and to prepare reports of these studies for publication. At the conclusion of the project it is planned to undertake clinical studies in geographic pathology on patients with cancers of the esophagus, stomach, and large bowel.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~519~~ 501(a)
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH /x/ ADMINISTRATION / /
REVIEW & APPROVAL / / TECHNICAL ASSISTANCE / /

12. Office of Biometry, Office of the Director, National Institutes of Health
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI ~~519~~ 501(a)
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Dunham, L. J. and Dorn, Harold F.
Techniques in the Geographic Pathology of Cancer
Schw. Zeit. für Allg. Pathologie und Bakteriologie, Vol. 18, No. 4, 1955
Printed in Switzerland.

16. None

HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Pathology Section — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~520~~ 501(b) — SERIAL NO.

6. Geographic Pathology. Experimental studies relating to the geographic pathology of mouth cancer.
PROJECT TITLE

7. Lucia J. Dunham
PRINCIPAL INVESTIGATOR(S)

8. Marvin S. Burstone
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Experimental studies on mouth cancer.

Objectives: The objective of this project is to examine the mucous membrane of the hamster cheek pouch for proliferative changes that may develop when test substances are chronically maintained in contact with these surfaces. Substances used are environmental materials suspected of being mildly carcinogenic for man.

Methods: The pellets which have been prepared for insertion in the cheek pouch of the hamster include the tobacco, lime, areca nut, and pepper leaf of the far Eastern "betel quid," the plastic material used in preparing "false" teeth, and tobacco tars and control materials. A technical assistant is retained part-time for help in the study.

Major findings: Since the project is a long-term one, there are no major findings at present. It is hoped to maintain the experimental animals for at least two years before gross and microscopic study of the pouches. The operations used for testing substances has so far been performed on 107 hamsters.

Significance to cancer research: The significance is to reach an improved understanding of chronic irritant factors as they may relate to cancer of the mouth in man.

Proposed course: The study will be extended to more animals and to a wider variety of suspected substances. Current experimental animals will be studied with care, when illness or death intervene. In addition, improved techniques of study will be attempted.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~520~~ 501(b)
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH /x/ ADMINISTRATION /_/

REVIEW & APPROVAL /_/ TECHNICAL ASSISTANCE /_/

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDIN FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

NO ENTRIES FOR 14, 15, or 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~527~~ 502 — SERIAL NO.

6. Histogenesis and histopathology of cutaneous neoplasms.
PROJECT TITLE

7. John H. Edgcomb
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

During the past year the project has included the following activities:

A. Studies of diseases of man (collaborative research).

i. Mycosis fungoides

The reaction of three patients with mycosis fungoides to electron beam therapy has been studied pathologically. This type of therapy causes remissions of cutaneous tumors in this condition.

ii. Melanomas

Naevi from the skins of patients with malignant melanomas have been examined. It appears that more naevi from such patients show junctional activity than is the case of naevi from control patients.

B. Studies of diseases in animals

i. Further studies are being made on C strain mice bearing Dr. R. Bryan's transplantable squamous carcinoma. Many of these mice develop severe leukocytosis. Attempts to transform this disease into granulocytic leukemia have been unsuccessful.

ii. An attempt to produce melanomas in guinea pigs is being continued. As yet (2 years) no melanomas have developed.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~527~~ 502
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

NO ENTRIES FOR 14, 15, or 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~508~~ 503 — SERIAL NO.

6. Accumulation and dissemination of information relating to the normal and pathologic anatomy of laboratory animals, particularly mice, with special emphasis on neoplasms.
PROJECT TITLE

7. Thelma B. Dunn
PRINCIPLE INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To accumulate information relating to the normal and pathologic anatomy of small laboratory animals, and to publish it in such a form as to be readily available to laboratory workers throughout the world.

Methods: Individual study of particular phases of pathologic anatomy of laboratory animals; collaboration and consultation with other scientists at the National Cancer Institute in projects requiring the review of histologic material obtained from experimental animals; review of material sent in for an opinion by scientists from outside laboratories; carrying in transplant of unusual neoplasms in mice, so they can be kept for continued study; and accumulation of literature and references relating to the subject.

Major findings: The present project has been carried on through a number of years. During the past year a paper has been published with Dr. Law describing the morphology of an unusual neoplasm of the parotid salivary gland in mice, with a review of previous reports of salivary gland tumors in mice. Several transplantable tumors are being carried, which have not been described before in the mouse. These are a uterine adenocarcinoma, a parathyroid neoplasm, an actively secreting adrenal cortical neoplasm, and a mucoid adenocarcinoma of the ovary.

Significance to cancer research: Since the mouse is used so regularly in cancer research, it is necessary that the normal and pathologic anatomy of this species be well understood, if reliable conclusions are to be drawn from cancer studies.

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

Proposed course of project: Work on this project will continue as it has in the past. Phases of the subject now under special study are:

a. Systematic review of the endocrine system of the mouse. Dr. Ross MacCardle is collaborating in this by carrying out studies related to the pituitary. A study of the endocrine organs of different inbred strains at different ages has been begun.

b. Continuing collaborative study with (1) Dr. Lloyd Law of mice receiving various filtrates, and of mice developing leukemia; (2) Review of similar material from Dr. Sarah Stewart; (3) Dr. W. E. Heston of crosses between strain C3H and C57BL in which a variety of tumors appeared; (4) Dr. H. B. Andervont on mammary tumors in mice, and the pathologic changes in wild mice, and (5) review of small numbers of slides from Dr. Margaret Deringer, Dr. Elizabeth Jones, Dr. Michael Potter, Dr. Vincent Price, and Dr. Robert Greenfield, and others.

c. Continuing review of unusual lesions sent from outside sources. Among those recently received were several from Dr. W. U. Gardner of Yale, Dr. Michael Klein of Florida, Dr. P. Loustalot of Switzerland, and Dr. H. I. Pilgrim of California. Opportunity to review these unusual lesions is of great value since it gives a chance for comparison with other laboratories.

d. Continuing observation of unusual transplantable tumors. Important information has often been gained by observing the behavior of transplanted tumors, especially where they have a hormonal effect.

e. Systematic review of literature relating to endocrine organs in mice has been started.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~508~~ 503
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH [x] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. Not known.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI ~~508~~ 503
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Dunn, T. B., Morphology and a Cure for Cancer. Journal of the American Medical Women's Association. Vol. 10: 101-109. April 1955.

Law, L. W., Dunn, T. B., and Boyle, P. J. Neoplasms in the C3H strain and in F_1 hybrid mice of two crosses following introduction of extracts and filtrates of leukemic tissues. J.N.C.I., 16: 495-539. 1955.

Dunn, T. B., Mammary Tumors in Laboratory Rodents. A lecture to a class in veterinary pathology in a course on Diseases of Laboratory Animals. Delivered December 5, 1955. This lecture to be published in a textbook related to the course.

Andervont, H. B., and Dunn, T. B. Transplantation of hepatomas in mice. Published in J.N.C.I., 15: 1513-1524, from Proceedings of the Conference on Experimental Hepatomas.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Elected Secretary-Treasurer of the Washington Society of Pathologists, 1955-56.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~517~~ 504 — SERIAL NO.

6. An electron microscopic investigation of normal and neoplastic tissues.
PROJECT TITLE

7. William G. Banfield
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To describe the ultra structure of selected neoplasms and to compare it with suitable normal and control tissues.

Methods: The usual electron microscopic methods will be used.

Major findings: The microscope is not yet in operation.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~517~~ 504
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 14, 15, or 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI 506 — SERIAL NO.
6. Pulmonary Tumor — PROJECT TITLE
7. C. Harold Steffee — PRINCIPAL INVESTIGATOR(S)
8. None — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: (1) To gain basic information on biologic aspects of cancer, using pulmonary tumors as a tool in this endeavor. (2) To gain information regarding the etiology and pathogenesis of human lung cancer, and to determine the carcinogenicity of various substances suspected as etiologic agents in human pulmonary cancer. This requires finding a method for producing similar tumors in animals.

Methods: (1) Biologic studies have been done using tumor transplantation and genetic techniques. (2) Attempts to induce experimental bronchogenic neoplasms in animals have used embryo transplantation techniques and also the intratracheal administration of known carcinogens and suspected carcinogens in adult mice.

Major findings: The study of the effect of size of the original lung tumor and the size of the injected fragment upon transplantability has yielded the following information. The data suggest that there is a critical size of pulmonary tumors; smaller neoplasms transplant poorly. When pieces of varying size of large tumors are transplanted the percentage of "takes" is essentially the same for all sizes except the very smallest. During the course of this experiment approximately 20% of the animals died over weekends and were too autolyzed for definitive autopsy. Because of this and also because the results obtained are of border-line statistical significance, it was decided to repeat this experiment, checking the animals on weekends. Insufficient time has elapsed with the second running of this experiment to permit accumulation of definitive data.

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

The hypothesis that malignant cells observed in the sarcomatous transformation of the alveologenic adenoma of the mouse are derived from host stroma has been tested. It has been found that this hypothesis is incorrect. (2) Previous studies with embryo mouse lung transplants, using methylcholanthrene as a carcinogen, produced 7 sarcomas, 1 squamous-cell carcinoma, and 2 adenocarcinomas in a total of 25 such transplants. Repetition of these studies with refinements in technique by which it is hoped more epithelial tumors may be obtained have not been completed as yet. A similar study using cigarette tar as the "carcinogen" has failed to produce any gross tumor in 13 such transplants.

An experiment in which several types of cigarette tars have been administered intratracheally to mice together with suitable control substances has not yet been completed. Mice which received intratracheal cigarette tars and died during the course of the experiment to date, have shown no increase in pulmonary tumors.

Collaborative studies on the carcinogenicity of various chromate fractions, carried on with Dr. Anne Baetjer of The Johns Hopkins University have not yielded gross tumors to date. Microscopic studies have been completed on approximately half of the animals so exposed, and these sections show chiefly a non-specific inflammatory reaction and rarely the formation of tiny granulomata.

Significance to cancer research: Study of the relationship of tumor size to transplantability is a problem in the fundamental nature of malignancy. Definition of the exact point at which benignity becomes malignancy will sharpen the focus for further biological and biochemical studies. The fact that the sarcoma cells which often arise on serial transplantation of mouse lung tumors are not derived from the host serves to eliminate this one possibility in the search for the source of these cells. The importance of this search to the general problem is one of understanding the fundamental cell behavior as well as an understanding of the occasional carcinosarcomas which are observed in the human. The other experiments performed in this project represent a more direct attack on the human cancer problem. Bronchiogenic carcinoma is now in many areas the commonest malignancy of men, and seems to be increasing in frequency. Many laboratories throughout the country are engaged in the search for etiologic agents but all of this work is seriously hampered by the lack of a suitable test object. Thus, we have placed much emphasis on trying to produce an analagous tumor experimentally. Simultaneously, we have studied the effects on experimental animals of two substances which have been implicated in human neoplasia, namely cigarette tars and chromates. Though these are but two of the many suspected etiologic agents, they at least represent a part of the attack on the vast problem of human lung cancer.

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

Proposed course of the project. Since the principal investigator is leaving the National Cancer Institute in December of 1955, many of these studies of an exploratory nature will be terminated. The animals bearing transplanted pieces of lung tumors in the study of the effect of transplant size and tumor size upon transplantability will be allowed to live out their life span in order that this data and this experiment can be completed. In the study on sarcomatous degeneration of transplanted lung tumors, no further transplants will be done even though all of the tumors have not yet undergone the degenerative changes. I believe sufficient data is already at hand to support the conclusions given above. Experiments on embryo transplant techniques and with intratracheal administration of cigarette tars will be terminated and the results prepared for publication if this seems warranted. The remaining slides in the collaborative study with Dr. Baetjer will be studied but no new experiments are planned in this area since the grant to Dr. Baetjer will terminate in 1956. Another collaborative study has been planned with the Medical Director, Randall B. Haas, U. S. Public Health Service Hospital, Manhattan Beach, to investigate other possible mechanisms of pulmonary carcinogenesis. These experiments will include intratracheal administration of carcinogenic hydrocarbons adsorbed on carbon particles, to tuberculous and non-tuberculous animals, to determine the lipoid solvent effect of caseous necrosis. These experiments will also include studies on certain enzymes which have been reported to cause squamous metaplasia of bronchial epithelium in humans. The animal work in these experiments will be done at Manhattan Beach; the pathologic material will be examined by the principal investigator in his new location. It is expected that this experiment will get underway shortly after the first of the next calendar year. We have prepared the carbon particles for these experiments, have tested their toxicity on guinea pigs, and will shortly ship them to Dr. Haas.

PROJECT REPORT FORM (Cont'd)

10. NCI 506
SERIAL NO.

11.
BUDGET ACTIVITY:
RESEARCH [X] ADMINISTRATION [ ]
REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

1) PHS Grant C-603, providing funds to Dr. Anne M. Baetjer, Department of Environmental Medicine, The Johns Hopkins University, 615 North Wolfe Street, Baltimore, Maryland, for experiments on inhalation and intratracheal injection of chromates.

2) U. S. Public Health Service Hospital, Manhattan Beach, Brooklyn 35, N.Y.

13. No similar research known to be in progress elsewhere.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

NO ENTRIES FOR 14, 15, or 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~502~~ 507 — SERIAL NO.

6. Mitochondrial changes in the liver of X-irradiated mice. — PROJECT TITLE

7. Ross C. MacCardle and Charles C. Congdon — PRINCIPAL INVESTIGATOR(S)

8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objective: To determine whether cytoplasmic elements are the primary focus of initial damage in X radiation injury, and to study the anatomical basis of recovery from X radiation injury that follows injection of bone marrow into irradiated mice, we studied mitochondria and other cytological structures by the usual methods at early periods postirradiation.

Methods:

Major findings: It was found that mitochondria of the liver certainly are altered long before the animal succumbs, and the mitochondria recover their normal state after bone-marrow treatment. This may be a factor in X radiation injury. This has led us to study the state of the hepatic and serum phospholipids and choline, the mitochondria after x-raying the liver only, and after shielding the liver.

Significance to cancer: Having found a definitive change in mitochondria, we now proceed to study the mitochondria of the reticulo-endothelial system in spleen, bone-marrow thymus, and lymph nodes. We are studying the thymus of C57Bl and LAF_1 mice in which X radiation produces lymphocytoma. This involves a cytological study also of thymectomized mice in which X radiation fails to produce tumors.

Proposed course: We wish to finish the study of the liver and bone-marrow after X radiation, and study the other cytological structures which we have already preserved in this project. We wish also to make a comparative cytological study of the thymus of various strains of mouse in respect to their susceptibility to formation of tumors at different ages.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~502~~ 507
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH /x/ ADMINISTRATION /_/

REVIEW & APPROVAL /_/ TECHNICAL ASSISTANCE /_/

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI ~~502~~ 507
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

The present part of this project in respect to tumors will be done probably in conjunction with Dr. Lloyd Law of NCI. The remainder of it is being done with Dr. Congdon who is now at Oak Ridge National Laboratory in Tennessee.

16. None
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~505~~ 508 — SERIAL NO.

6. Comparative study of the macroscopic form and cytological structure of normal human epidermis and human hair follicles to evaluate changes in pathologic states such as in basal-cell tumors, squamous-cell tumors, intraepidermal tumors, acne, mycosis fungoides, and other diseases
PROJECT TITLE

7. Ross C. MacCardle with Dr. E. J. Van Scott of NIH
PRINCIPAL INVESTIGATOR(S)

8. ____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Wooden models are being made of whole hair follicles in health and disease that reveal striking differences in size and pattern. Minerals and other cytological constituents will be studied at different levels of the follicle. This study of models has not been attempted previously.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~505~~ 508
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, or PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 14, 15, or 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~504~~ 509(2) — SERIAL NO.

6. Relationship of Nadi oxidase (cytochrome oxidase) granules to mitochondria in normal and malignant tissue
PROJECT TITLE

7. Ross C. MacCardle
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

During the past two years, it was discovered by the use of ultracentrifugation that the Nadi oxidase granules and mitochondria resident in the living cytoplasm are anatomically independent of each other in situ in the normal intact cell of epithelium of the epididymis, kidney and liver of mouse. The form of the Nadi oxidase granule is distinctly different from that of the mitochondrion. It has now been found that in hepatoma of the mouse they are also anatomically separate. However, in the case of the malignant dependent thyrotrophin-secreting transplant tumor of the pituitary gland, the Nadi oxidase granules can be dislodged by ultracentrifugation to one pole of the cytoplasm along with the mitochondria, whereas the granules of the normal pituitary gland cannot be moved by the same centrifugal force required to move the mitochondria -- the mitochondria of a normal gland thus being moved to one pole of the cell leaving the Nadi granules unmoved. This may not necessarily mean that the mitochondria and Nadi oxidase granules of a tumor are anatomically associated, however. It may indicate that the viscosity of the malignant protoplasm is less than in normal cells, thus permitting the granules to be moved with greater ease, or it may mean that the weight of the granules of this tumor differs from that of the granules of the normal gland.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~504~~ 509(2)
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 14, 15, or 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-~~500~~ 509(a) — SERIAL NO

6. Intracellular cytology of thyrotrophin-secreting tumors of the anterior lobe of the pituitary gland of radiothyroidectomized mice, and of human pituitary tumors.
PROJECT TITLE

7. Ross C. MacCardle
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Object: The object of this study is to ascertain and define the earliest morphological alterations that occur in cells of tissues in their change from the normal to the malignant state of protoplasm in respect to a possible analysis of the underlying cause of the malignant transformation. This tumor of the mouse represents one of the most valuable experimental tumors because it resembles pituitary tumors of man. It clearly consists of a single, histologically-identifiable type of tissue. One or another of the constituent cells of the anterior lobe proliferate in different states of hyperplasia and malignancy. The gland is stimulated to become hyperactive in producing the thyroid-stimulating hormone for a thyroid gland that has been essentially destroyed by treatment with radioiodine. Finally, the hyperplastic tissue becomes malignant to produce 10 times as much hormone as the normal gland. The amount and kind of hormone produced by a tumor can be determined, and the cytological alterations of the same tumor can be identified and related to the function.

Methods: Cytological methods can detect alterations in cellular minerals (microincineration), mitochondria, Golgi apparatus, nucleoproteins, cytochrome oxidase granules.

Findings: The major findings so far indicate that the Golgi apparatus first becomes hypertrophied and more readily metallophilic as more cytoplasmic iron becomes deposited in the vicinity of it. The staining properties of the mitochondria of the tumor are different so that

PROJECT REPORT FORM (cont'd)

9. PROJECT DESCRIPTION (Cont'd)

they cannot be destained as easily as those of the normal gland. Most of the cells of the malignant thyrotrophin-secreting tumor appear to originate from alpha cells, thus suggesting that TSH may be formed in these cells. At least in the malignant state. The cellular constituents of the hyperplastic tissue have not been identified with production of TSH, and there is no evidence that alpha cells of the normal gland produce thyrotrophin.

Proposed Course: The proposed course of the project is to study the fetal, adult and hyperplastic gland to compare the relation of cytological changes to production of hormones with those of primary and transplant malignant tumors. It is clear that a study of the effect of TSH on the cells of an already developed TSH-tumor should be made to determine the cellular organoids first affected when the activity of the tumor begins to diminish in the presence of TSH. Comparative cytological studies of human and animal pituitary tumors should be accomplished. It is proposed to study the effect of radioiodine on young and adult dwarf homozygous mice that lack pituitary alpha cells.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~500~~ 509(a)
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 195;

13. No
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI ~~500~~ 509(a)
SERIAL NO.

15.
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 195

MacCardle, R. C., and C. C. Congdon. 1955:
"Mitochondrial changes in hepatic cells of X-irradiated mice."
Am. J. Path. 31: 725-745

MacCardle, R. C. 1955:
"Characteristics of mitosis in tumor cells."
In press: Annals of N. Y. Academy of Sciences.

16. None
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~501-~~ 509(b) — SERIAL NO.

6. Writing a brief Monograph entitled: "The Tumor Cell: Characteristics of Malignant Protoplasm" as part of a series of volumes of An International Treatise on Physiology of Protoplasm "Handbuch der Protoplasmaforschung" being edited by Heilbrunn and Weber.
PROJECT TITLE

7. Ross C. MacCardle
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Object: The object of this monograph is to summarize and correlate our knowledge of the properties and possible identification of a malignant cell of a tumor.

Method: By reviewing the literature and relating some new data found by the author, it is clear that although a single white blood cell of the

Major findings:
circulating blood of a leukemic mouse can upon injection into a normal host produce leukemia (notwithstanding the serum injected with the cell), such a cell possesses no anatomical features by which it can be distinguished from its normal cell of origin. The most malignant tumor constitutes several types of cells, some of which may have nothing to do with malignancy and none of which can be detected as a malignant cell. The transplantability of the malignant tumor cells is discussed, and the cytology of several tumors is described for the first time.

Significance to cancer: This paper will bring together old and new data in an effort to evaluate the status of our knowledge of the mechanism of the transformation of normal protoplasm to malignant protoplasm.

Proposed course: I propose that it now be printed in book form, and it is nearly ready to go to the printers. I was invited to write it, and I understand that it was accepted in advance.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~501~~ 509(L)
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 14, 15, or 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~503~~ 509(c) — SERIAL NO.

6. Intracellular cytology of human and animal tumors. — PROJECT TITLE

7. Ross C. MacCardle — PRINCIPAL INVESTIGATOR(S)

8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Object: Little is known of the basic intracellular cytology of the cytoplasm of human or animal tumors. Most of the modern studies deal with electron microscopy (a method limited to tissue fixed in one or two ways) and certifugal isolation of mitochondria of a polyglot tumor (hepatoma) of the mouse (the "mitochondria" being of questionable integrity and identify after crushing the cell).

Method: Using light microscopy and ordinary cytological methods, I have been studying the minerals, Golgi apparatus, mitochondria and mitotic figures of various tumors in an attempt to evaluate some of the remarkable features found by electron microscopy in tumors that have not been studied by ordinary methods. I have been making a comparative cytological study of white blood cells of circulating blood in human leukemia in relation to the structure of cells of the bone marrow. I have found that the Golgi apparatus is in the form of widely dispersed dictyosomes in the argyrophobic cytoplasm of the young transplant Sarcoma 37 in mice, whereas it is a small compact network in richly argyrophilic cytoplasm of the older vascularized transplant tumor. This seems to indicate a detectable difference in slowly-growing and rapidly-growing malignant tissue. I am also studying basal-cell tumors, squamous-cell carcinomas, superficial intraepidermal tumors, Bowen's disease, and Xeroderma pigmentosum in the skin of man.

Proposed study: I expect to continue this study along the same lines.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~503~~ 509(c)
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 14, 15, or 16.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Pathology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~522~~ 510 — SERIAL NO.

6. Mechanisms of tumor invasiveness, and the influence of chemical agents upon them.
PROJECT TITLE

7. Joseph Leighton
PRINCIPAL INVESTIGATOR(S)

8. Murray Belkin (Laboratory of Chemical Pharmacology), Ira Kline (Laboratory of Chemical Pharmacology)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: (1) The further development of quantitative methods for evaluating a) the destructive invasion of normal human tissues by human cancers in tissue culture, and b) the interaction in vitro of host cells and tumor cells.

(2) The study of the mechanisms of local invasion and of metastases in tissue culture with special emphasis on the metabolic requirements of these malignant processes using as experimental tools biochemical agents such as cortisone, hyaluronidase, stilbesterol and analogues of metabolites.

(3) The appraisal of the influence of chemical agents on tumor invasiveness in which the pharmacologic effect is considered in terms of a) altered rate of invasiveness, b) changes in the organization and differentiation of the tumor, and c) the responses of the host tissues which may tend to limit the spread of the tumor.

(4) The discovery of the conditions which must be supplied for the tumor in tissue culture so that progressive growth may be obtained from a large percentage and a wide variety of types of clinical cancers, making chemotherapeutic studies possible.

(5) Cultivate tumors from the operating room to observe the extent of differentiation in vitro as a supplementary aid to the evaluation of sections made of biopsy and autopsy material.

Methods: Sponge matrix methods for tissue culture are being used in this work.

Major findings: (1) Tissue culture studies on the destructive invasion of normal tissues by cells of human cancer have been further extended. Invasion by several cancer cells has been observed including a) strain HeLa (Gey), which arose from an epidermoid carcinoma of the

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

cervix, b) strain KB (Eagle) which arose from a carcinoma of the mouth, c) strain J-96 (Osgood) which arose from a monocytic leukemia. A variety of normal human tissues are invaded and replaced by these tumor cells.

(2) Further studies on variations of sponge matrix methods have been developed which permit the measurement of the speed of the invasive process.

(3) The spontaneous transformation of normal human connective tissue cells into a histologically malignant tumor has been observed. A paper describing this event is in press in Science. Studies are in progress on the biologic malignancy of this cell line in cultures where it is combined with normal human tissues and in heterologous animal transplantation.

(4) A study on one potential chemotherapeutic agent, NCI #3022, a water soluble podophyllotoxin derivative has been completed and a manuscript describing our experiments is being prepared.

(5) Several "normal" cell lines originating in other laboratories have been examined in sponge matrix. In some instances these "normal" cells are morphologically indistinguishable from highly malignant cancer. The biologic properties of these cells in terms of their invasive capacity is being studied.

Significance: The productivity of this project has in large measure developed from the nature of its organization. It has been one of close collaboration and integration of efforts of personnel of two laboratory departments, Pathology and Chemical Pharmacology.

Methods have been developed which permit the experimental study of the invasive powers of human cancer so that the metabolic requirements of invasion as well as potential inhibitors of invasion can be studied.

Observations have been made in combinations of tumor with normal tissues that may contribute directly to our understanding of the invasive process. Cells growing out of several normal tissues invade tumor cell masses to an extent comparable to the invasion by the tumor of other normal tissues. This raises the possibility that in some instances normal cells in the body may enter and divide a tumor nodule giving rise to several new centers of tumor growth. A second observation which may be significant is a negative one. In invasion experiments, one almost never sees degenerating forms among the normal cells in the areas being invaded by tumor. It is as if the normal cells disappear completely, either by migration or very rapid dissolution.

Proposed course: The principal investigator has planned to accept a position at the University of Pittsburgh in May, 1956, where he will have an opportunity to teach as well as to continue his research work. The project will be pursued there, and the facets of it that are of direct interest to the Laboratory of Chemical Pharmacology will continue to be studied at the N.I.H. It is planned and expected that many aspects of the fruitful collaboration existing up to now between Dr. Belkin, Mr. Kline, and the principal investigator will continue, regardless of the distance between cities.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~522~~ 570
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. No similar research is known to be in progress elsewhere.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

PROJECT REPORT FORM (Cont'd)

14. NCI 570
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Major contributor to An Introduction to Cell and Tissue Culture, by the staff of The Tissue Culture Course, Cooperstown, New York, 1949-53, Burgess Publishing Company, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

Guest Lecturer and Consultant on Tissue Culture to the Department of Pathology, New England Deaconess Hospital, July 11 & 12, 1955.

Lectures at Camp Detrick, Maryland; Naval Medical Research Center, Bethesda; and Tissue Culture Course at Cooperstown, New York.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI 511 — SERIAL NO.

6. Effect of neoplastic diseases and of various stresses on the pathophysiology and morphology of certain endocrine organs in man and in animals.
PROJECT TITLE

7. E. M. Nadel
PRINCIPAL INVESTIGATOR(S)

8. None (See Item #12)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Quantitative measurement of polar reducing corticosteroids in man and in animals by the use of recently developed paper chromatographic fractionation techniques.

Objectives: To quantitatively measure the polar corticosteroids present in the blood, urine, and tissues of man and animals (normal, stressed, and tumor-bearing) and to correlate where possible the clinical course with the adrenal and other morphologic alterations and with specific corticosteroid excretion patterns.

Methods: The term stress is used in its widest concept for the purposes of this report and includes dietary deficiency and excesses as stresses, hormonal excesses, and deficiencies as stresses, x-ray irradiation effects, stress by physical and microbial agents, carcinogens, and the effect of carcinoclastic and potentially carcinostatic drugs.

Animals: Stressed animals, normal controls and tumor-bearing animals are prepared biologically and the clinical course followed. Tissues, blood, and urine are collected during the course of the observation period and at post-mortem examination, for chemical, histochemical, and histopathological study. Extraction, purification and analysis are done by specific applications of chromatographic and spectrophotometric methods.

Man: Clinical patients from the Leukemia Service, NCI, Surgical Service, NCI, and Psychosomatic Service, NIMH, are being studied in addition to non-patient controls. Analyses for polar corticosteroids are performed on the urine of patients before, during, and after the administration of ACTH.

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

Major findings: The pattern of excretion of the new polar corticosteroids has now been studied extensively in guinea pigs with leukemia, with liposarcoma, following stilbesterol treatment, following ACTH administration, following x-radiation, etc. (1) There are quantitative differences in the excretion of 6-β hydroxycortisol, Steroid IIa, and cortisol in neoplastic disease. (2) There is a decrease in 6-β hydroxylation in scurvy in guinea pigs. (3) Stilbesterol treated scorbutic guinea pigs respond to ACTH less than do control guinea pigs. (4) There are strain differences in the response of guinea pigs to ACTH. (5) There are 5 polar blue-tetrazolium reducing corticosteroid zones in the urine of man in contrast to 3 such zones in the urine of guinea pigs as determined by paper chromatography after ethyl acetate extraction. (6) The response of clinical patients to ACTH is by an increased extraction of both free and of conjugated corticosteroids. The principle known free corticosteroids are 6-β hydroxycortisol and tetrahydro-F and cortisol (F).

Significance to cancer research: The presence of compounds, more polar than cortisol in the urine of man and of guinea pigs, indicates: (1) Conclusions based on the excretion of corticosteroids without specific reference to their identity must be re-evaluated, e.g. the principle corticosteroid in the albino Hartley guinea pig is cortisol, while the principle corticosteroid in Strain 2 and Strain 13 guinea pigs is not cortisol. (2) When hydrolyzed urines are extracted and their glycogen potency compared with that of extracted unhydrolyzed urine there is little difference in the biologic activity of the 2 extracts. Inasmuch as the free unconjugated polar urinary corticosteroids are the biologically active materials responsible for nearly all of the glycogenic activity of hydrolyzed or unhydrolyzed urine it now becomes important to study these free corticosteroids in some detail. (3) Up to the present insufficient attention had been given to these new polar corticosteroids for 2 reasons: a) they were not known to be present prior to their recent discovery by us in the urine of guinea pigs and of man; and b) previous methods of extraction actually destroyed them.

Proposed course: It was thought prior to 1950 that Vitamin C deficient (scorbutic) guinea pigs had poor adrenal function. We showed that adrenal activity was actually excessive in scurvy by (a) measuring the corticoid in the urine, (b) measuring it in the blood, (c) demonstrating the biological activity of purified extracts, (d) isolating and crystallizing the major product, (e) identifying it as hydrocortisone (compound F) and (f) by histological study. These studies exploded previous speculations and served to set up new problems.

Despite a high circulating blood titre of glucocorticosteroids, the glycogen in the liver of Vitamin C deficient guinea pigs is paradoxically low. It was found that the guinea pig cannot deposit glycogen in its liver in the absence of Vitamin C, even though cortisol were administered together with glucose or fructose. This is being further explored.

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

Further studies on the guinea pig indicated six other unsuspected urinary corticosteroids were present. All have since been isolated and all but one identified. One of these is a previously unknown compound, 6 BOH cortisol. This new corticosteroid has now been found in human urine together with a new series of $C_{21}O_6$ compounds. Isolation of these new steroids is in progress to provide sufficient amounts for biologic testing.

The quantitative pattern of the corticosteroid excretion is currently being investigated in guinea pigs under various stress combinations, such as x-radiation, dietary deficiencies, drug effects, neoplastic diseases, environmental alterations, and an attempt is being made to correlate adrenal morphology with the excretion of specific corticosteroids. This type of investigation will later be extended to other endocrine organs.

A clinical study developing from the observations in guinea pigs and utilizing Clinical Center material from the Leukemia Service and Surgical Service, NCI, and from the Psychosomatic Service of the NIMH has been started. The purpose is to study the alteration in the excretion of specific corticosteroids during the course of leukemia, e.g. in remission, in relapse, under therapy, while intervening infection, during active disease, and to note whether the pattern changes which the adrenal is challenged with ACTH. These patterns are compared with those of non-neoplastic patients and patients with neoplastic diseases other than leukemia.

Autopsy material from the patients who have died with leukemia is being collected for a histopathological study of the effect of leukemia on various endocrine organs.

Biosynthesis of steroids in normal and tumor-bearing animals is being studied in vivo with a Visiting Scientist. New methods of detection are being developed with the aid of radioisotopes. In vitro studies utilizing a simple perfusion apparatus are contemplated.

A "library" of frozen urine aliquots and bloods from clinical patients and animals is being collected for future study.

In addition, a long-term study is planned on the corticosteroid patterns occurring following the use of methylcholanthrene, total body radiation, and other potentially carcinogenic agents, in order to detect any specific quantitative and qualitative changes in corticosteroid excretion pattern in carcinogenesis. A related study on humans exposed to radiation has been proposed.

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

A continued study on the effect of various stresses (see Methods above) is contemplated, on the guinea pigs.

The effect of various potentially therapeutic agents on the course of cancer in the guinea pigs is being studied with the hope of picking up alterations in the specific corticosteroid excretion patterns associated with carcinostasis.

A continued study on carbohydrate metabolism in the guinea pigs is being pursued with the hope of applying this fundamental knowledge to the in vitro perfusion studies that are planned when facilities become available.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~523~~ 511
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. E. Frei, Clinical Center, NCI, L. Cramer, Clinical Center, NCI.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. None to my knowledge.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

PROJECT REPORT FORM (Cont'd)

14. NCI 511
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Effect of neoplasms and of stresses on the excretion of specific corticosteroids in the guinea pig.
Nadel, Burstein, and Dorfman.
Clin. Res. Proc., Feb. 1955 (Proc. Am. Fed. Clin. Res.).

Urinary excretion of cortisol, 6-β hydroxycortisol and an unidentified steroid (Steroid IIa) by guinea pig with leukemia or with liposarcoma.
Nadel, Burstein, and Dorfman.
Proc. Am. Assoc. Cancer Research 2: 37, 1955.

Corticosteroids in the urine of normal and scorbutic guinea pigs: isolation and quantitative determination.
Burstein, Dorfman, and Nadel.
J. Biol. Chem. April 1955.

Isolation of polar reducing corticosteroids from human urine.
Nadel, Burstein, and Dorfman.
Arch. Biochem. & Biophysics., Accepted October 7, 1955.

Dissimilarity in alkaline phosphotase staining of Keratohyaline granules.
Nadel and Wodinsky.
J. Histochem. & Cytochem. Sept. 1955.

Non-parallel changes in changes in cholesterol and ascorbic acid in the adrenals of malaria parasitized chicks.
Taylor, Greenberg, Josephson, and Nadel.
J. Clin. End. & Met. June 1955. (Proc. Endocrine Society)

On the defect in glycogen deposition in the livers of scorbutic guinea pigs.
Nadel, Mulay and Saslaw.
Endocrinology May 1955.

16. None
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI 5124 — SERIAL NO.

6. Effect of microbial and other agents on the development and course of neoplastic disease in animals.
PROJECT TITLE

7. E. M. Nadel
PRINCIPAL INVESTIGATOR(S)

8. None (See Item #12)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

The present project title includes 3 sub-projects gathered under the project title of: (a) Mutual aspects of the pathophysiology of leukemia and malaria in animals. When it became obvious that the project on perfusion studies could not begin because of the administrative uncertainty of space, facilities and moving date to the Clinical Center laboratories, 2 other projects related to a) were undertaken.

These projects include: (b) The effect of certain viruses on the course of cancer in animals and (c) the mode of action of certain living and chemical agents on the development and course of cancer in animals.

Objective: (1) To explore the activity of certain potentially carcinostatic and carcinogenic agents. (2) To explore certain immunogenetic and endocrine aspects of resistance to malaria infection and to viruses helpful in a study of the carcinostatic potentiality of these microbial agents. (3) The mode of action of certain virucidal and malariacidal drugs as carcinostatic agents.

Methods: The overall problem is studied by the combined use of techniques in pathology, microbiology, hematology, genetics, chemical pharmacology, and immunology. Inbred, hybrid and specific backcross animals are studied for resistance to micro-organisms such as the malaria parasite, neurotropic viruses, etc., and the effect of these on the course of transplantable tumors is studied. Combinations of drugs and of viruses are studied.

Major findings: (1) Guinea pigs with leukemia lived significantly longer after they were experimentally infected with the virus of lymphocytic choriomeningitis. (LCMV). (2) Guinea pigs made immune to the

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

LCM virus were afforded no protection when the virus was injected after transplantation of the tumor. (3) Four drugs (ethionine, amethopterin, and a purine and pyrimidine analogue) were studied to find that dose at which each individual drug was ineffective. Combinations of these drugs at their individually ineffective dosages successfully prolonged the life of tumor-bearing mice, more than 100% in comparison to controls. (4) Repeated therapy with the combination of 4 drugs to tumor-bearing mice resulted in the development of a resistant tumor. This resistant tumor is now being used as a means of studying the mode of action of other potentially carcinostatic drugs.

Significance to cancer research: (1) A neurotropic virus (LCMV) made relatively benign by repeated passage to mice (300 generations) was able to prolong the life of leukemia guinea pigs from 19 to over 30 days. The virus did not of itself appear lethal to guinea pigs though other strains of the virus apparently are. This increase in survival is greater than that previously reported from this project, on the inhibitory effect of malarial infection on the course of leukemia in mice. This type of study has therapeutic implications for use in man. (2) By judicious use of a tumor, resistant to four different types of chemotherapeutic agents, the mode of action of potentially carcinostatic drugs, with different chemical structure can be studied.

Proposed course: Attempts to enhance the duration of the inhibitory effect of the microbial agents under study are being made. These include the judicious use of hormones, x-ray irradiation, drugs and multiple sequential use of microbial agents, various pyrogenic materials (e.g. polysaccharides, etc.). The possibility of the use of viral therapy in the form of the LCM virus for leukemia in man must be considered. Continued studies on the possible similarities in the genetics of resistance in mice to malaria and to leukemia, and the role of endocrines in such resistance are underway.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~524~~ 512
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

Cooperating personnel assigned to the staffs of Dr. Greenberg, Chemotherapy Section, Laboratory of Tropical Diseases, NMI, Dr. Haas, Office of the Director, NMI, Dr. Jay, Laboratory Aids Branch, Office of the Director, NIH.

13. None to my knowledge
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

PROJECT REPORT FORM (Cont'd)

14. NCI ~~524~~ 572
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Effect of malaria on leukemia in mice.
Nadel, Greenberg, Coatney.
J. Infectious Diseases.

Differences in survival of several inbred strains of mice and their hybrids infected with plasmodium berghei.
Greenberg, Nadel, Coatney.
J. Infectious Diseases.

Synergistic inhibitory action of amethopterin and a diaminopyrimidine on leukemia L 1210 in mice.
Nadel, Greenberg.
Cancer Research.

Increased resistance to malaria of certain inbred strains of mice, their hybrids and backcrosses.
Nadel, Greenberg, Coatney, and Jay.
Am. J. Path.

Resistance to quadruple combination therapy in leukemia L 1210 in mice.
Nadel and Hilgar.
Am. J. Path., June 1955. (Proc. Am. Assoc. Path. & Bact.)

Inhibitory effect of lymphocytic chromomeningitis virus in the course of leukemia in guinea pigs.
Nadel and Haas.
Fed. Proc. 14: March 1955. (Proc. Am. Soc. Exp. Path.)

Backcross studies on the genetic of resistance to malaria in mice.
Nadel, Greenberg, Jay, and Coatney.
Genetics, Sept. 1955.

16. None
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~525~~ 573 — SERIAL NO.

6. Use of isolated organ perfusion techniques in a study on the pathologic physiology of tumor-bearing animals.
PROJECT TITLE

7. E. M. Nadel
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: Study changes in physiology accompanying tumor growth to learn how tumors divert energy sources away from the host, as a means towards the control of tumor growth.

Methods: The perfusion methods used have been used successfully in the hands of Dr. Leon Miller, while the micro-methods to be used have been described by Drs. Scholander, Kirk, and Natelson.

Major findings: There have been no major findings inasmuch as work on this project has been delayed by unforeseen circumstances.

Proposed course: It is hopefully anticipated that work can be started on this project shortly after the contemplated move from Building 8 to Building 10.

PROJECT REPORT FORM (Cont'd)

10. NCI 573 ~~525~~
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

NO ENTRIES FOR 14, 15, or 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc, & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~510~~ 514 — SERIAL NO.

6. Induction of adrenocortical tumor in the rat and study of the adreno-cortical steroids of the tumors, thus induced. Also, the effect of these steroids on the steroid metabolism of the host.
PROJECT TITLE

7. A. S. Mulay
PRINCIPAL INVESTIGATOR(S)

8. W. H. Eyestone
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To find out if the steroids produced and the enzyme systems involved, in the adrenal cortical tumor differ either qualitatively or quantitatively from those in the adrenal gland.

Methods: Transplanting and harvesting of the adrenal tumors. Determining activities of enzymes like choline esterase, adenosine triphosphate, phosphomonoesterase, and alkaline phosphotase. Quantitative and qualitative metabolism of steroids by tumor slices under different given conditions, on fresh tumor tissue. Extracting steroids from harvested tumors stored in deep freeze (when enough material is collected), purifying the extract, separation of various steroid fractions by chromatographic methods, and then their characterization by various physical and chemical methods.

Major findings: Adreno-cortical adenocarcinoma has been induced in 9.5% of the female Osborne-Mendel rats, under experimental treatment. This adrenocortical tumor has pronounced effect on the adrenal glands of the host. Considerable quantity of formaldehydogenic steroids have been found in the extracts of these tumors.

Significance: Successful conclusion of this program may give us some insight of chemical and enzymatic changes which transform normal adrenal glandinto adrenocortical adenocarcinoma.

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

Proposed Course: A considerable portion of the next calendar year will be devoted to separation and identification of the steroids in this tumor. This involves growing and collecting the tumor material, extracting the steroids, separating different steroid fractions chromatographically, and collecting enough of each fraction for further separation and then physical and chemical characterization. At the same time a technician is being trained in enzymologic and metabolic procedures for working on fresh tumor tissue.

PROJECT REPORT FORM (Cont'd)

0. NCI ~~510~~ 514
SERIAL NO.

1. _____
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

2. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

3. As far as I know, it does not.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI 514
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Mulay, A. S. and Schlyen, S. M.
Lesions induced in C57BR mice with gallium citrate and methylcholanthrene.
Am. J. Pathol. In press.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

Elected to membership in Society for Experimental Biology and Medicine (National)

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~512~~ 515 — SERIAL NO.

6. Sex difference in the incidence of hepatomas in rats, fed a carcinogenic azo dye, and study of hormonal and other factors responsible for this difference.
PROJECT TITLE

7. A. S. Mulay
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To determine the role played by sex hormones on the incidence of hepatomas induced in rats fed carcinogenic azo dyes, and see what part, if any, is played by other so-called "residual sex differences."

Methods: Male and female rats, intact, gonadectomized, and with or without estrogen or androgen treatment, are fed a synthetic diet of known composition containing p-dimethylaminoazobenzene. All groups of rats thus treated are kept under identical conditions, and a few animals from each group are sacrificed at definite time intervals. Livers of these animals are examined for macroscopic and microscopic hepatomas. From these observations, effect of the treatment on the relative induction time and the incidence is calculated.

Major findings: Intact male and female Osborne-Mendel rats fed a carcinogenic azo dye in multiple deficiency synthetic diet of known composition, for 10 months, showed a marked sex difference in the incidence of hepatoma.

Significance: Evidence thus gathered will forge a link in the chain of environmental factors necessary to induce neoplasm in animals.

Proposed course: Experiments on various treatments to different groups of animals are just starting and will run through the next calendar year.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~512~~ 515
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH /x/ ADMINISTRATION / /

REVIEW & APPROVAL / / TECHNICAL ASSISTANCE / /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. As far as I know, it does not.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI 513
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Symeonidis, A. and Mulay, A. S.
Histopathology of the adrenal glands of rats fed a low protein, low riboflavin diet alone, or with p-dimethylaminoazobenzene.
J. Nat. Cancer Inst. In Press.

16. None
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~518~~ 516 — SERIAL NO.

6. Induction, Pathogenesis and Morphological Alteration of Tumors Arising in Bone
PROJECT TITLE

7. Albert W. Hilberg
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To develop a consistent method of producing bone tumors in animals which are similar, morphologically, to those in man and to study the events leading to tumor formation or alteration of tumors as affected by transplantation.

Methods: Animals have received injection of beryllium oxide by various routes such as intravenous, intramedullary and intratracheal. At intervals skeletal surveys by x-ray examination and blood serum phosphatase levels are determined. All control animals and injected animals are autopsied and bony tissues examined grossly and microscopically. Special studies of tissues are made using histochemical and x-ray techniques.

Transplantation of spontaneous bone tumors into various sites such as liver, kidney, subcutaneous tissue and intraperitoneal spaces is carried out to study effects on morphology of tumors.

Major findings: The rabbits injected with beryllium have developed a lymphoma, 2 bone sarcomas, and 1 bone sarcoma developed in a mouse and is being successfully carried in serial transplantation.

Morphological variations in spontaneous bone tumors by selective site transplantation into kidney have revealed a differentiation of undifferentiated cells into bone after bone had failed to develop in subcutaneous transplants. This represents a possible redifferentiation of anaplastic cells.

To date some 40 spontaneous tumors primary in bone in mice have been found and classified by morphological type. A detailed description is being completed.

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

Significance to cancer research: Bone tumors are said to be among the most common neoplasms in children. These tumors develop at any age and represent a major problem since cures are rare and treatment usually mutilating. Attempts to learn more about the development of bone tumors, including contributing factors in development and the biological and morphological behavior of these tumors can give valuable information toward the goal of prevention, treatment, and cure of bone tumors. Information concerning specific substances, such as beryllium, which produce bone tumors, may be of value because of the industrial uses of beryllium and its compounds.

Proposed course: Continuation of the study of animals injected with beryllium oxide. Continuation of selective site transplantation studies of spontaneous tumors arising in bone, with particular emphasis on transplantation to the liver. The classification and description of all spontaneous tumors arising in bone in mice is a continuing process as these tumors are received from many sources within the various laboratories of the National Institutes of Health.

Collaboration in beryllium studies in intratracheal injections and in studies of Rous sarcoma will continue until completion.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~518~~ 516
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. None to my knowledge
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None to my knowledge
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI ~~518~~ 516
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 195

Hilberg, Albert W.
Morphological Variations in an Osteogenic Sarcoma of the Mouse when Transplanted to the Kidney
J. Nat. Cancer Inst. In press.

16. None
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Pathological Technology Sect. — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~515~~ 517 — SERIAL NO.

6. Pathological Technology Section
PROJECT TITLE

7. Mr. J. M. Albrecht
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Stained tissue sections are the fundamental basis of all clinical and experimental studies of cancer. The Section prepares histological sections for all the investigators of the National Cancer Institute. It makes available all the established routine and special stains and in addition develops and provides the current experimental methods of tissue preparation such as enzyme stains and specific histological stains.

January 1, 1955 - December 31, 1955

Number of Investigators	67
Number of Pieces of tissue	105,361
Number of Bottles of tissue	15,921
Number of blocks cut	52,356
Blocks cut serially	464
Frozen blocks cut	722
Number of autopsies	14,635
Number of recuts	920
Number of slides stained H & E	67,562
Number of slides stained special	13,701
Number of unstained slides	7,805

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

Photographic Service rendered to the Laboratory of Pathology, January 1, 1955, to December 31, 1955

GROSS

Black & White	84
Color	94

MICROS

Black & White	380
Color	333

LANTERN SLIDES

Black & White	103
Color	195

PRINTS

4 x 5	1548
8 x 10	32
14 x 17	5
Mounted	288

PROJECT REPORT FORM (Cont'd)

10. NCI ~~515~~ 517
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI ~~515~~ 517
SERIAL NO.

15. None
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955.

16.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Willie D. Morgan received cash award June 1955 for a vacuum device and sipho-vacuum cap which aids in the filling of small utility bottles from large cans or bottles or reagents.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~507~~ 518 — SERIAL NO.

6. Heterologous Transplantation — PROJECT TITLE

7. C. Harold Steffee and Katharine C. Snell — PRINCIPAL INVESTIGATOR(S)

8. None — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To produce neoplasia of human tissues.

Methods: Human fetal tissues have been transplanted into the cheek pouch of the hamster, the host animal being treated with cortisone.

Major findings: The transplanted fetal tissues generally persist for a reasonable time in the hamster cheek pouch but no evidence of neoplasia of these tissues has been obtained. A major obstacle in this program has been the development of dissecting aneurysm in many of the cortisone-treated hamsters with premature death of the animal resulting from hemothorax or hemoperitoneum. Similar lesions have been found in the control animals treated with cortisone but not given any fetal transplants. That this finding is not limited to hamsters is evidenced by the discovery of degenerative changes in the aorta of each of two mice bearing functional adrenal cortical carcinomas (Dr. Thelma B. Dunn).

Significance to the program of the Institute: The original objective of these studies was to produce human carcinoma in organs which are difficult to study in experimental animals because of the lack of analagous neoplasms in the animal. These sites include the lung and the gastrointestinal tract. Had we succeeded in this endeavor we would have had an extremely valuable tool for testing a number of suspected carcinogens for these tissues, and a tool which would have been much more comparable to the human situation than is otherwise possible. Perhaps additional studies will permit us to attain this objective. The significance of the aortic lesions to cancer research is at best rather nebulous. It may, however,

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

provide us with further insight into the fundamental effects of adrenal cortical hormones on the connective tissues of the body. This, then, is an area in which we are seeking fundamental knowledge with no immediate applicability to the cancer problem.

Proposed course of the project: A number of hormones related to cortisone will be tried in an attempt to attain our original objective. Additional studies will also be carried out to try to define the precise relationship of cortisone to the development of the aneurysm.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~507~~ 519
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 14, 15, OR 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~511~~ 519 — SERIAL NO.

6. Chemical and histopathological changes induced in the adrenal glands of rats fed a deficiency diet. (This diet is usually used in conjunction with azo dye feeding for the induction of hepatoma in rat.)
PROJECT TITLE

7. A. S. Mulay and E. M. Nadel
PRINCIPAL INVESTIGATOR(S)

8. A. Symeonidis
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To find the factor or factors absent in the deficient diet, which are responsible for the induction of the histopathological changes observed in the adrenal glands of rats kept on this deficient diet, and others deficient only in one or two known factors. To study the chemical and metabolic changes in the adrenal glands of these animals, concomitant with the histopathological changes.

Methods: Starting with a complete synthetic diet of known composition, different diets are prepared from which a known factor or factors are withheld. Such diets are fed to different groups of rats and the chemistry and histopathology of their adrenal glands is compared with that of the adrenal glands of rats on complete diet of known composition.

Major findings: Histopathology of the adrenal glands of rats fed a multiple deficiency diet of known composition (one used in connection with hepatoma induction in rats with azo dye feeding) is described in our preliminary paper "Histopathology of the adrenal glands of rats fed a low protein, low riboflavin diet alone or with p-dimethylamino-azobenzene" in J.N.C.I. In press.

Significance: The role played by the adrenal gland in the induction of hepatoma in rats fed multiple deficiency diet containing carcinogenic azo dye, has been demonstrated in our laboratory (J.N.C.I. 14: 805-817, 1954, and Endocrinol. 57: 550-558, 1955) and in others

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

(Richardson: Cancer: 6: 1025-1029, 1953, and Griffin: Cancer Research 13: 77-79, 1953). Knowledge of the factor or factors responsible for these changes will take us a step nearer to the understanding of the neoplastic process.

Proposed course: Critical experiments to confirm our first observations on the altered histopathology of the adrenal glands of rats on multiple deficiency diet of known composition are in progress. Better part of the year will be taken up in confirming the histopathology picture and analyzing for concomitant chemical changes in these altered adrenal glands. Experiments with complete diets of known chemical composition, deficient in only one or two known factors have just been started.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~511~~ 519
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. As far as I know, it does not.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI ~~511~~ 519
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Nadel, E. M., Mulay, A. S., and Saslaw, L. D.
On the failure of glycogen deposition in the livers of scorbutic Guinea Pigs.
Endocrinol, 56: 584-589, 1955.

Symeonidis, A., Mulay, A. S., and Trams, E. G.
Effect of prolonged pretreatment with desoxycorticosterone on the liver of hepatectomized rats.
Endocrinol. 57: 550-558, 1955.

Mulay, A. S. and Eyestone, W. H.
Transplantable adenocortical adenocarcinoma in Osborne-Mendel rats fed a carcinogenic diet.
J. Nat. Cancer Inst. 16: 723-739, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~513~~ 520 — SERIAL NO.
6. Tissue Culture of Mouse Leukemias. — PROJECT TITLE
7. C. J. Dawe — PRINCIPAL INVESTIGATOR(S)
8. Michael Potter and Joseph Leighton — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Development of methods for isolation and continuous cultivation in vitro of mouse leukemia cells.

Methods: Methylcholanthrene-induced leukemias in ascites form in DBA mice are used as a source of leukemia cells which are propagated alternately in gelfoam matrix in roller tubes and in mice. By inoculating the tissue cultures intraperitoneally into mice at successively increasing time intervals after explantation, it is possible to determine the survival time of leukemia cells in tissue culture and to perpetuate the cell time, which otherwise dies out in tissue culture alone.

Major findings: In the four months during which this project has been underway, it has been possible to carry one line of lymphatic leukemia through 6 tissue culture passages and an equal number of mouse passages. During this time the maximal survival time in tissue culture has increased from 7 to 13 days. The possibility that a morphologic change may have occurred in the leukemia cells is under study.

Significance to cancer research: For studies of growth characteristics, nutritional requirements, and susceptibility to therapeutic agents, it is desirable to be able to cultivate pure populations of leukemia cells in relatively controlled environment such as is obtainable in tissue culture. The purpose of this project is to determine whether leukemia cell lines can adapt to continuous tissue culture propagation as a result of

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

repeated exposure to tissue culture environment. If this proves to be so the method can then be applied more generally to the isolation of various types of leukemia cells in tissue culture. At the present time, no satisfactory method of achieving this end is available.

Proposed course: The method described will be continued for at least a year, at which time the results and possibilities of the approach will be evaluated. Additional cell lines will be added to the experiment.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~513~~ 520
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 14, 15, or 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI 521 (~~514~~) — SERIAL NO.

6. Tissue culture in a perfusion chamber designed to use whole blood as a source of nutrient.
PROJECT TITLE

7. C. J. Dawe
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objective: To determine the applicability of a new type perfusion chamber to special problems in tissue culture.

Methods: A lucite perfusion chamber has been designed and constructed to permit culture of tissue explants in a flowing plasma medium derived by membrane filtration of whole blood, circulated through the chamber under physiological pressures in a gravity system. The chamber permits continuous microscopic observation of the cultured cells.

Major findings: This apparatus has not yet been tested in relation to its effects on all viability and growth. The hydraulics have been shown to function satisfactorily as outlined above.

Significance to cancer research: Using fresh heparinized whole blood as the circulating medium it becomes possible to bathe tissue explants in an environment presumably very similar to that provided by the plasma component of the circulating blood in vivo. Oxygen tensions of the medium can be controlled by varying the oxygenation of the circulating red cells. The investigator's objective in developing this chamber is to study the effects of the medium so derived on the survival and proliferation of leukemia cells in vitro. The extent to which leukemia cells can proliferate while actually within the plasma of the peripheral vascular system is at present unknown. Other applications of the apparatus are apparent. For example, it would be possible to observe the effects of metabolic products of one cell type on another cell type by connecting the chamber in series.

Proposed course: The growth promoting or inhibiting effects provided by this system will be studied primarily on mouse and human leukemia cells, using human blood as the circulatory element initially.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~514~~ 521
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI ~~514~~ 521
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Cytologic studies of sputum, secretion, and serous fluids of patients with malignant lymphoma.
C. J. Dawe, T. B. Woolner, E. M. Parkhill, and J. R. McDonald.
Amer. J. Clin. Path. 25: 480-488, May 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Certified in Pathologic Anatomy by American Board of Pathologists (April 1955) Completed all requirements, including thesis and examinations for degree of Ph.D. in Pathologic Anatomy from University of Minnesota. Degree to be conferred March 1956. Thesis title: "Hodgkin's Disease and Its Interrelationships with Other Disorders."

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~516~~ 522 — SERIAL NO.

6. An investigation of the collagenous connective tissues.
PROJECT TITLE

7. William G. Banfield
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The immediate objectives are:
1. To elucidate the age changes in collagen.
2. To uncover mechanisms affecting the usual state of the collagen in the body.

Methods: 1. Collagen from human skin, scars and Achilles tendons are subjected to dilute acetic acid in order to establish more definitely the normal limits for the age-swelling pattern of Achilles tendon and to measure the amount of soluble collagen present in these tissues. Variations in the reaction of skin and Achilles tendon from the normal are investigated through the patient's history and possible causes of such variation are subject to animal test. The maturation of scars is studied with respect to changes in acid soluble collagen and histology.
2. Hamsters are tested for changes in skin collagen after hormone treatments or gland removal.
3. A successful search has been made for a stain which will react differently to collagen from a young Achilles tendon than to collagen from an old Achilles tendon.

Major findings: 1. A possibility has been found that chorionic gonadotropin and an adrenal cortical hormone each may increase the solubility of human skin collagen.
2. A method for differentially staining collagen in young and old tendons and in young and old scars has been developed.
3. Recent scars contain a large amount of acid soluble collagen whereas old scars do not.

Significance to cancer research: A knowledge of collagen metabolism including collagen maturation, may help in the understanding of connective tissue tumors. Such knowledge should also contribute to an evaluation and possible control of the stroma which accompanies and even

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

seems necessary for the continued growth of many tumors. Staining techniques, especially if placed on a histochemical basis, would be invaluable for studying the collagenous components of tumors.

Proposed course: The measurement of the acid soluble collagen in scars will be continued until all stages in the maturation of a scar can be represented. The scar specimens will be studied using the newly-developed differential staining technique for collagen and conventional histologic stains. The screening of skin and tendons for their content of acid soluble collagen will be continued and leads will be followed by animal experimentation. Animal experiments to determine possible effects of gland removal on collagen development will be continued. The mechanism by which old and young collagen is differentially stained will be investigated and an attempt will be made to improve the method. The electron microscope will be used to further the project.

PROJECT REPORT FORM (Cont'd)

10. NCI-~~516~~ 522
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI ~~516~~ 522
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Banfield, W. G.
Width and length of collagen fibrils during the development of human skin, in granulation tissue and in the skin of adult animals.
Journal of Gerontology 10: 13-17, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

None.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~528~~ 523 — SERIAL NO.

6. Effects of Ionizing Irradiation with emphasis on the morphologic alterations and neoplasms
PROJECT TITLE

7. Richard L. Swarm
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The collection of additional information regarding the development of neoplasms in man and laboratory animals following exposure to radiation injury is one general objective of this study. Some interest in the type of irradiation change other than that which is manifested in the formation of neoplasms is maintained. The different effects produced by different types of x-radiation, Alpha irradiation and Beta irradiation are of particular interest. A knowledge of the changes effected by different types of radiation in existing tumors will be sought.

Methods: Three types of approach have been made. (1) Participation in the Surgical Pathology and Post-Mortem pathology division of the Pathologic Anatomy Department of the Clinical Center. Here a review of accessioned specimens is made possible by actual participation in the study and diagnosis of specimens as they are received. An effort is made to select and categorize anatomic material showing radiation change. (2) Detection of neoplasms and other changes in animals who have been given whole body irradiation of varying amounts at significant intervals prior to anatomical study. (3) A study of the distribution and effects of some radioactive isotopes given by different routes to animals and therapeutically or for diagnostic purposes to man will be made. Here the emphasis has been on the effect of I-V administered colloidal thorium dioxide.

Major findings: A new project. Of interest, however, is one human case which was studied during life by the General Medicine Branch of NCI and which was later studied anatomically. Study revealed the presence of neoplasms in the liver, spleen, and bone of a patient who had stored thorium dioxide in the reticulo-endothelial system for many years. Studies of this are as yet incomplete, however, from the observations made in this case and other reported cases, an etiologic relationship between the storage of thorium and the development of neoplasms in man seems to exist.

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION (Cont'd)

Proposed course: (1) An interest in the morphologic effects of radiation on human tissue and in particular on previously existent neoplasms will be maintained and further developed. Particular emphasis will be placed on the study of the effects of different types and energies of irradiation. Study of human case material (specimens) from the Clinical Center will be the important phase of this activity. (2) The anatomic study of animals exposed to whole body irradiation will be continued. (3) The study of the distribution and morphologic changes effected by the administration of colloidal thorium dioxide given intravenously to man for diagnostic purposes and experimentally to animals will be continued. Knowledge of the pattern of storage and effects in man will be furthered by a proposed study of human material at the Clinical Center, The Armed Forces Institute of Pathology, and of material submitted by Dr. William Looney.

PROJECT REPORT FORM (Cont'd)

10. NCI 523-528
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

(1) The work up of anatomic specimens from patients who have received irradiation in the Clinical Center will complement the work of the clinical services particularly that of the Radiation Therapy Branch. (2) The anatomic study of laboratory animals given whole body irradiation has been limited to hamsters supplied by Dr. W. Smith, Radiation Branch, NCI. Other pathologists have assisted Dr. Smith in this study. (3) Research on the morphology of damage in animals produced by I-V administered thorium dioxide has been made by many investigators in this country and abroad. Only two European investigators have succeeded in producing neoplasms in animals following I-V administration of colloidal thorium dioxide. Experiments designed to confirm or refute these findings are contemplated. To my knowledge, no study of the morphology of thorium aggregates in human tissues like that proposed has been undertaken elsewhere.

PROJECT REPORT FORM (Cont'd)

14. NCI ~~528~~ 523
SERIAL NO.

15. None
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

Elected to Fellowship in the American Society of Clinical Pathologists and to membership in the Washington Society of Pathologists and in the D. C. Section of the Society for Experimental Biology and Medicine.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~529~~ 524 — SERIAL NO.

6. Morphologic and histochemical study of a human carcinoma of the floor of the mouth in material from the patient, from long term tissue culture of the tumor and from heterologous transplantation of tissue culture material to the cortisone treated rat.
PROJECT TITLE

7. Alan S. Rabson
PRINCIPAL INVESTIGATOR(S)

8. Gerald Suskind
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to determine whether or not there are significant morphologic and histochemical differences in tumor cells after prolonged growth in tissue culture and in heterologous hosts.

Methods Employed: In 1954, a tumor of the floor of the mouth was excised from a patient at the Clinical Center and cells from this tumor have been grown in large quantity in tissue culture as recently reported by Eagle (Strain K-B). The original histologic sections of this tumor as well as subsequent recurrences are available in the files of the Pathologic Anatomy Branch) for morphologic and histochemical study. The cells in tissue culture will be studied on cover-slip preparations and in sponge matrix tissue culture. Solid tumors in cortisone treated rats have been produced by injection suspensions of the cells in tissue culture, and are being studied with a variety of fixatives and stains.

Major Findings: The project has only been in progress for a short time and no significant findings are available.

Significance to Cancer Research: The potential value of cell lines of human tumor cells in biological and chemotherapeutic studies has been questioned on the grounds that the cells are variants of the original tumor and have only a limited relationship to it. It would seem to be of considerable interest if it could be demonstrated that cells grown for many generations in tissue culture and subsequently in heterologous hosts are morphologically and histochemically similar to the original tumor from which they were derived.

Proposed Course of Project: As described above under Methods Employed, the morphology and histochemistry of the original carcinoma of the floor of the mouth will be compared morphologically and histochemically with the tumor cells in tissue culture and in cortisone treated rats.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~529~~ 524
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI ~~529~~ 524
SERIAL NO.

15.
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Pretest of Forms and Field Techniques For Use in the Detroit-Windsor Air Pollution Study by A.F.W. Peart, C.P. Anderson, A.S. Rabson and W.L. McEwen, AMA Arch. of Indust. Health 11: 47, 1955.

The Effect of Gamma Globulin on Subclinical Infection in Familial Associates of Poliomyelitis Cases II. Serological Studies and Virus Isolations from Pharyngeal Secretions. G.C. Brown, A.S. Rabson, and J.H. Schieble, J. of Immunology 74: 71, 1955.

C-Reactive Protein in Serum of Patients with Leprosy. A.S. Rabson, International J. of Leprosy 23: 155, 1955.

16.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI ~~530~~ 523 — SERIAL NO.

6. Attempt to induce carcinoma of the bladder in hamsters by infection with Schistosoma haematobium
PROJECT TITLE

7. Dr. Eloise Cram and Dr. Louis B. Thomas
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

This research project is in collaboration with Dr. Eloise Cram, NMI, and consists of studying chronic infection with S. haematobium (Gold Coast strain) in hamsters; particularly with reference to the induction of bladder carcinoma.

(a) 217 hamsters were exposed to approximately 300 cercariae each early in 1955.

(b) Approximately 177 infected hamsters are still living and will be allowed to live as long as possible.

(c) Forty infected hamsters have died after 162-305 days after exposure to cercariae. These animals have all had heavy Schistosomal infection of the intestines and liver, but only slight infection of the bladder. Pathological study of these animals is incomplete at this time. No lesions suggestive of neoplastic change in the bladder have been seen.

Significance to cancer research: S. haematobium infection has been found associated with bladder carcinoma in several parts of the world and is thought possibly to be a cause of bladder carcinoma. This chronic infection study is possible because of Dr. Cram's work in getting S. haematobium established in hamsters.

Proposed course of the project:

(1) A continuation of the study of Gold Coast strain S. haematobium in hamsters.

(2) Similar study of chronic infection with Egyptian strain S. haematobium when infected snails become available in Dr. Cram's laboratory.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~530~~ 525
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

NO ENTRIES FOR 14, 15, or 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI 526 — SERIAL NO.

6. Metabolism of cortisol into higher hydroxylated derivatives in guinea pigs with and without neoplastic disease.
PROJECT TITLE

7. E. M. Nadel and S. Burstein
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: This title includes three sub-projects: (1) Isolation and elucidation of structures of new cortisol metabolites in vivo (Dr. S. Burstein). (2) Study of enzymatic transformations involved in specific tissues. (3) The incorporation of C^{14} acetate into cortisol and metabolites.

Methods: The methods previously utilized include, extraction, paper and column partition chromatography, infra-red spectrometry, ultra violet spectrophotometry, incubation techniques using slices and homogenates of liver, adrenals, and other organs, use of radioisotopic techniques.

Major findings: (1) 6-β hydroxycortisol and Steroid IIa (as yet unidentified) have been isolated from the urine of guinea pigs. (2) 6-β hydroxycortisol, Steroid 2 (as yet unidentified) tetrahydrocortisol have been isolated from the urine of man. (3) Radioacetate is incorporated in increased amounts into cortisol and corticosterone in the adrenals of scorbutic guinea pigs, and both steroids have been identified for the first time in the tissue of the guinea pig.

Significance to cancer research: This work provides the background material for the continuation and extrapolation of similar studies on the tissues of tumor-bearing guinea pigs.

Proposed course: In this collaborative project we will endeavor to complete such studies during the coming year. Such a study is now feasible because of the closer association with Dr. S. Burstein on the reservation as a Visiting Scientist from the Worcester Foundation for Experimental Biology.

PROJECT REPORT FORM (Cont'd)

10. NCI 526
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

NO ENTRIES FOR 14, 15, or 16

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induc. & Pathogenesis — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI 527 — SERIAL NO.

6. Morphology, pathogenesis and transplantability of spontaneous neoplasms within the canine species.
PROJECT TITLE

7. Louise S. Lombard
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to study the morphology and pathogenesis of spontaneously occurring neoplasms within the canine species and to obtain transplantable canine neoplasms which could be utilized in morphologic, chemotherapeutic, metabolic, endocrinologic and irradiation studies.

Methods: Through the use of cortisone and/or total body irradiation, a spontaneous anaplastic thyroid carcinoma was transplanted by various routes in heterologous puppies and studied morphologically.

Major findings: The transplantable canine thyroid carcinoma was grown in a wide variety of tissues with growth occurring in almost 100% of the irradiated heterologous puppies. The morphology of the tumor remained essentially unchanged throughout the 35 serial transplant generations. Lymph node and lung metastases were found in irradiated animals receiving either fresh or frozen tumor tissue (the thyroid carcinoma was preserved by storage at a -60 to -70°C. for several months).

Significance to cancer research: Transplantable malignant neoplasms in the dog would offer tumors in a larger host for morphologic, metabolic, biochemical, hormonal, and irradiation studies, as well as including another species for the testing of chemotherapeutic substances. The transplantable canine thyroid carcinoma, intracerebrally inoculated, is now being used as a test tumor for the efficacy of radioactive boron in the treatment of brain tumors (Univ. of Penna.).

Proposed course: The transplantable thyroid carcinoma will be transplanted to untreated, closely inbred puppies and in irradiated puppies receiving cysteine and bone marrow. Morphologic studies and transplantation experiments will be performed utilizing other spontaneous canine tumors, especially leukemia.

PROJECT REPORT FORM (Cont'd)

10. NCI ~~521~~ 527
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH /x/ ADMINISTRATION /_/

REVIEW & APPROVAL /_/ TECHNICAL ASSISTANCE /_/

12. School of Veterinary Medicine, University of Pennsylvania Facilities, Personnel (Dr. Mark Allam, Dean) 1956

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 57

13. None

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

PROJECT REPORT FORM (Cont'd)

14. NCI ~~521~~ 527
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Urbain Leblanc
Early Veterinary Pioneer in Cancer Research
J. Amer. Vet. Med. Assoc., 126: 363-365, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

Society of Phi Zeta, May, 1955.
American College of Veterinary Pathologists, Nov., 1955.
Washington Society of Pathologists, Nov., 1955.
Conference of Research Workers in Animal Diseases in North America, Dec., 1955.

Note: Collaborative research is being done with Drs. H. L. Stewart and H. B. Andervont on adenomatous gastric lesions in strain I mice; with Dr. W. R. Bryan on rapid and slow growing Rous Sarcoma; with Dr. H. P. Morris on experimentally produced pituitary adenomas and hepatomas in rats.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. 624 (SERIAL NO.)

6. Description and Treatment of the High-Radiation-Dose Syndrome
PROJECT TITLE

7. H. L. Andrews and K. C. Brace
PRINCIPAL INVESTIGATOR(S)

8. H. Gump
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: 1. To characterize the syndrome produced by x-ray doses up to about 100 times that lethal in 30 days. Emphasis in this project is on function rather than on pathology. 2. To study agents capable of modifying the high-dose syndrome. 3. To study the effect of dose rate and fractionation on the high-dose syndrome.

Methods employed: Doses from 1000 r upward are administered with the 3 Mev x-ray generator. The guinea pig is the animal of most interest because of the sharp break in signs of radiation injury at 6,000 r. Typical studies made before and after radiation are blood counts, blood electrolytes, pain threshold, pinna reflex, electrical impedance of body tissues. Survival times are carefully recorded.

Major findings: Using 200 KVP x-rays at 55 r per minute we have found in the guinea pig:

1. At 6,000 r there is a sharp change from a 5 day death in depression to a death in less than 24 hours with marked signs of increased central nervous system excitability.

2. When barbiturates are given prior to irradiation, irradiation of even 15,000 r produces only the depression normally seen with less than 5,000 r and the survival time is about 4 days instead of the 1 day or less obtained without medication. A series of depressant and anti-convulsant drugs are without effect on survival time although some prevent the appearance of the high-dose syndrome.

Significance to cancer research: Any increase in knowledge of the biological effects of radiation are of potential value in radiation therapy. It is of interest that the large doses used in this project are not large in terms of local doses delivered for therapeutic purposes.

Proposed course of project: Much of the work already done will be repeated using the 3 Mev generator to obtain a greater relative radiation dose to underlying structures in the central nervous system. The high dose-rates obtainable with this generator will be studied for biological effectiveness and will permit an extension of studies of agents modifying the high-dose syndrome.

10. N6I- 624
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[X]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION(IF OTHER THAN BETHESDA)) 5. NCI-627 (SERIAL NO.)

6. Change in Tissue Constituents by Radiation
PROJECT TITLE

7. H. L. Andrews and E. J. Liljegren
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine changes in amino acid concentrations produced by x-irradiation in various body tissues of the guinea pig.

Methods employed: Assays of various tissues were made by separation columns and paper chromatography. Normal animals were compared with those receiving various doses of x-rays.

Major findings: Experimental work has been completed and the results are being analyzed. There are changes in amino acid concentrations but it is premature to discuss them in detail now.

Significance to cancer research: Any increase in our knowledge of the biological effects of radiation is of potential importance in radiation therapy.

Proposed course of project: Unless the data are more striking than presently appears this project will be terminated with publication of the findings.

10. NCI-627
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[X]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957.

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-628 (SERIAL NO.)

6. Dosimetry of High Energy Radiations
PROJECT TITLE

7. H. L. Andrews
PRINCIPAL INVESTIGATOR(S)

8. R. E. Murphy
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To develop methods and instruments for determining the radiation doses delivered to tissues at energies up to 3.5 Mev and at high dose rates.

Methods employed: The usual types of ionization chambers used for radiation dosimetry are not designed to read correctly at the x-ray energies available from the 3.5 Mev generator and are probably incapable of measuring the high intensities anticipated when the generator is run at full output. Chemical dosimeters appear to have a response independent of photon energy, and to be capable of accurately recording high dose rates. Work has concentrated on two chemical systems, the production of HCl from chloral hydrate and the oxidation of ferrous iron to ferric.

Major findings: As an extension of the main problem the chloral hydrate system has been adapted to depth-dose determinations by the addition of a gelling agent. With this dosimeter a beam of radiant energy can be visualized and measurements of local radiation doses made with a probing pH electrode. The gel absorbs radiation almost exactly as does water, and hence its response will be a good indicator of tissue dose in complex structures not amenable to calculation or to measurement with other methods.

Significance to cancer research: A most basic requirement for good radiation therapy is that the tumor dose be made as high as possible relative to the dose delivered to healthy tissue. Any method which can improve the measurements of dose delivered to deep body structures should improve the ability of the therapist to keep the tumor/tissue dose ratio high.

Proposed course of project: Measurements at high dose rates have not been made because of target failures when attempts were made to operate the generator at high power. As this difficulty is overcome chemical dosimetry will be applied to high dose rates and to short pulses of both x-rays and

NCI-628
SERIAL NO.

PROJECT DESCRIPTION (CONT.)

electrons. These measurements will then provide a basis for the extension of research in radiation biology into a dose-rate range never before reached in the laboratory.

10. NCI-628
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[X]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-629 (SERIAL NO.)

6. The effect of ionizing radiation on amino acids
PROJECT TITLE

7. Charles R. Maxwell
PRINCIPAL INVESTIGATOR(S)

8. Dorothy C. Peterson
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to determine the mechanism of the chemical reactions induced by ionizing radiation in aqueous solutions of amino acids. This is part of a long range program to accumulate information on simple systems of biological interest so that general principles may be ascertained and applied to complex systems which are not amenable to thorough, direct investigation.

Methods employed: Solutions of amino acids are irradiated with ionizing radiation and then analyzed for the products formed. Since the products are sensitive to radiation it is necessary to determine the yield of each product as a function of dose to as low a dose as possible and to extrapolate these values to obtain the initial yield of the product at very low doses before secondary reactions distort the picture.

Most of the study is done with 50 KV X-rays as the ionizing radiation. The effect of dose rate and ion density is investigated by irradiating with electrons and alpha particles. Protons and neutrons will probably be used in the future.

Major findings: Earlier work has shown that x-rays induce four reactions in aqueous solutions of both glycine and alanine. One of these reactions was shown to result directly from the absorption of energy by the dissolved molecule; the other three were shown to be indirect, e.g., the result of energy absorbed by the water which is generally considered to produce H and OH free radicals and H_2O_2.

The work this year has been concentrated on studying the role of the above active intermediates.

1) Irradiations in the presence of dissolved oxygen have shown that the

PROJECT REPORT FORM (Cont.)

reductive deamination of glycine to acetic acid is the action of the H free radical.

2) Experiments using OH free radicals produced chemically from Fe^{++} and H_2O_2 have shown that the oxidative deamination of glycine to glyoxalic acid is the action of the OH free radical.

3) Failure to observe the formation of formaldehyde as the result of OH radicals distributed uniformly in the solution by the reaction of Fe^{++} and H_2O_2 has lead to the conclusion that the reaction producing formaldehyde is peculiar to the high local concentrations of radicals along the discrete tracks of ionizing particles.

4) Investigation of the effect of alpha particles from Po^{210} upon aqueous solutions of glycine has shown that this densely ionizing particle induces the same reactions as does x-rays and electrons but in a different ratio. The relatively high yield of formaldehyde is in agreement with finding 3).

5) The kinetic measurements associated with obtaining result 3) have provided considerable insite as to the mechanism of the "catalytic" action of Fe^{++} ion on the action of H_2O_2 on amino acids and will result in a separate publication.

Significance to cancer research: Although not directly connected with Cancer research, it is of great interest because it seeks an understanding of the mechanism for the effects of radiation which is used empirically as a tool in the clinical treatment and laboratory study of cancer.

Proposed course of project: Continuation of the work will be directed to completing the glycine and alanine investigation and extending the work to the effect on more complicated amino acids.

Specifically:
1) The mechanism for the large effect of very small concentrations of dissolved oxygen upon the glycine reactions will be studied.
2) Measurements upon the influence of glycine concentration and of temperature upon the relative frequency of the various reactions will be refined.
3) Studies upon the effect of pH upon the reactions will be made.
4) Studies upon the effect of x-rays on phenylalanine and tyrosine will be initiated.

PROJECT REPORT FORM (Cont.)

10. NCI-629
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[x]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont.)

14. NCI-629
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The Effect of Ionizing Radiation on Amino Acids

II. The Effect of X-Rays on Aqueous Solutions of Alanine. N. E. Sharpless, A. E. Blair and C. R. Maxwell, Radiation Research 2, 135-144 (1955)

III. The Effect of Electron Irradiations on Aqueous Solutions of Glycine. C. R. Maxwell, D. C. Peterson, and W. C. White, Radiation Research 2, 431-438 (1955)

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION(IF OTHER THAN BETHESDA)) 5. NCI-634 (SERIAL NO.)

6. Late effects of irradiation in animals protected from early death by bone marrow or antibiotic treatment
PROJECT TITLE

7. Willie W. Smith, pathological studies by George Brecher (Clinical Center), Katherine Snell, Richard Swarm
PRINCIPAL INVESTIGATOR(S)

8. Ilo M. Alderman, Ruth Gillespie
OTHER INVESTIGATOR(S)

9. PROJECT DESCRIPTION

Objectives: To determine the extent to which life expectancy is reduced in animals which have been exposed to severely damaging radiation and then treated in such a way as to prevent early death from infection, hemorrhage or anemia and to determine where possible, the cause of death in these animals.

Methods employed: Animals exposed to radiation which causes death in the absence of treatment are treated with bone marrow or streptomycin and are then observed for the remainder of their lives. At death autopsies are performed and histopathological studies made. Various treatments are applied with the object of prolonging life.

Major findings: (1) In hamsters surviving the first month following exposure to x-radiation the median survival time appears to be linearly related to the radiation dose rather than to the proportion surviving the first month. Treatment with streptomycin or marrow appears to prevent early death but not to promote total recovery. (2) Three treatments applied to hamsters surviving a month after irradiation have failed to alter significantly the survival time. (3) Histopathological studies have shown a high incidence of gastric ulcer, pneumonia, vascular and renal changes and neoplasm, depending upon the initial radiation dose and the time of death.

Significance to Cancer Research: (1) An evaluation of the total efficacy of treatments which may be used to reduce the deleterious effects of therapeutic treatment with x-ray; (2) an estimate of the incidence and type of neoplasm which may be expected after whole-body exposure to varying doses of radiation; (3) an estimate of the physiological condition of the animal after exposure to varying doses of radiation.

PROJECT REPORT FORM (CONT.)

Proposed course of project: In addition to continuing this project along the lines indicated above, functional tests will be applied to the animals surviving various exposures and treatments. These will probably include resistance to experimental infection, ability to mobilize leucocytes, ability to respond with polycrythemia to repeated exposures to hypoxia, resistance to toxins and possibly liver and kidney function tests.

PROJECT REPORT FORM (CONT.)

10. NCI-634
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[x]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

Dr. George Brecher, Clinical Center Pathologist.

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

PROJECT REPORT FORM (CONT.)

14. NCI-634
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

X-Irradiation in Hamsters and Effects of Streptomycin and Marrow-Spleen Homogenate Treatment, W. W. Smith, R. I. Marston, L. Gonshery, I. M. Alderman and H. J. Ruth, Am. J. Physiol. 183, 98 (1955).

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-636 (SERIAL NO.)

6. The Lethal Effects of Visible Radiation on a Strain of Haploid Yeast
PROJECT TITLE

7. Dr. Mortimer M. Elkind
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objectives of this investigation are to delineate the parameters which control the sensitivity of yeast cells to visible radiation, and, where possible, to correlate the effects observed with processes within the cell.

Methods employed: Actively growing (log phase) yeast cells (haploid Saccharomyces cerevisiae) are harvested from a liquid growth medium (yeast extract plus dextrose), washed by centrifugation, and resuspended in a potassium phosphate buffer. Immediately after resuspension, the cells are essentially insensitive to the emission from a 300 watt, incandescent lamp slide projector (350 m/u to 750 m/u). With time, the population becomes increasingly light sensitive to this emission as measured by the ability of the cells to grow into visible colonies when plated on agar containing growth medium. The cells are irradiated in the same buffered solution and are kept at a temperature of from 1.0-2.0° C during irradiation.

Major findings: A. For a given composition of the buffer solution and a given temperature of storage, with time the cells become progressively more light sensitive. B. For the same length of time of storage the sensitivity is strongly dependent on the temperature of storage. For instance, 2 hrs. of storage at 30° C will produce the same sensitivity as about 28 hrs. storage at $1\frac{1}{2}$° C. C. For the same length of time and temperature of storage, the sensitivity is strongly dependent upon the pH of the buffer solution and to a lesser extent upon the molarity of the buffer solution. D. Suppression of oxygen tension of the buffer solution at the time of irradiation decreases the sensitivity. E. Compared to log phase cells, resting cells do not develop appreciable light sensitivity in the course of storage.

PROJECT REPORT FORM (Cont.)

Significance to cancer research: This research is a phenomenological study of a process present in a biological system which, if at all, has not received much attention in the past. In addition to the technical need for a knowledge of the scope and extent of this effect as it might present itself as an artifact in other radiation studies with this organism, this work is related to cancer research as basic biological research in general is so related.

Proposed course of project: Optimumly the culmination of this work would probably consist of an identification of the biological processes responsible for light sensitivity. With this in mind it is planned to complete the exploration for the apparent pertinent parameters involved, and to examine the action and absorption spectra of these cells within the limitations of available equipment and techniques.

10. NCI-636
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[x]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

14. NCI-636
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Mortimer M. Elkind and Carl A. Beam, "Variation of the Biological Effectiveness of X-Rays and Alpha-Particles on Haploid Saccharomyces cerevisiae," Radiation Research, 3, 88-104 (1955).

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION(IF OTHER THAN BETHESDA)) 5. NCI-637 (SERIAL NO.)

6. Development of High-Intensity X-ray Source
PROJECT TITLE

7. H. L. Andrews
PRINCIPAL INVESTIGATOR(S)

8. R. E. Murphy
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To obtain from the 3 Mev generator the x-ray output which it should be capable of producing.

Methods employed: The 3 Mev Van de Graaff generator given to NIH by the Liggett and Meyers Tobacco Co. is capable of producing x-rays at intensities never before reached in the laboratory. It had never been used as an x-ray generator and when attempts were made to utilize its capabilities x-ray targets failed by melting. Failures occurred at only about 1/4 of full power and hence the possible generator capability is seriously restricted. Since target failure allows cooling water to enter the accelerating tube each failure represents a minor disaster. Pending remedial steps the generator has been operated conservatively to insure continuity of service. Improved operation is to be expected from: 1. higher voltage operation, 2. high frequency target scanning, 3. use of targets of high atomic number, 4. improved target cooling.

Major findings: A study of failures of the gold targets, and the general theories of x-ray production have suggested the following:

1. Since the efficiency of x-ray production increases with voltage operation at the highest possible voltage will give increased output for equal target heating. By careful attention to details we have raised the routine operating voltage from 3.0 Mev to 3.5 Mev. For special purposes operation at 3.8 Mev appears possible but can not be counted on for daily use.

2. All target failures appear to be due to a burst of high current lasting for perhaps one microsecond. If this is the case sweeping the incident electron beam over the face of the target at very high speed should reduce local heating. Preliminary experiments with 300 kilocycle scanning indicate its feasibility and indicate the direction for future equipment design.

NCI-637
SERIAL NO.

PROJECT DESCRIPTION (CONT.)

3. Since x-ray production is proportional to the atomic number of the target material steps have been taken to replace the gold (Z=79) targets with thorium (Z=90). Thorium targets have been obtained from the AEC, and will be installed when suitable welding techniques are proven satisfactory.

4. Delivery of cooling water to the back surface of the target has been improved and steps are planned to reduce the trauma caused by target failure.

Significance to cancer research: With the high outputs potentially available from this generator radiation doses can be administered at dose rates never before available. It is possible that quite different biological results will be obtained when a given radiation dose is given at say 10,000 r/min rather than at the more usual 50-100 r/min.

Proposed course of project: These were covered under "major findings." This project moves slowly to avoid disruption of existing radiation schedules but within 6 months operation at full generator power can be expected.

10. NCI-637
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[X]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-638 (SERIAL NO.)

6. A study of the neuropathology of massive doses of x-rays in the guinea pig
PROJECT TITLE

7. Kirkland C. Brace
PRINCIPAL INVESTIGATOR(S)

8. Howard L. Andrews
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the effect of massive doses of x-rays on the central nervous system of the guinea pig.

Methods employed: Guinea pigs are exposed to various doses of x-rays and the brains removed at various intervals after the exposure. The tissues are examined microscopically to determine the time course of and type of pathology that could be observed.

Major findings: The only readily observable changes with doses of less than 25,000 r of x-rays are in the granule cells of the cerebellum. Doses of less than 6,000 r do not show any changes in the central nervous system. Doses of in excess of 6,000 r produce a peculiar pyknosis of the granule cells which appears about an hour after the exposure at a dose rate of 50 r per minute. Almost 90 per cent of the cells are involved after 8 hours. Death occurs at about 24 hours. The neurological symptoms observed are closely associated with the amount of pyknosis present.

Significance to Cancer Research: It has long been reported that the non-dividing cells of the central nervous system are highly resistant to radiation. It appears from this data that the cerebellum is particularly radiosensitive in the guinea pig. This may explain the particular sensitivity of the medulloblastoma which is derived from the same anlage as the granule cells.

Proposed course of project: With the availability of new equipment we expect to repeat this study at much higher dose rates to determine if there is any change. We also plan to extend the study to some other species of animal.

PROJECT REPORT FORM (cont.)

10. NCI-638
SERIAL NO.

11. BUDGET ACTIVITY:

Research	☒	Administration	☐
Review & Approval	☐	Technical Assistance	☐

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

National Institute of Mental Health

(This project was reported last year by NINDB. At that time Dr. Alvord was principal investigator along with Dr. Brace. Dr. Alvord has left NIH.)

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-639 (SERIAL NO.)

6. A study of ~~the neuropathology~~ of life span of the nucleated red cell
PROJECT TITLE

7. Kirkland C. Brace
PRINCIPAL INVESTIGATOR(S)

8. Paul D. Altland, NIAMD
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: A study of the life span of nucleated erythrocytes of the birds, reptiles, and amphibians using C^{14}.

Objectives: A better understanding of the remarkable resistance of the lower forms and particularly the poikilotherms to the effects of ionizing radiation.

Methods employed: The erythrocytes of various species of animal are labeled in vivo by the intravenous or intraperitoneal injection of glycine-2-C^{14}. The methyl carbon is incorporated into the hemoglobin of the erythrocyte and is carried with the cell until it is destroyed. The determination of the life span is based upon the interpretation of the fall in the specific activity of the hemoglobin following injection of the glycine.

Major findings: The life span of the bird erythrocyte has been determined by several other methods. We have repeated the determination to check the validity of our method. The value of 42 days we obtained for the duck erythrocyte confirms previous work. The specific activity of the hemoglobin of the box turtle remains almost unchanged after 500 days after injection and the specific activity of the hemoglobin of the toad remains unchanged after 270 days after injection. We can only interpret this data as showing that these cells are extremely long lived.

Significance to Cancer Research: An understanding of the effects of ionizing radiation on a cell depends on knowing the normal physiology of the cell. These extremely long lived cells of the poikilotherms may partially explain the resistance of these cells to effects of radiation and could explain the general radio resistance of the animal.

PROJECT REPORT FORM (cont.)

Proposed course of project: The work on the turtle and toad has been set up to continue for several more years. We hope to determine if a finite life span of these cells does exist. If possible, this study will be extended to some other species especially the fish.

PROJECT REPORT FORM (cont.)

10. NCI-639
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[x]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

National Institute of Arthritis and Metabolic Diseases

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Kirkland C. Brace and Paul D. Altland, Red Cell Survival in the Turtle, The American Journal of Physiology 183, 91 (1955),

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION(IF OTHER THAN BETHESDA)) 5. NCI-640 (SERIAL NO.)

6. The effect of x-radiation on the anaphylactic response in mice
PROJECT TITLE

7. Falconer Smith
PRINCIPAL INVESTIGATOR(S)

8. Marie M. Grenan, (Hazel P. Gump, not presently attached to this project)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: a) Obtain a quantitative expression in terms of combining ratios of antigen and antibody for the increases susceptibility of the irradiated mouse to anaphylaxis. b) Improve the tissue specificity of leucocyte and other tissue antigens and study the effects of their homologous antibodies on irradiated mice.

Methods employed: The responses of passively immunized, irradiated mice to varying concentrations of antigen are tested using hen's egg albumin and its homologous antiserum (rabbit) as test preparations. In addition, tests are carried out with mice using intravenous injections of mouse-leucocyte antiserum and erythrocyte preparations.

Major findings: Rabbit, mouse leucocyte antiserums (prepared from peritoneal exudates) given intravenously is more harmful to irradiated mice than to their nonirradiated controls. Since a similar result was observed with hen's egg albumin antiserum, additional studies of a quantitative nature, using this preparation will be made.

Significance to Cancer Research: Anaphylaxis is a common response to protein by appropriately sensitized mammalian tissue and is apparently enhanced by radiation. It is considered possible that specific tissue sensitivity can be obtained which in turn may produce an additive effect when combined with x-radiation. In addition, studies of the effects of x-rays on the anaphylactic response provide information on the biochemical behavior of the reactive tissue.

Proposed course of project: Improvement in specificity of tissue antigens by the isolation of specific cell types, the preparation of antisera to these and testing of the antisera will occupy a major portion of the calendar year assigned to this project.

PROJECT REPORT FORM (cont.)

10. NCI-640
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[x]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

No other units cooperating.

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No knowledge of parallel studies elsewhere.

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Radiation Branch
LABORATORY OR BRANCH

3. Radiation Biology Section
SECTION OR SERVICE

4. _____
LOCATION(IF OTHER THAN BETHESDA)

5. NCI-641
SERIAL NO.

6. The Effect of Irradiation on Susceptibility to Viral Infections
PROJECT TITLE

7. Willie W. Smith, Bernice Eddy (L.B.C.)
PRINCIPAL INVESTIGATOR(S)

8. Ilo M. Alderman, Ruth Gillespie
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine whether or not exposure to x-radiation alters susceptibility to various viral infections.

Methods employed: The response to challenge with poliomyelitis or influenze virus in mice given just sublethal irradiation is compared with that of controls.

Major findings: The results thus far indicate that mice exposed to just sublethal radiation are no more susceptible to the challenging injection of polio virus than are controls. Experiments with influenza are in progress.

Significance to Cancer Research: To promote a more complete understanding of the effects of irradiation and to enable one to anticipate possible deleterious effects of irradiation used therapeutically.

Proposed course of project: This project will be continued along the lines indicated. In addition, we plan to study the effects of sublethal radiation on response to several other noxious agents.

PROJECT REPORT FORM (Cont.)

10. NCI-641
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[x]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

Dr. Bernice Eddy, L. B. C.

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-642 (SERIAL NO.)

6. Antibody production in irradiated mice: Effect of fractional body shielding on hemolysin production and of radiation dose rate on hemolysin production
(PROJECT TITLE)

7. Falconer Smith
(PRINCIPAL INVESTIGATOR(S))

8. H. Jeanette Ruth
(OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to determine the effect of x-radiation administered under the indicated conditions on the immune response in mice.

Methods employed: Peak anti sheep erythrocyte hemolysin titers of individual mice are determined at weekly intervals following exposure to x-rays, radiation dose rate being varied in some instances while in others the volume of dose of x-rays is varied.

Major findings: It was recently shown that bone marrow or spleen homogenate was without effect on radiation damage to the immune response. Shielding varying fractions (60-90%) of the body provides varying degrees of protection to the immune response at a given total body dose as determined by serum hemolysin titers at one to three weeks after x-ray exposure.

Significance to Cancer Research: Antibody production reflects one aspect of protein synthesis by living tissue and is therefore directly related to cell metabolism, a problem of much interest in cancer research.

PROJECT REPORT FORM (cont.)

10. NCI-642
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[x]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR O HER ORGANIZATI ONS, PRO IDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

No other units cooperating.

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No knowledge of parallel studies elsewhere.

PROJECT REPORT FORM (CONT.)

14. NCI-642
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Falconer Smith and H. Jeanette Ruth, Hemolysin Production in Irradiated Mice Given Spleen or Bone Marrow Homogenate, Proceedings of the Society of Experimental Biology and Medicine 90, 187 (1955).

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-643 (SERIAL NO.)

6. Long Term Survival and Tumor Incidence Following Acute or Chronic Irradiation
(PROJECT TITLE)

7. Joanne Hollcroft
(PRINCIPAL INVESTIGATOR(S))

8. Eliza Miller, Charles C. Congdon
(OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives:

A. To study long term survival and carcinogenesis of:

1) Chronic irradiation of guinea pigs with or without injections of bone marrow and determine the effect of bone marrow treatment in aleukemic leukemia.

2) Acute radiation doses when the animals are protected from the acute irradiation effect.

B. Methods employed:

1) Family 2 guinea pigs were exposed to 8.8 r per day gamma irradiation, when their hematocrit number dropped to about 25 they were removed from the radiation field. At this time half the pigs were given intravenous injections of bone marrow. Survival and tumor incidence were studied.

2) C_3H_b mice were x-irradiated under the following conditions:

(a) 400 r at birth,

(b) 400 r with sham spleen shielding,

(c) 400 r with spleen shielding,

(d) 900 r with spleen shielding, and

(e) 900 r with chemical protection of cystiene and anoxia

NCI-643
SERIAL NO.

PROJECTION DESCRIPTION (CONT.)

C. Major findings:

1) Intravenous injections of bone marrow suspensions decreased the number of early deaths following limited chronic irradiation. These injections may have prevented some aleukemic leukemia in male guinea pigs but seemed to have no effect on aleukemic incidence in female guinea pigs. The hematocrits of the animals developing aleukemic leukemia appeared to drop lower after removal from the radiation field than animals which recovered more completely. Of the animals which recovered from the chronic irradiation the bone marrow treated animals seemed to have a longer survival time.

2) Survival time following acute exposures was not influenced by spleen protection or the fact that the animals were irradiated at birth. The mice receiving cystiene and anoxia protection, lived longer than the spleen shielded animals. In the mice irradiated with 900 r an increase in the following lesions appeared:

(a) Adrenal tumors,

(b) ovarian tumors,

(c) glomerularsclerosis,

(d) myelofibrosis as noted in the sternum,

(e) lens damage to the eye. An increase in the number of reticular endothelial neoplasms was noted in the animals protected chemically and an increase in miscellaneous carcinomas and sarcomas was seen in female spleen shielded mice. In both groups pyelonephritis occurred less frequently than in the controls.

Significance to cancer research: Irradiation is a well known carcinogen. The long-term effects of acute doses of total body irradiation are of consequence in considering the role of radiotherapy in treatment of cancer.

Proposed course of project:

1) Influence of hematocrit number and white blood count as well as total dose on development of aleukemic leukemia.

2) Completion of histologic studies.

10. NCI-643
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[x]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. It is hoped that Dr. Congdon of the Oak Ridge National Laboratory will continue with the pathologic diagnoses on these studies.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

13. None.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH.

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-644 (SERIAL NO.)

6. Modification of X-irradiation Treatment of Localized Tumors.
PROJECT TITLE

7. Joanne Hollcroft
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine in what manner the tumor reacts to ionizing radiation and in what manner the radiosensitivity may be increased.

Methods employed: Vary the physiology of the host prior to, during or following irradiation of a transplanted lymphosarcoma L-1, follow tumor growth.

Major findings:

1. Attempts to change blood supply to tumor by administration of adrenalin, histamine, priscoline or diathermy did not greatly alter the response of the tumor to x-irradiation.

2. When a single dose of cortisone was administered either prior to or following irradiation the increase in response was found to be additive.

3. Thiotepa may increase the response if given at the proper time prior to irradiation.

4. Fractionation of a dose of 4000 r into 4 equal doses given 2 minutes apart produced the same regression as the same dose given daily but if the dose is given at 2 day intervals the effect is less.

Significance to Cancer Research: To find methods of increasing usefulness of radiotherapy.

Proposed course of project: Continued studies on fractionation and comparison of fractionation treatment of radiosensitive tumors with that of radioresistant tumors. Studies on radiation induced radioresistance. Use of antimetabolite and cytotoxic poisons to potentiate radiation effects.

10. NCI-644
SERIAL NO.

11. BUDGET ACTIVITY:

Research	[X]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-645 (SERIAL NO.)

6. Effect of X-irradiation on Disseminated Lymphosarcomas, L#2 and L_2C.
PROJECT TITLE

7. Joanne Hollcroft
PRINCIPAL INVESTIGATOR(S)

8. Charles C. Congdon
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the LD 100 g. L-2 tumor and devise a feasible treatment schedule. Treatment of L_2C guinea pig leukemia.

Methods employed: A/HeN males bearing L-2 lymphosarcoma were irradiated starting on the 10th day after transplant. A bio-assay of the liver spleen and tumor from these mice was performed by innoculating A/HeN males subcutaneously with these tissues at various times following treatment.

A/HeN males bearing L-2 tumors were x-irradiated and treated subsequently with bone marrow.

Family 2 guinea pigs bearing L_2C lymphocytic leukemia were treated with x-irradiation plus bone marrow; thiotepa, x-irradiation plus bone marrow or thiotepa alone. (This work was started by Dr. Congdon.)

Major findings: With doses greater than 2000 r the greatest number of "takes" occurred when the tumor fragments were transplanted 1, 2 or 3 days after treatment. Few transplants grew from tissues taken immediately or 4 hours after treatment. Two daily doses of 1000 r or 4 daily doses of 500 r were found equally effective in inducing the transplantability of tumor fragments 1 day after the end of treatment. No tumor fragments grew after transplantation from animals receiving 4000 r. When tumor fragments were irradiated in vitro with 4000 r 15% of the tumor transplant grew.

In treating mice bearing L-2 tumor with irradiation followed by bone marrow longest survival time (11 days) occurred when the animals were irradiated with 5000 r to the tumor and 900 r to the body given in 2 equal doses 4 hours apart.

NCI-645
SERIAL NO.

PROJECT DESCRIPTION (CONT.)

Family 2 guinea pigs bearing L2C lymphocytic leukemia have been treated successfully with 5 x 400 r x-irradiation plus bone marrow, thiotepa or a combination of thiotepa and x-irradiation.

Significance to Cancer Research: Radiotherapy of leukemia.

Proposed course of project: Use of the bio-assay technic to study radiosensitivity of other disseminated neoplasms. Study of the number of cells innoculated vs time to death in hopes to extrapolate this information to the effectiveness of radiation treatment. Continuation of radiation treatment of guinea pig leukemia.

10. NCI-645
SERIAL NO.

11. _____
BUDGET ACTIVITY:

Research	[x]	Administration	[ ]
Review & Approval	[ ]	Technical Assistance	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITH UT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Therapy Service (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-650(C) (SERIAL NO.)

6. Service Radiation Therapy
PROJECT TITLE

7. J. Robert Andrews, M.D., and Philip Rubin, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Robert W. Swain
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To provide radiation therapy for those patients requiring such in the course of the disease for which they are being investigated by research groups other than the Radiation Branch.

Methods employed: Consistent with contemporary radiotherapeutic practice employing a wide range of photon or other energies, radium and radioisotopes as indicated.

Patient material: Those patients present with a wide variety of neoplasms for which radiation therapy might be of palliative or other value.

Major findings: Variable as would be expected from the diverse nature of the material studied and the widespread diseases generally present.

Proposed course of project: To continue.

PROJECT REPORT FORM (Cont.)

10. NCI-650(C)
SERIAL NO.

11. BUDGET ACTIVITY:

Research	☐	Administration	☐
Review & Approval	☐	Technical Assistance	☐

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 & 16.

. PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Radiation Therapy Service — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-651(C) — SERIAL NO.

6. Electron Beam Radiation Therapy — PROJECT TITLE

7. J. Robert Andrews, M.D. — PRINCIPAL INVESTIGATOR(S)

8. Philip Rubin, M.D., and Robert W. Swain — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To deliver effective doses of ionizing radiation to the epidermis and corium by means of controlling energies and character of the ionizing radiation to limit the effects to this zone.

Methods employed: Ionizing radiation is an effective treatment for a variety of multiple, malignant, superficial, cutaneous neoplasms including mycosis fungoides, Bowing's disease and, possibly, Kaposi's sarcoma. Ionizing radiation may be administered as x-ray therapy but in this case there is generally such absorption of radiation in deeper tissues, including the radiosensitive bone marrow, as to limit the amount of radiation to less than an effective dose. The absorptions of electrons in the energy range (< 2.0 Mev) available is, however, limited to less than 1 cm. of tissue. This makes possible the administration of effective doses of radiation to superficial neoplasms without affecting deeper structures.

The physical factors associated with the production, dosimetry, and clinical use of an electron beam are being thoroughly studied. Clinical studies of skin erythema and skin blistering doses are being performed and compared with x-ray effects as to relative biological effectiveness. The effects of various doses on superficial cutaneous neoplasms are being studied. In addition to clinical observations, appropriate biopsy material is being obtained and all observations are being carefully documented.

Patient material: Cases of mycosis fungoides, Bowing's disease, Kaposi's sarcoma and other primary or secondary superficial malignant neoplasms.

Major findings: This project was initiated only in the last six months. The response of the limited number of patients so far available for study indicates that this type of ionizing radiation is clinically effective.

Proposed course of project: As indicated in methods above.

PROJECT REPORT FORM (cont.)

10. NCI-651(C)
SERIAL NO.

11. BUDGET ACTIVITY

Research	☐	Administration	☐
Review & Approval	☐	Technical Assistance	☐

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Radiation Therapy Service — SECTION OR SERVICE
4. _____ — LOCATION(IF OTHER THAN BETHESDA)
5. NCI-652(C) — SERIAL NO.

6. The Relative Biological Effectiveness (RBE) Factor for the Destruction of Human Carcinoma
PROJECT TITLE

7. J. Robert Andrews, M.D., Philip Rubin, M.D., and Robert W. Swain
PRINCIPAL INVESTIGATOR(S)

8. Eugene J. Van Scott, M.D., and Richard P. Reinertson, M.D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the relative biological effectiveness (RBE) factor for the destruction of human carcinoma.

Methods employed: Human multiple, superficial cutaneous neoplasms are treated with a range of doses by a variety of ionizing radiations (including low voltage unfiltered x-rays, medium voltage filtered x-rays, 2 Mev x-rays, the gamma rays of radium, and 0.9 and 1.5 Mev electrons) and the cancerocidal dose determined for each. In addition to grossly observable clinical effects, microscopic histological studies have also been undertaken.

Patient material: Cases of multiple, superficial, epithelial cancers of the skin.

Major findings: Major findings can not be reported at this time because this project was initiated only within the last six months.

Proposed course of project: As indicated in methods above.

PROJECT REPORT FORM (cont.)

10. NCI-652(C)
SERIAL NO.

11. BUDGET ACTIVITY

Research	☐	Administration	☐
Review & Approval	☐	Technical Assistance	☐

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Therapy Service (SECTION OR SERVICE) 4. _____ (LOCATION(IF OTHER THAN BETHESDA)) 5. NCI-653(C) (SERIAL NO.)

6. The Influence of Oxygen in Modifying Radiation Response

7. J. Robert Andrews, M. D. (PRINCIPAL INVESTIGATOR(S))

8. Philip Rubin, M. D., Robert W. Swain (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION:

Objectives: To investigate the possibility of modifying response of neoplastic cells to ionizing radiation by altering the oxygen content of blood.

Methods Employed: Appropriate patient material (see below) is irradiated over bilaterally more or less symmetrically enlarged lymph nodes with equal doses of ionizing radiation (250 KV X-rays) under different conditions of oxygen saturation of hemoglobin and dissolved blood oxygen. One bilaterally symmetrical area is irradiated under conditions of normal atmospheric pressure and oxygen tension. The other bilaterally symmetrical area is irradiated under conditions of normal atmospheric pressure with 100% inspired oxygen under which conditions the differential oxygen tension is increased by a factor of approximately 5.

If a differential effect is observed it is proposed to extend the experiment by irradiation of similar patient material under conditions of reduced oxygen tension (normal atmospheric pressure but reduced differential oxygen tension) and under conditions of increased oxygen tension to above normal atmospheric pressure by radiation in a pressure chamber. It is further proposed to investigate directly the oxygen tension in the tissues exposed to ionizing radiations by the use of appropriate instruments.

Patient Material: Cases of Hodgkin's disease or lymphosarcoma with bilaterally and symmetrically enlarged lymph nodes in previously untreated areas.

Major Findings: Major findings can not be reported at this time because this project was initiated only within the last six months.

Proposed Course of Project: As indicated in methods above.

10. NCI-653(C)
SERIAL NO.

11. BUDGET ACTIVITY:

Research	☐	Administration	☐
Review & Approval	☐	Technical Assistance	☐

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Therapy Service (SECTION OR SERVICE) 4. _____ (LOCATION(IF OTHER THAN BETHESDA)) 5. NCI-654(C) (SERIAL NO.)

6. Time as a Modifier of Clinical Radiation Response

7. J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR(S)

8. Philip Rubin, M. D. and Robert W. Swain
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To evaluate the prolongation of treatment time as a modifier of clinical response to ionizing radiations.

Methods Employed: Irradiation of appropriate patient material (see below) to tumor doses confirming with the equation,

$$D = 2750\ t^{0.23}$$

over approximately a 100 day treatment period.

Patient Material: Cases of squamous cell carcinoma of the head, neck, larynx and uterine cervix in Stages II and III.

Major Findings: Major findings can not be reported at this time because this project was initiated only within the last six months.

Proposed Course of Project: As indicated in methods above.

10. NCI-654(C)
SERIAL NO.

11. _____
BUDGET ACTIVITY:

Research	☐	Administration	☐
Review & Approval	☐	Technical Assistance	☐

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER, 1956 or 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (cont.)

14. NCI-654(C)
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

J. R. Andrews and Joe M. Moody, The Dose-Time Relationship for the Cure of Squamous Cell Carcinoma: Presented at the Inter-American Congress of Radiology, Washington, D. C., 1955; Accepted for publication by the American Journal of Roentgenology and Radium Therapy.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Therapy Service (SECTION OR SERVICE) 4. _____ (LOCATION(IF OTHER THAN BETHESDA)) 5. NCI-655(C) (SERIAL NO.)

6. Determination of Radiation Tolerance to the Brain by Means of Electroencephalographic Studies
(PROJECT TITLE)

7. Philip Rubin, M.D., and J. Robert Andrews, M.D.
(PRINCIPAL INVESTIGATOR(S))

8. Dr. Cosimo Ajmone Marsan
(OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives: The use of electroencephalographic studies while treating various brain tumors will be employed to determine if any damage has been done to normal brain tissue. It is assumed that physiological changes reflected in the electroencephalogram may precede the severe structural anatomical changes. No attempt has been made to correlate electroencephalographic changes in definite areas of the brain with isodose curves which will give exact dosage to these points.

Methods employed: Serial electroencephalographic studies will be done during the course of treatment, beginning with a base line study. Aberrations in brain waves in certain areas will be correlated with the dosage level at these points. With the sharp beam alignment and available isodose curves this can be calculated with sufficient accuracy to have considerable correlative values.

Patient material: Appropriate case material which includes a wide range of brain tumors which have proved to be inoperable, but show some evidence of radioresponsiveness on the basis of the literature.

Major findings: Major findings can not be reported at this time because this project was initiated only within the last six months.

Proposed course of project: It is proposed that in the next year this study will be considerably amplified in conjunction with an expanded activity in brain tumor research in the NINDB.

PROJECT REPORT FORM (cont.)

10. NCI-655(C)
SERIAL NO.

11. BUDGET ACTIVITY:

Research	☐	Administration	☐
Review & Approval	☐	Technical Assistance	☐

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Therapy Service (SECTION OR SERVICE) 4. _____ (LOCATION(IF OTHER THAN BETHESDA)) 5. NCI-656(R) (SERIAL NO.)

6. Attempt at Radiotherapy with S^{35}-Sulfate
PROJECT TITLE

7. Philip Rubin, M. D. and J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR(S)

8. J. A. Hollcroft, Marion Matthews, Raymond Gottschalk, M.D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: S^{35} given as sulfate selectively concentrates in cartilage and tissues containing abundant sulfated mucopolysaccharides. Since the beta radiation of S^{35} is very weak (in the neighborhood of 50 kv.) it must be determined first whether this can destroy cartilage cells. The S^{35} is laid down in the ground substance of cartilage, a chondroitin sulfuric acid polysaccharide. It is postulated that the path of the emitted electrons will be adequate to reach the cells. To date, no evidence of cell destruction has been shown with the dosage used in adult cartilages. It seems essential before one embarks on the use of large amounts of this radioisotope which will mean considerable expense, that evidence of some radiotoxic effect should be shown on cartilage.

Methods employed: The following animal experiment was therefore decided upon and is being carried out as outlined below:

1. Thirteen litters of white suckling rats were utilized weighing between 8 to 25 gms.
2. Varying amounts of radioactive sulfate were injected ranging from the toxic level (2.0 Mc/gm.) to the postulated therapeutic level (0.1 Mc/gm.)
3. The animals that have died during the procedure were bottled in 10% formalin and are to be examined at a future date by Dr. Gottschalk for the pathology and tissue assays which are pertinent to the procedure.
4. Weights of the animals will be taken every week to obtain a growth curve.
5. X-rays of all the bones in the body will be made every two weeks to determine the effect of the administered S^{35} on epiphyseal centers and bone growth.

Patient material: Application to Patients with Chondrosarcoma: If the above work suggests that growing cartilage can be destroyed by radiosulfur,

PROJECT REPORT FORM (cont.)

an attempt will be made to utilize this agent on a clinical basis. Only patients with advanced chondrosarcoma who have been treated with surgery and external radiation previously will be candidates for this treatment.

Major findings: Definite disturbances in the epiphyseal and metaphyseal growth pattern of long bones have been observed. This remains to be correlated with tissue assay and radiomicrographs of tissue sections.

Significance to Cancer Research: A new method of treating chondrosarcoma if the animal experiments prove successful.

Proposed course of project: Continuation as outlined above until studies are completed which will be in three months.

10. NCI-656 (N)
SERIAL NO.

11. BUDGET ACTIVITY:

Research	☐	Administration	☐
Review & Approval	☐	Technical Assistance	☐

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

PROJECTS WHICH ARE BEING DISCONTINUED

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. Nutrition and Metabolism (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-701(C) (SERIAL NO.)

6. A. Meticorten: Metabolic and clinical effects of massive doses of Prednisone in subjects with malignant disease (PROJECT TITLE)

7. Donald M. Watkin (PRINCIPAL INVESTIGATOR(S))

8. _____ (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives: To quantitate the effect of massive doses of Prednisone, a synthetic steroid reputedly low in undesirable side effects, in malignant disease not amenable to more conventional therapy.

Methods Employed: Metabolic balance technique I^{131} labelled human serum turnover.
Renal function studies.
Electrophoretic analysis of serum protein.

Patient Material: Two women, one with multiple myeloma and one with lymphosarcoma, and three men, one with Hodgkins Disease, one with multiple myeloma, and one with prostatic carcinoma.

Major Findings: All subjects demonstrated negative nitrogen balance; all demonstrated retention of sodium and chloride. Theoretical phosphorus balances could not be reconciled with calcium and nitrogen balances. Potassium balances were negative. Uric acid excretion was slightly elevated in three and dramatically elevated in two patients. Marked negative phosphorus and nitrogen balances and marked elevated urinary uric acid excretions were associated with objective diminution in tumor size in lymphoma and Hodgkins Disease. I^{131} albumin turnover rates were accelerated by Prednisone therapy in one patient. Renal function studies before and during treatment indicated a marked reduction in renal plasma flow apparently due to reduction in cardiac output, an example of forward failure. Electrophoretic studies of plasma proteins revealed for the first time a change in the characteristic pattern in a patient with multiple myeloma.

Numerous side effects including acute psychosis, congestive heart failure, paroxysmal tachycardias, systemic moniliasis, pulmonary embolism and severe acne were observed during massive Prednisone therapy.

Significance to Cancer Research: Prednisone, because of its potency greater than Cortisone and freedom from side effects when given in smaller doses, suggested a means of giving enormous corticoid doses to patients with hopeless malignancy. The reported studies indicate that in massive doses the hormone has numerous undesirable side effects, that it is effective against malignancies in the lymphoma group, and that it can induce some changes hitherto unreported in multiple myeloma. Except for experimental purposes, however, Prednisone in massive doses cannot be recommended as a therapy for malignant disease.

Proposed Course of Project: The discrepancies between theoretical and actual phosphorus balances, changes in myeloma proteins, changes in resistance to disease, and alterations in intracellular electrolytes, especially in the myocardium, are avenues which may be followed as suitable patients present themselves and as new synthetic hormones are developed.

B. Metabolism of nitrogen, calcium, phosphorus, electrolytes in metastatic prostatic carcinoma.

Objectives: Accumulation of metabolic data in patients with extensive metastatic prostatic malignancy before, during and after various therapeutic maneuvers.

Methods Employed: Metabolic balance technique. Red cell turnover determinations.

Patient Material: In 1954, two patients were studied before and after orchiectomy. In 1955, one of these returned one year after operation for a follow-up metabolic study. In addition, two other patients with extensive metastatic disease, both several years post-orchiectomy, were admitted for study of the effect of hormone administration.

Major Findings: The beneficial effects of orchiectomy in reducing calcium and protoplasmic loss were maintained one year after operation. Stilbestrol administration was of equivocal value. Massive Prednisone dosage superimposed on stilbestrol administration in one patient produced a temporary clinical improvement, but in the long run resulted in marked wasting of bone and protoplasm.

Incidental Findings: The patient treated with Prednisone demonstrated a unique afibrinogenemia which was followed throughout his course and which seemed to improve under Prednisone therapy. This was not due to prostatic fibrinolysin but to a lack of fibrinogen production.

CI-701(C)
ERIAL NO.

PROJECT REPORT FORM (cont'd)

Significance to Cancer Research: Quantitative evaluation of the natural course of the disease together with quantitative measurements of the effects of therapy.

Proposed Course of Project: Continued search for more patients with extensive prostatic disease, follow-up on patients already under study, further investigation of the relationship of prostatic malignancy to afibrinogenemia.

C. Metabolism of fluoride in leukemic subjects with and without chronic fluorosis

Objectives: Investigation of possible role of fluoride in genesis of leukemia.

Methods Employed: Metabolic balance technique.

Patient Material: During 1954 two leukemic patients with fluorosis, one leukemic without fluorosis and one non-leukemic without fluorosis were studied. In 1955, three patients without fluorosis, two normals and one with multiple myeloma, were studied.

Major Findings: On an intake of distilled water, patients with fluorosis excrete fluoride in the urine. Patients with and without fluorosis excreted a fixed percentage of fluoride intake. Leukemia or multiple myeloma did not alter the fluoride excretion pattern. Balances of nitrogen, potassium, phosphorus, calcium, sodium and chloride were not significantly altered by fluoride administration at levels of 5 mg. per day.

Significance to Cancer Research: The essentially negative results of these studies should help allay the fears of many laymen that fluoride in drinking water may prove harmful. No evidence has been obtained in these studies to indicate any harmful effects of fluoride administration. These studies of course do not rule out fluoride as a carcinogen but do demonstrate the absence of any measurable toxic effect.

Proposed Course of Project: Any further pursuit of this subject will depend on the ability of NIDH to perform fluoride assays. At the present, no extension of the program has been planned as far as NCI is concerned.

D. Role of B complex vitamins in tissue anabolism

Objectives: To observe the utilization of B complex vitamins in tissue anabolism induced by steroids or hyperalimentation.

NCI-701(C)
SERIAL NO.

PROJECT REPORT FORM (cont'd)

Methods Employed: Metabolic balance technique. Bioassay for vitamins in food and urine.

Patient Material: One patient studied initially in 1954 was carried over into 1955.

Major Findings: No direct relationship was noted between vitamin retention and tissue anabolism. The small quantities of vitamins required and the inaccuracy of the bioassay technique may account for the negative findings.

Significance to Cancer Research: The demonstration of the essentiality of certain vitamins in the growth of normal or tumor tissue could lead to development of effective antimetabolites.

Proposed Course of Project: The bioassay procedures originally conducted by the Endocrinology Branch are no longer available, hence, no continuation of this project is contemplated.

E. Metabolism of human serum albumin administered intravenously in large doses to subjects with malignant disease.

Objectives: Quantitative measurement of the metabolism of large amounts of human serum albumin given intravenously.

Methods Employed: Metabolic balance technique.
Electrophoretic analysis of serum.
Turnover of I^{131} labelled human serum albumin.

Patient Material: One normal, one subject with Hodgkins Disease, one patient with multiple myeloma, one with lung cancer and one with face cancer.

Major Findings: Balance data indicate that the albumin was gradually utilized over a period of about 4 weeks. Isotope studies show an increased rate of albumin turnover during and after albumin administration.

Significance to Cancer Research: Albumin is readily available plasma constituent frequently low in patients with advanced cancer.

Proposed Course of Project: Further evaluation of the data compiled in these studies. No new studies planned at this time.

PROJECT REPORT FORM (cont d)

10. NCI-701(C)
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16

PROJECT REPORT FORM

1. N. C. I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. _____ (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI 702(C) (SERIAL NO.)

6. Metabolic Study of Serum Proteins
PROJECT TITLE

7. J. L. Fahey, J. L. Steinfeld, D. M. Watkin, H. A. Sober and E. A. Peterson
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9A. PROJECT DESCRIPTION: Electrophoretic Studies of the Serum Proteins in Neoplastic Disease States. (J. L. Fahey)

Objectives: The objective of this project is to electrophoretically quantitate the serum protein changes occurring in neoplastic states and to correlate these changes with specific aspects of the clinical status of the patients.

Methods Employed: Development and refinement of a quantitative method of zone (paper) electrophoresis was completed during the year. Systematic follow-up of patients admitted to the Metabolic, Acute Leukemia and Solid Tumor Services was instituted so that serum protein data can be correlated with a clinical evaluation of the type and extent of disease, disease activity, nutritional state of the host and therapy employed. Sequential observations in the same patients are an important aspect of this study.

Patient Material: Patients admitted to the Clinical Center for other projects were utilized in this study.

Major Findings: Zone (paper) electrophoresis can be utilized for quantitative measurement of serum proteins.

Long term patient follow-up was instituted. A study of the serum protein changes during immune response to antigen challenges in leukemic and control patients (in collaboration with the Acute Leukemia Group) revealed no striking correlation of gamma globulin changes in preliminary evaluation. However, the study is not yet complete.

General assistance to all clinical groups has been utilized to evaluate serum proteins of patients with multiple myeloma and of patients in which a gamma globulinemia was suspected. None were found, however.

PROJECT REPORT FORM (Cont'd)

Significance to Cancer Research: Delineation of the sequence of plasma protein changes in neoplastic disease is needed, for this is an aspect of tumor-host relationship which lends itself to quantitation. Also, the effects upon the serum proteins of anti-tumor therapeutic regimens is generally unknown.

Proposed Course of Project: Emphasis will continue to be upon serial determinations throughout the course of illness in the patients studied, and upon correlation with clinical data. The immune studies will be completed. Evaluation of the effects of cortisone, similar steroids and other chemical agents will continue.

9B. PROJECT DESCRIPTION: Measurement of Albumin Turnover and Total Exchangeable Albumin in Patients with Cancer, Control Patients with Weight Loss and Volunteer Normal Subjects. (J. L. Steinfeld)

Objectives: To determine whether the decreased serum albumin concentration found in patients with cancers is due to an increase in plasma volume or failure of albumin production to keep up with albumin degradation or metabolism - and further to ascertain if the metabolism of albumin in cancer patients proceeds at a normal, increased or decreased rate.

Methods employed: Commercially available (Abbott Laboratories) I^{131}albumin is checked for free radioactivity and satisfactory preparations (one milligram of albumin nitrogen and 100 I^{131}) are injected intravenously into patients on relatively constant caloric and nitrogen intake. Frequent serial sampling of serum radioactivity as well as daily determination of total urinary radioactivity permits calculation of total exchangeable albumin and albumin turnover.

Patient Material:		
Adult female	11 admissions	308 patient days
Adult male	5 admissions	140 patient days
Out patients	3 admissions	

Major Findings: The major findings of this project during the past year have been significantly decreased total exchangeable albumin found associated with relatively normal plasma volumes and the decreased or low normal turnover rates of albumin in cancerous patients. This is evidence against a much increased albumin turnover with failure of albumin production to keep pace with the increased destruction being the factor responsible for the low serum albumin concentrations seen clinically.

Significance to Cancer Research: In order to understand the physiological relations between host and neoplasm it is necessary to investigate the possible sources of tumor food supply. Since serum albumin concentration is low in cancerous patients, one might hypothesize trapping of serum albumin by tumor and use of that albumin for metabolism or tumor growth. It is of

PROJECT REPORT FORM (Cont'd)

importance to learn which nutrients tumors can and cannot use since such understanding may contribute to the development of effective anti-tumor agents.

Proposed Course of Project: In collaboration with Dr. Robert Milch, the tissue distribution of I^{131} albumin in tumors as compared with normal tissues will be determined through the counting of I^{131} albumin found in various tissues obtained at operation or at autopsy. These determinations will be corrected for the I^{131} blood content of the various tissues.

Also the distribution and turnover of serum albumin and other plasma proteins in cancer patients will be investigated using endogenously labeled plasma proteins as with S^{35} or C^{14} amino acids.

9C. PROJECT DESCRIPTION: Fate of Intravenously Administered Albumin.
(J. L. Fahey)

Objectives: The objective of this project is to characterize the disappearance rate and distribution of the products of albumin loads administered intravenously to patient in various neoplastic and nutritional states.

Methods Employed: Complete metabolic balance techniques have been utilized to follow nitrogen and, in certain instances, calcium and phosphorus balances in selected pateints. Intravascular distribution of albumin and other serum proteins have been measured by electrophoresis and radioactive iodinated albumin.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult Male	4	35 days
	Adult Female	2	25 days

Major Findings: Intravenously administered albumin is partially available as a source of nitrogen for tissue and tumor needs.

Significance to Cancer Research: Patients with neoplasm develop low serum albumin levels. The cause of this phenomen is obscure. Determination of the fate of intravenously administered albumin loads under several conditions should help to clarify this problem. Nutritional evaluation of intravenously administered albumin can be undertaken at the same time.

Proposed Course of the Project: Early completion of analytic data is anticipated. Correlation and evaluation of findings can then be completed.

PROJECT REPORT FORM (Cont'd)

9D. PROJECT DESCRIPTION: Chromatography of Serum Proteins. (J. L. Fahey)

Objectives: The objective of this project is to further characterize the serum proteins from normal and tumor-bearing patients by application of new methods of protein separation.

Methods Employed: Drs. Sober and Peterson of the Laboratory of Biochemistry, NCI, have designed systems of substituted cellulose columns on which protein components move differentially under pH and salt gradients in controlled temperature conditions. Individual serum protein components are identified by electrophoretic and spectrophotometric means.

Major Findings: Within the past year equipment has been assembled and the chromatographic procedure established in the clinical area. Further work on the method has resulted in some reduction in the time and complexity of the procedure required to carry out an analysis.

Significance to Cancer Research: The alteration of body protein metabolism in neoplastic states is reflected by serum protein changes. These have as yet been only grossly defined by other analytical means. Further identification and characterization of the serum proteins themselves are necessary in order that the causes of protein abnormality may be studied

Proposed Course of the Project: Further effort will be devoted to development of a modified, more rapid adaptation of the present column chromatographic technic. When this is achieved a clinical survey will be feasible in which sera from patients with a variety of neoplasms and in various states of disease activity will be tested.

9E. PROJECT DESCRIPTION: Evaluation of Protein Metabolism by Means of Isotope Labelled Amino Acid. (J. L. Fahey)

This study has been delayed because of the basic decision to change the method of protein fractionation from the cold-alcohol method of Cohn to the substituted-cellulose column technic of Sober and Peterson. However, this method requires further development before it can be efficiently applied to the requirements of such a study. Work with the method is already underway as noted in report.

PROJECT REPORT FORM (Cont'd)

10. NCI-702(C)
SERIAL NO.

11. A, B, C, D and E
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

A. The Acute Leukemia, Solid Tumor and Surgical Services of the NCI have made available blood samples from patients admitted to study on those services and have collaborated generously in providing the clinical information necessary for adequate interpretation of the serum protein data.

B. None.

C. None.

D. Drs. Sober and Peterson of the Physical-chemistry Section of the Laboratory of Biochemistry, NCI, have been responsible for the development of this chromatographic method and are actively collaborating in the application of this technic to clinical studies.

E. None.

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

A. None.

B. None.

C. None.

D. None.

E. None.

No entires for items 14, 15 and 16.

PROJECT REPORT FORM

1. N.C.I.
INSTITUTE

2. General Medicine
LABORATORY OR BRANCH

3. Metabolism
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NCI 703(C)
SERIAL NO.

6. Estimation of Red Cell Survival In Patients With Advanced Neoplastic Disease Before and After Treatment.
PROJECT TITLE

7. J. L. Steinfeld
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine if measurement of red cell life would be a useful parameter for ascertaining the value of a proposed therapy for cancer by first correlating such studies of red cell life span with approved methods for clinical and laboratory evaluation of cancer therapies.

Methods employed: 100 micro-curies of Sodium Chromate^{51} in 8cc of ACD solution are incubated with 50cc of patient's blood and after washing three times with saline to remove free radioactivity, the labelled red cells are reinjected into the patient from whom serial samples of blood are taken, radioactivity measured and red cell life span is estimated.

Patient Material:

Admissions	Adult male	3	90 pt-days
	Adult Female	1	30 pt-days
	OPD Visits	20	

Major Findings: Twenty studies in eighteen patients have shown that red cell life span is variably decreased in cancer patients. In only two instances was the patients' status stable enough to permit two full studies of one month each to be carried out before and after therapy. No conclusions may be drawn at this time as to value of this technique for judging efficacy of cancer therapy.

In a collaborative effort with Drs. H. Chaplin and P. Schmidt of the Clinical Center Blood Bank, red cell survival of blood stored and frozen at -45°C for varying periods of time was found to be in the normal range.

PROJECT REPORT FORM (Cont'd)

Significance to Cancer Research: Objective measures of judging the destructive effects of cancer therapy on cancers and beneficial effects on the host are urgently required at the present time. Such techniques as this - although involved and time consuming - would be of value in select cases if correlation were high between red cell life span and clinical status.

Proposed Course of Project: To continue as outlined above and to pursue further with Drs. V. Price and R. Greenfield studies of the tissue distribution of labeled red cell components at surgery and at autopsy, comparing normal and cancerous tissues after correction for the blood content of the tissues.

PROJECT REPORT FORM (Cont'd)

10. NCI-703(C)
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for items 14, 15 and 16.

PROJECT REPORT FORM

1. N.C.I. — 2. General Medicine Branch
INSTITUTE — LABORATORY OR BRANCH

3. ____ — 4. ____ — 5. NCI-704(C)
SECTION OR SERVICE — LOCATION (IF OTHER THAN BETHESDA) — SERIAL NO.

6. The Influence of Delta Amino Levulinic Acid and Some of Its Analogues on Tumor Growth.
PROJECT TITLE

7. D. P. Tschudy
PRINCIPAL INVESTIGATOR(S)

8. ____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Since delta amino levulinic acid is an intermediate in porphyrin synthesis and one carbon transfers we are attempting to demonstrate a growth promoting action of this compound in tumors, along with direct demonstration in tumors of the enzyme which converts delta amino levulinic acid to porphobilinogen. We are attempting to inhibit tumor growth by means of chemical analogues of this compound.

Methods employed: Organic synthesis, tumor transplantation, tumor growth rate methods, paper chromatography, extraction of enzymes from tumors, pharmacological methods and histologic methods.

Patient Material:

Major Findings: Delta amino levulinic acid has been synthesized by a new method. A number of chemical analogues of this compound have been prepared, some of which had never previously been synthesized. Pharmacologic and enzyme studies in animals are not completed.

Significance to Cancer Research: If inhibition of "one carbon fragment" utilization can be attained by this method along with partial inhibition of porphyrin synthesis, antitumor activity may result. Synergistic increase of anti-tumor activity of folic acid antagonists may also be produced.

Proposed Course of Project: If we are successful in demonstrating the enzyme synthesizing porphobilinogen, we may attempt to demonstrate its intracellular localization. Growth inhibitory activity of any of the compounds will be studied in other systems including human tumors.

PROJECT REPORT FORM (Cont'd)

10. NCI-704(C)
SERIAL NO.

11.
BUDGET ACTIVITY

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for items 14, 15 and 16.

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-706(C) (SERIAL NO.)

6. Study of the Value of a Skin Test Using Polysaccharide in Cancer and Non-malignant Diseases (PROJECT TITLE)

7. John H. Tuohy and Murray Shear (PRINCIPAL INVESTIGATOR(S))

8. (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION:

Objectives: To examine the sensitivity and specificity of the test for human malignancy in which a small measured amount of bacterial polysaccharide is injected intradermally into the skin of humans with and without malignant disease.

Methods Employed: As indicated above.

Patient Material: None.

Major Findings: This study has been in abeyance during the reporting year due to the requirements of the Solid Tumor Chemotherapy Program.

Significance to Cancer Research: Implicit in the objectives listed above.

Proposed Course of Project: This study may be abandoned in view of the increased activity in the Solid Tumor Chemotherapy Program.

PROJECT REPORT FORM (cont'd)

10. NCI-706(C)
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] T AL ASSISTANCE [ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 195

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16

PROJECT REPORT FORM

N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

(SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5.NCI-707(C) (SERIAL NO.)

Evaluation of Blood and Plasma Water Content in Patients with Malignant Neoplasms.
PROJECT TITLE

John H. Tuohy
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives: To determine the relative difference, if any, between the moisture content of the blood of patients with malignant disease, patients with other forms of acute and chronic illness, and normal individuals.

Methods Employed: The chemical determination of moisture content of whole blood, serum, plasma, and tissue by the use of the Carl Fischer reagent.

Patient Material: None.

Major Findings: This project has been temporarily abandoned and was not active during the reported year.

Significance to Cancer Research: Carried to completion with a positive result, this would furnish a diagnostic test for malignant disease.

Proposed Course of Project: Indicated above.

PROJECT REPORT FORM (cont'd)

10. NCI-707(C)
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES - ITEMS 14, 15 & 16

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. Dermatology (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-708(C) (SERIAL NO.)

6. Dermatologic Research
PROJECT TITLE

7. E. J. Van Scott, Richard Reinertson, C. B. McCall, Jack Waite, Ross McCardle
PRINCIPAL INVESTIGATORS

8.
OTHER INVESTIGATORS

9. A. PROJECT DESCRIPTION - Statistical Incidence of Types of Pigmented Lesions on the Palms and Soles - E. J. Van Scott, Richard Reinertson, and C. B McCall

Objectives: To determine the incidence of pigmented lesions on the palms and soles, and to determine histological types of those occurring on these sites.

Methods Employed: Examination of palms and soles, of randomly selected group of individuals, for presence of pigmented lesions. Biopsy removal of random group of such lesions.

Patient Material: Random selection from patients in Clinical Center, employees, juvenile home.

Major Findings: Approximately 25% of over 700 individuals examined have been positive for pigmented lesions on the palms or soles. Approximately 45 lesions have been excised from 40 individuals and have been examined histologically. Over 50% of these lesions have been found to be "junction nevi."

Significance to Cancer Research: Since Pack reported that the incidence of nevi on the palms and soles is less than 1%, and since 4 to 12% of melanomas are said to occur on the foot, an accepted current therapeutic procedure is to prophylactically excise any pigmented lesion from the palms and soles on the rationalization that such lesions are unduly prone to develop into melanomas.

The data of this project questions the validity of such reasoning and opens for re-evaluation the role of the junction nevus in the development of melanoma.

Proposed Course of Project: This project is completed, pending the final compilation of the data and its review by statistician.

9. B. PROJECT DESCRIPTION - Relationship of Junctional Activity in Pigmented Nevi to Development of Melanomata - E. J. Van Scott and Jack Waite

Objectives: To determine incidence of activity, junctional or otherwise, of nevi in patients with and without melanoma.

Methods Employed: Nevi on the backs of three groups of patients will be numbered by a constant method. The three groups of patients will be:

a) Normals (patients without melanoma).
b) Patients with metastatic melanoma.
c) Patients with primary operable melanoma.

Randomly selected nevi will be excised from the three groups of patients for histological examination.

Patient Material: Patients hospitalized in the Clinical Center or followed in Admission and Follow-up Clinic.

Major Findings: Nevi from three individuals have been excised to date. No findings to report at this time.

Significance to Cancer Research: This project attempts to establish whether or not there is some generalized bodily influence which promotes malignant change in "benign nevi" found on the skin of patients with melanoma.

Proposed Course of Project: Continuation as outlined above.

9. C. PROJECT DESCRIPTION - Histologic and Histochemical Anatomy of Certain Skin Lesions, as Correlated with Gross Anatomy - E. J. Van Scott and Ross McCardle

Objectives: To establish the gross and microscopic anatomical changes which occur in the skin and its appendages under normal and pathological conditions.

Methods Employed: Punch biopsy specimens are serially sectioned and stained with different stains. Sections are examined microscopically. Balsa wood models of cutaneous structures are made from projections through a microscope of these structures; resultant models are 72 times normal size.

ICI-708(C)
SERIAL NO.

PROJECT REPORT FORM (cont'd)

Patient Material: Skin specimens obtained from patients in Clinical Center, Employee Health Service.

Major Findings: Reconstruction models thus far made:

Condition	No. of models
Normal hair follicle of back	2
Follicle involved with acne (back)	4
Follicle involved with basal cell carcinoma (back)	1
Normal follicle of beard	2
Beard involved with alopecia areata	2
Follicle of normal scalp	1
Follicle involved with alopecia areata (scalp)	2
Follicle involved with early male baldness	2
Total number of models	16

Correlation of microscopic changes with gross changes concurrently being made.

Significance to Cancer Research: This study relates to:

1. Elaboration of anatomical changes taking place in the skin at the time of development of skin cancer.
2. The study of controlled growth and quiescence of the germinative cells in the hair bulb.
3. The effects of the hormonal milieu on the growth of hair and sebaceous glands.
4. Suggests the possibility of the study of hair growth in tissue culture as a means of studying certain conditions which may affect cellular mitosis.

Proposed Course of Project: Continuation as outlined above.

9. D. PROJECT DESCRIPTION - Amino Acid Content and Enzymatic Activity of Skin and Mucous Membranes - E. J. Van Scott and Richard Reinertson

Objectives: Quantitative determination of certain amino acids and arginase activity in the various layers of normal and pathologic skin.

Methods Employed: Tissue to be analyzed includes epidermis and corium, mucous membrane of upper esophagus and vagina, and liver, obtained from autopsy material; also includes scales from patients with exfoliating skin diseases, malignant tissues from skin of patients with either primary or metastatic disease; hair, nails.

NCI-708(C)
SERIAL NO.

PROJECT REPORT FORM (cont'd)

Chemical methods:

1. Arginase: Method of Krebs and Henseleit, modified.
2. Cystine: Okuda titration of hydrolyzed tissue.
3. Arginine: Sakaguchi method, as improved by Sakaguchi.
4. Other amino acids: Paper chromatography of hydrolyzed tissue, elution of color from spots on paper and quantitative estimation by colorimeter.

Patient Material:

Major Findings: Determinations of arginase activity, arginine, cystine, and free sulfhydryl have been made on several specimens of psoriatic scales, plantar calluses, epidermis, and scales from exfoliative dermatitides. The amount of arginine in psoriatic scales has been found to be significantly less than that found in plantar calluses.

Method for analyses by paper chromatography is being refined.

Significance to Cancer Research: The amino acid pattern of total whole skin of animals has been reported to change when the skin undergoes malignant change. The amino acid content of the various layers of the skin is not known and particularly is it unknown for human skin. This project attempts to partially define the values for various chemical constituents in discrete layers of normal and abnormal skin. Changes in the chemical composition of cancerous skin may be accounted for by volume changes of certain layers of the skin rather than elementary changes brought about by the malignant growth per se. On the other hand, the actual chemical composition of specific cellular elements, or layers, may change under pathological conditions. This project attempts to clarify such points.

Proposed Course of Project: Continuation as above.

9. E. PROJECT DESCRIPTION - Chemotherapy of Skin Cancer and Skin Diseases by Iontophoresis - E. J. Van Scott

Objectives: Evaluation of local effect of drugs in skin tumors and skin diseases of substances introduced by electric current.

Methods Employed: Introduction of substances into skin by iontophoresis apparatus; observations of gross changes.

Patient Material: Incidental procedure on patients hospitalized for other major purposes.

Major Findings: No demonstrable effect has been shown to occur in normal skin, psoriatic skin, and in tumors of mycosis fungoides

NCI-708(C)
SERIAL NO.

PROJECT REPORT FORM (cont'd)

following the iontophoretic introduction of thallium, manganese, cobalt, methotrexate.

Significance to Cancer Research: Project attempted to illicit any direct effect of substances introduced locally into skin tumors.

Proposed Course of Project: Discontinuation as a discrete project.

PROJECT REPORT FORM (cont'd)

10. NCI-708(C)
SERIAL NO.

11.
BUDGET ACTIVITY

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16

PROJECT REPORT FORM

1 N.C.I. (INSTITUTE) 2 General Medicine Branch (LABORATORY OR BRANCH)

3 Acute Leukemia (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-709(C) (SERIAL NO.)

6. The Chemotherapy of Acute Leukemia
PROJECT TITLE

7 Emil Frei, III
PRINCIPAL INVESTIGATOR(S)

8 E. J. Freireich, James Stengle, Richard T. Silver, G. Lennard Gold
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION·

Objectives· To study the effects of chemical compounds on the course of acute leukemia.

Methods employed·

1. All patients referred to the Clinical Center with the diagnosis of acute leukemia are admitted to the study. (for participating hospital units see #12).
2. Objective criteria for the evaluation of disease activity have been developed and applied
3. One of the following methods for evaluating drug effect will be applied depending upon the degree of known antitumor activity·
   a) Pilot studies in a few patients with varying dosages for drugs of potential antitumor activity.
   b) Sequential analysis i e., administration of the drug to the number of patients necessary to establish with confidence limits that it is, or is not, influencing the course of the disease and.
   c) comparative studies of drugs of proven human antitumor activity in an effort to determine whether significant differences exist
4. Combinations and varying dosage schedules will be studied when laboratory evidence or theoretical factors suggest possible increased antitumor activity.

PROJECT REPORT FORM (cont'd)

Major Findings· 1. A preliminary review of our data indicates that combined Methotrexate and 6 Mercaptopurine therapy is not superior to either one above.

2. The evidence as to whether the administration of Methotrexate at less frequent intervals alters the therapeutic toxic ratio is inconclusive.

3. Pilot studies using Methotrexate at intervals frequent enough to maintain constant blood levels have revealed enhanced therapeutic effect

Significance to Cancer Research· Trial in humans of potentially effective chemical compounds is of obvious importance. Quantitative comparative studies designed to detect slight but statistically significant differences are essential to direction of drug development into profitable areas.

Proposed Course of Study· The most intense activity will obviously focus on the drugs that show major activity in the animal screen. Leads that develop as a result of studies by the pharmacology group such as dosage schedules routes of administration etc. will be exploited.

In an effort to increase the number of clinical trials other hospital units have been included in the study and it is hoped that more will be added.

PROJECT REPORT FORM (cont'd)

0 NCI-709(C)
SERIAL NO.

1.
BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

2.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 957

Department of Pediatrics
University of Buffalo
Buffalo, N Y.

Roswell Park M moria Institute
Buffalo 3, New York

3. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH.

NO ENTRIES FOR ITEMS 14 15 & 16

PROJECT REPORT FORM

1. NCI — INSTITUTE
2. General Medicine Branch — LABORATORY OR BRANCH
3. None — SECTION OR SERVICE
4. None — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-710(C) — SERIAL NO.

6. Intermediary Metabolism in Man
PROJECT TITLE

7. Charles G. Zubrod, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Alton Meister, M.D., Donald Tschudy, M.D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Intermediary Metabolism in Man

Objectives: At various times members of the nonclinical branches collaborate with the clinical group in an attempt to make precise biochemical studies on patients with known defects in intermediary metabolism, or to explore possible disturbances in intermediary metabolism in patients with neoplastic disease.

Methods: Such patients are admitted to the general medicine service and the details of the programs, and their integration with problems of patient care, are worked out by consultation between members of the general medicine staff, and the nonclinical investigator. The specific laboratory methods utilized are rather variable, and depend on the type of problem under study. In general, they have followed the pattern of simple exploration of the presence or absence in blood and urine of products of intermediary metabolism.

Patient Material: (1955 calendar year) At times it is possible to undertake studies in patients already on the service. At other times special recruitment is needed. For example: The desire of Dr. Meister to study the amino acid patterns in phenylpyruvic oligophrenia was led to such special recruitment.

Admissions:	No.	Average Stay Days
Children male	1	28
Children female	1	28

PROJECT REPORT FORM (Cont'd)

Major findings: These are reported by the separate laboratories collaborating in the studies.

Patients, known to have phenylpyruvic oligophrenia, were admitted on February 28, 1955 for studies to be carried out by members of this Section in collaboration with Dr. Samuel Bessman (Associate Professor of Pediatrics, University of Maryland Medical School, Baltimore) and Dr. Sidney Udenfriend (National Heart Institut

Administration of glutamine (7.6 mMole per kilo) led to a dramatic drop in phenylpyruvic acid excretion--to one-third to one-fourth of the control level.

a. Oral administration of glutamine and asparagine (7.6 mM/kilo) does not lead to dangerously high levels of blood ammonia nor to very great excretion of ammonia..

b. Blood levels of glutamine, 5 times the normal level, were maintained for about 3 hours.

c. Glutamine administration does not appear to have affected the excretion of phenylacetylglutamine, or of creatinine.

It may tentatively be su gested that the striking decrease in phenylpyruvic acid excretion after glutamine administration is due to transamination of this keto acid to phenylalanine.

Significance to CANCER Research: This program is of importance to the National Cancer Institute program because it represents an attempt to utilize directly the newest developments in the nonclinical branches, in the study of the natural history of neoplastic disease. In this way, the theory and the technique of biochemistry, tissue culture, biology of growth and radiobiology can be made available to the clinical studies at an early stage of development. It is hoped that from such active collaboration will come new leads as to the pathogenesis of neoplasia.

Proposed course of project: At the present time no specific projects are being carried on, since the ones proposed last year have either been completed or developed into full projects of their own.

PROJECT REPORT FORM (Cont'd)

0. NCI-710 (C)
SERIAL NO.

1. _____
BUDGET ACTIVITY:

RESEARCH	☐	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

2. From time to time patients will be studied in cooperation with other Institutes at the National Institutes of Health, but without specific allocation of other research funds.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

3. Does not apply
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. Solid Tumor Chemotherapy (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-711(C) (SERIAL NO.)

6. Assay of Compounds for Antitumor Effect by Injection into Skin Metastases
PROJECT TITLE

7. C. O. Brindley
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To develop a method of assay of compounds for antitumor effect in humans by the injection of these materials into subcutaneous tumor masses.

Methods employed: Needle and syringe injection of lesions; determination of antitumor effect by direct caliper measurement, comparative photographs and histological study. A patient having a remission of her malignant disease was studied by endocrinological survey and a search for immune response to the tumor was made by serological tests and by transfusion of this patient's blood or plasma to other patients with similar malignancy.

Patient material:

		No.	Average Stay Days
Admissions:	Adult males	0	52
	Adult female	7	
Outpatient:	Number of patients	10	
	Number of visits	20	

Major findings: The study so far has shown that (1) extensive histological change may be caused by antitumor agents before changes in size can be demonstrated by caliper measurement or changes in appearance occur, and (2) the quantity of antitumor agent which must be injected locally into tumor masses in order to cause regression in size of these masses is relatively large and approaches the systemically tolerated dose in some cases.

PROJECT REPORT FORM (cont'd)

One patient in the study had a complete regression of all metastatic lesions.

Significance to Cancer Research: For many agents the effects on biological systems and the antitumor effects in animals are not closely correlated with antitumor effects in the human. The number of agents to be tested is large. So a method whereby agents can be safely tested within a short period of time in humans would be useful. The possibility that the injection of subcutaneous metastases could be developed into such a method should be explored.

Proposed course of the project: For the past several months suitable patients for this study have not been available. The project has been temporarily stopped for this reason. When such patients become available in quantity, it is planned that the project will be resumed. The patient having the complete remission of her disease will continue to be studied for possible causes of this remission.

PROJECT REPORT FORM (cont'd)

10. NCI-711(C)
SERIAL NO.

11.
BUDGET ACTIVITY

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 195

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Project 700C was a similar study as carried on by
Dr. R. D. Sullivan.

NO ENTRIES FOR ITEMS 14, 15 & 16

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. Solid Tumor Chemotherapy (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-712(C) (SERIAL NO.)

6. A. Controlled Studies in the Chemotherapy of Solid Tumors
B. Preliminary Studies of Chemotherapeutic Agents on Human Solid Tumors
PROJECT TITLE

7. A. C. O. Brindley, B. I. Shnider, J. H. Tuohy
B. C. O. Brindley, B. I. Shnider, J. H. Tuohy
PRINCIPAL INVESTIGATOR(S)

8. None
None
R INVESTIGATORS

9. A. PROJECT DESCRIPTION - Controlled Studies in the Chemotherapy of Solid Tumors

Objectives: These are two-fold: (1) To test the validity of the experimental design of a cooperative randomized controlled study of chemotherapeutic agents on a comparative basis, and (2) to determine the relative effectiveness of a test chemotherapeutic agent against a standard drug in the treatment of a number of selected solid tumors in man.

Methods employed: (1) Determination of experimental design including the format of the cooperative study.
(2) Selection of patients.
(3) Selection of test and standard drugs and their administration according to an established protocol.
(4) Antitumor effect is judged only on the basis of objective measurements.
(5) Collection and synthesis of data from all cooperating groups.

Patient material: 1955 (April-November)

		No.	Average stay days
Admissions:	Adult male	20	90
	Adult female	31	90
	Children, male	1	7
	Children, female	0	--

NCI-712 (C)
SERIAL NO.

PROJECT REPORT FORM (cont'd)

Outpatients:
In Admissions & Follow-up Department
Patients 77
Visits 122

In home or outside hospital
Patients 20
Visits 20

Major findings: The study is now in its formative stage. At present Triethylene Thiophosphoramide as the test drug and HN_2 as the standard are being compared according to the design indicated above. Twenty-nine (29) patients have been, or are now in the study.

Significance to Cancer Research: By matching patients and the drugs being compared in a completely randomized manner, and by basing anti-tumor effect solely on objective criteria, this study represents an entirely new departure in the field of cancer chemotherapy. It is the first major effort to transfer the experience in experimental therapeutics in other fields to study of chemotherapeutic agents in malignancy. The cooperative endeavor of which this project is a part has achieved acceptance by the Clinical Panel of the National Cancer Chemotherapy Center. It is being suggested as a pattern of study for other cooperative groups. There is hope that in this way drugs showing promise in more preliminary studies can be critically and rapidly evaluated in large numbers of human cases.

Proposed course of project: In the course of the next calendar year, it is anticipated that five other institutions will join in this study following the experimental design originated by the Solid Tumor Chemotherapy Group of NCI at the Clinical Center.

Our own activities here will consist in increasing our experience and participation in this study by expanding our out-patient activities and bed capacity.

9. B. PROJECT DESCRIPTION - Preliminary Studies of Chemotherapeutic Agents on Human Solid Tumors

Objectives: To test rapidly agents which have shown promise in other studies (animal screening, pharmacological, etc.) for their toxicity and antitumor effect in humans.

Methods employed: Agents will be studied in selected patients using sequential analysis and objective criteria.

NCI-712(C)
SERIAL NO.

PROJECT REPORT FORM (cont'd)

Patient Material: (Estimated - calendar year 1956)
8 patients per agent x 5 agents = 40 patients
Average days/patient = 60 days

Patients participating in "A" may also be included in this study upon completion of their participation in the Controlled project.

Major Findings: None

Significance to Cancer Research: A logical part of a drug development program and sequel to animal screening is a rapid preliminary method for the assay of an agent's toxicity and anti-tumor activity in humans. It is a necessary preliminary to the final critical evaluation of any chemotherapeutic material.

Proposed course of project: As agents are furnished from intramural and outside sources, they will be systematically evaluated by this technique.

PROJECT REPORT FORM (cont'd)

10. NCI-712(C)
SERIAL NO.

11. A & B
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

A. Cooperative Groups:

By virtue of requested grants it is expected that the following 5 institutions will contribute to the cooperative study of which this project is a part.

Department of Medicine, University of Miami, Miami, Florida
Roswell Park Memorial Institute, Buffalo, N. Y.
Lemuel Shattuck Hospital, Boston, Mass.
Johns Hopkins Hospital, Baltimore, Md.
Georgetown University Medical Division, D. C. General Hospital, Washington, D. C.

B. None

13. A & B - None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (cont'd)

14. NCI-712(C)
SERIAL NO.

15. A & B -
PUBLIC OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

16.
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

A. Acceptance by Clinical Panel, National Cancer Chemotherapy Center and its establishment as model for other such projects.

B. None

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-713(C) (SERIAL NO.)

6. Energy Metabolism and IV Fat Emulsion
PROJECT TITLE

7. C. B. McCall
PRINCIPAL INVESTIGATOR(S)

8. Dr. Donald Watkin
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To measure the effect of hyperalimentation with intravenous fat emulsion on respiratory quotient and energy expenditure in patients with inoperable cancer.

Methods employed: After suitable training period, by an open circuit method employing high velocity low resistance values and Douglas bags, measure O_2 and CO_2 in expired air and calculate RQ, oxygen consumption, and calories/square meter/hour during three periods: 1) control, 2) while receiving IV fat, and 3) follow up after IV fat discontinued.

Patient material: Patients on Dr. Watkin's metabolic service admitted for IV fat study.

Major findings: Five individuals have been studied to date. There has been no consistent pattern during control period, precluding any statement as to the effect of the extent of the tumor.

Two patients during the second period (i.e. receiving IV fat) showed an abnormally low RQ to a level suggesting ketone body formation. There was no consistent BMR change. In one case fasting RQ was relatively high with subsequent fall while receiving IV fat.

One individual in follow up showed normal RQ.

PROJECT REPORT FORM (cont'd)

Significance to Cancer Research: This study may help in part to answer the question, whether one can protect a patient from his cancer by supplying calories and how is IV fat emulsion used by body.

In general, this type study might prove useful on a number of metabolic patients under a variety of conditions, such as, type alimentation, extent of tumor, and nutrition.

Proposed course of project: Finish the patients already started and add others until we have information as outlined complete on 6 patients.

PROJECT REPORT FORM (cont'd)

10. NCI-713(C)
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. NONE
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16

PROJECT REPORT FORM

1. N.C.I. — INSTITUTE
2. General Medicine Branch — LABORATORY OR BRANCH
3. Leukemia Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-714 (C) — SERIAL NO.
6. Iron Metabolism. — PROJECT TITLE
7. James Stengle — PRINCIPAL INVESTIGATOR(S)
8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: a) Does the iron metabolism of the patient with malignant neoplastic disease differ from that of the normal?
b) Is the leukemic cell or solid tumor competing for iron at the expense of normal body needs and may this be a partial explanation for the anemias characteristic of these diseases?

Background: Recent work in the National Cancer Institute (Price) indicates that certain mouse tumors have a high iron content. It is commonly noted in bone marrow observation that stainable iron is lacking during an acute exacerbation of leukemia but may reappear during remission. It has been reported (Simmons and Everett) that normal rat leukocytes incorporate considerable amounts of radioactive iron administered orally or parenterally.

Methods Employed: In leukemic patients serial serum iron and leukocyte iron determinations will be made and correlated with hemoglobin and leukocyte counts.

In vitro studies of normal and leukemic leukocyte uptake of radioactive iron will be made.

In solid tumor patients serum iron and hemaglobin values will be followed and surgical and autopsy specimens analysed for iron content.

Some of the above work will be carried out in cooperation

PROJECT REPORT FORM (cont'd)

10. NCI - 714 (C)
SERIAL NO.

11. _____
BUDGET ACTIVY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. _____
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. _____
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUND), IDENTIFY SUCH RESEARCH:

No entries for items, 14, 15, and 16.

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. Leukemia Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-715(C) (SERIAL NO.)

6. The Hemorrhagic Diathesis Associated with Acute Leukemia.
PROJECT TITLE

7. E. J. Freireich
PRINCIPAL INVESTIGATOR(S)

8. Emil Frei, III
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: a) To identify factors responsible for precipitating gross hemorrhage in already thrombocytopenic patients.
b) To study the effectiveness of fresh blood, platelet transfusion, and other blood products in controlling gross hemorrhage in leukemic patients.

Methods Employed: In vivo tests of bleeding tendency, in vitro tests of blood coagulation, clinical observation and measurement of gross bleeding.

Patient Material:

Major Findings: Major effort thus far has been directed toward setting up techniques and procedures.

Significance to Cancer Research: Hemorrhage ranks with infection as the two major causes of death in acute leukemia. At present no effective prophylaxis or therapy for thrombocytopenic hemorrhage in leukemia is available. Many of the new chemotherapeutic agents for leukemia as well as other tumors cause thrombopenia and bleeding. Any effective therapy of thrombopenic bleeding could greatly prolong life of acute leukemia patients as well as improve the effectiveness of chemotherapeutic agents.

Proposed Course of Project: Observations of coagulation mechanism will be made periodically in acute leukemia patients in an attempt to define any changes associated with the onset of clinical hemorrhage. Patients suffering from hemorrhage will be given fresh blood transfusions and other blood products and the effect on the clinical bleeding and coagulation mechanism assayed.

PROJECT REPORT FORM (Cont'd)

10. NCI-715(C)
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. Division of Biologics Control Blood Bank
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for items 14, 15 and 16.

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. Leukemia Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-716 (C) (SERIAL NO.)

6. Nutritional Aspects of the Therapy of Acute Leukemia
PROJECT TITLE

7. Emil Frei III and Emil Freireich
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To determine the effects of nutritional factors contained in animal tissues on the course of human acute leukemia.

Methods Employed: Various animal tissues are emulsified, analyzed for chemical composition and presence of infectious agents, and if tolerated by experimental animals, are administered to to acute leukemia patients. The course of the disease is followed by clinical observation of the patient and various hematological determinations on blood and bone marrow.

Patient Material: None.

Major Findings: Emulsified material has been prepared from hog tissues and stored at -50°C. Studies in animals are in progress and no evidence of toxicity has been found to date.

Significance to Cancer Research: The major approach to the therapy of neoplastic diseases has been and continues to be the use of cytotoxic agents. As a new approach, based on the assumption that the neoplastic cells are capable of maturing and returning to normal, nutritional non-cytotoxic factors will be used in the treatment of acute leukemia. The discovery of a factor or factors that would promote the maturation of leukemic cells would have immense significance.

Proposed Course of Project: The pig tissues now available, if found satisfactory after screening in animals will be administered to acute leukemia patients that have been proven refractory to conventional forms of therapy. This will be

PROJECT REPORT FORM (Cont')

administered by nasogastric tube and the course of the disease carefully observed. If any changes occur, further separation of component tissues will be used to identify the responsible factor. If no changes occur, other tissues and other routes of administration will be attempted.

PROJECT REPORT FORM (Cont'd)

10. NCI -716(C)
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. U. S. Department of Agriculture - Meats Research Division, Beltsville, Md.
Dr. Ellis and Dr. Hiner supplied and prepared hog tissues.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for items 14, 15 and 16.

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. Acute Leukemia (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-717(C) (SERIAL NO.)

6. Infections, Fever, and Host Resistance in Acute Leukemia (PROJECT TITLE)

7. Richard T. Silver (PRINCIPAL INVESTIGATOR(S))

8. Dr. John Utz, NMI Dr. Emil Frei Dr. John Fahey; Dr. Grace Beal, NMI: Dr. Robert Kolb L.B.C. (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives· 1. To obtain data relating to the natural history of fever and infections in acute leukemia.
2. To define the mechanisms of impaired host resistance to infection in acute leukemia.

Methods employed· Fevers of known and unknown origin have systematically been studied according to a previously defined protocol in over 30 patients with acute leukemia. Comprehensive bacterial and viral data have been collected and analyzed in conjunction with a group in the National Microbiological Institute.

In order to define the capability of leukemic granulocytes to phagocytize opsonocytophagic determinations have been made in 11 patients. The antibody response to 6 challenge antigens has been measured in 11 leukemic patients and in 7 "normal controls." Alterations in serum proteins during the immunization procedure have been measured by paper electrophoresis.

Major Findings: The tabulation of the relative incidence of fever of known and unknown origin in the leukemic patient has not been completed. Of major importance in problems of patient care is the finding that many fevers previously ascribable to leukemia per se, have been demonstrated to be due to a complicating and remedial bacterial infection. The marked susceptibility to bacterial infection does not seem to apply to viral diseases.

The opsonocytophagic tests have been concluded. No difference in the phagocytic activity of the mature polymorphonuclear cell in leukemic

PROJECT REPORT FORM (cont'd)

blood during active disease, bacterial infection or in hematological remission (as compared to normal) was noted. Phagocytosis by the more immature cells of the granulocytic series of both leukemic and normal blood is minima. This confirms previous reports in the literature. The susceptibility to infection does not bear a direct relationship to the absolute mature polymorphonuclear count, but rather to a ratio of mature poly/total WBC Significant differences in the response to challenge antigens and in the electrophoretic pattern of the serum proteins have not been observed, although final results must await completion of the immunization procedures in the remaining normal controls, and statistical analysis.

Significance to Cancer Research: In spite of the voluminous leukemia literature, there have been no comprehensive observations relating to fever and infection in these patients. Although there have been many individual studies pertaining to phagocytosis antibody response and electrophoretic patterns in leukemia there has been no study to the best of our knowledge, which has attempted to correlate all these parameters with serial clinical observations. Thus, it is hoped that information obtained from this study will augment our understanding of the natural history of acute leukemia and aspects of host resistance in general, and aid in the further improvement in the clinical management of the leukemic patient.

Proposed Course of Study: The present study should be concluded within the next 6 months.

PROJECT REPORT FORM (cont'd)

0 NCI-717(C)
SERIAL NO.

1
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

2.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

3
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH).

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. _____ (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-718(C) (SERIAL NO.)

6. Pulmonary Physiology
PROJECT TITLE

7. C. B. McCall
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION - Ventilatory studies on patients with airway, lung, or pleural involvement by neoplasm.

Objectives: To study and correlate changes in tumor size with pulmonary function and blood gas studies and clinical course.

Methods: Obtain baseline determinations of total vital capacity, timed VC, maximum breathing capacity, residual air, total lung volume, nitrogen rinse out, and fluoroscopy. These determinations will be repeated at suitable intervals and correlated with X-ray size of tumor, sputum, wheezes, chest pain and dyspnea.

Patient material: All patients on Solid Tumor Chemotherapy Program with suitable pulmonary lesions.

Major Findings: A major portion of my time in past five months has been devoted to first, learning techniques and determinations; second, learning their proper interpretation and evaluation and thirdly, calibrating instruments and developing more accurate methods. In addition, during this time I have answered 42 consults from NCI - these tests being especially valuable in patients before and after pulmonary surgery.

As to the above project, I have to date only baseline determinations from which no conclusions can be drawn.

PROJECT REPORT FORM (cont'd)

Significance to cancer research: Such studies may shed some light on the effect of various tumors and their growth on pulmonary physiology, particularly ventilation, and possibly provide some clue as to the cause of dyspnea.

Proposed course of project: Perform studies as indicated on all suitable patients admitted to Solid Tumor Chemotherapy Program.

PROJECT REPORT FORM (cont'd)

10. NCI-718(C)
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. Acute Leukemia (SECTION OR SERVICE) 3. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-719(C) (SERIAL NO.)

6. A Study of the Clinical Manifestations of Acute Leukemia
PROJECT TITLE

7. Emil Frei, III
PRINCIPAL INVESTIGATOR(S)

8. Richard T. Silver, Richard Swarm, E. J. Van Scott, Charles Wells
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To delineate etiologic and pathogenetic factors of the various manifestations of acute leukemia with particular emphasis on the oral, neurologic, hepatic, renal, and osseous lesions and the abnormal uric acid metabolism.

Methods employed: The comprehensive application and correlation of clinical, hematological, radiologic, chemical, microbiologic, and histologic methods to the above manifestations in patients with acute leukemia.

Major Findings: Though data concerning the above has been collected, it has not as yet been analyzed and correlated. It is apparent that multiple factors may contribute to these manifestations, e.g. the leukemic process per se, antimetabolite therapy, steroid therapy, infection, anemia, etc.

Significance to Cancer Research: A study of the natural and modified history of acute leukemia and its complications is of obvious importance to cancer research.

Proposed Course of Project: It is anticipated that some of the information gained from this study will have practical application in the clinical management of this disease. Therefore, at least portions of the study will continue indefinitely. Moreover, additional diagnostic and experimental procedures will be applied when it is apparent that the present methods are inadequate to explain the observed manifestations.

PROJECT REPORT FORM

10. NCI-719(C)
SERIAL NO.

11.
BUDGET ACTIVITY

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16

PROJECT REPORT FORM

1. N.C.I. — INSTITUTE
2. General Medicine Branch — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-720(C) — SERIAL NO.

Evaluat — PROJEC — LE: Amino Acid Metabolism in Human Subjects by Utilization of Parenteral Routes of Administration

John L. Fahey
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION -

Objectives: The general objective is to study the human metabolism of intravenously administered amino acids in normal and disease states. Specific objectives are (a) to establish the identity and amount of individual amino acids essential to achieve positive nitrogen balance, and (b) to approach the problem of determining the role of individual amino acids by observation of the effects of incomplete amino acid mixtures.

Methods Employed: Optically-pure l-amino acids prepared by the methods of Greenstein and obtained from him have been placed in aqueous solution and administered intravenously at a constant rate of infusion. By means of complete collection techniques metabolic balance data have been obtained.

Total nitrogen, ammonia nitrogen, urea nitrogen and alpha amino nitrogen have been determined on appropriate samples of urine, stool or plasma.

Patient material:

	No.	Total Stay
Admissions:	2	300

Major Findings: 1. Mixtures of pure l-amino acids containing all of the "essential" amino acids (Rose) were adequate to achieve nitrogen balance in the single malnourished adult maintained on a balance study.

PROJECT REPORT FORM (cont'd)

2. Omission of l-Arginine from the intravenous mixture was capable of producing convulsions and coma and, in preliminary dog experiments, has been lethal.

3. Administration of the toxic (arginine-deficient) solutions resulted in a marked rise in blood ammonia level of the dogs which coincides with the development of convulsions and coma. The rise in blood ammonia can be prevented by coincident or prior administration of l-arginine.

Significance to Cancer Research: Further information on amino acid disposition and nitrogen transfer within the intact subject will provide a better base from which to investigate tumor metabolism and the metabolism of the tumor-containing host. Clinically, development of means of managing the toxic effects of elevated blood ammonia levels would be important in the management of hepatic damage states and in conditions of alkalosis in which ammonium chloride administration is indicated. Safe, profitable intravenous administration of amino acids is important in the general medical care of many patients with neoplasm.

Proposed Course of the Project: A. Further investigation of the conditions determining l-Arginine requirement (i.e. anesthesia, rate of amino acid administration or ammonium administration, and possible similar protective action of other amino acids) are to be continued in preliminary dog studies. Extension of these observations to human subjects in normal and pathologic states is planned with special interest in hepatic dysfunction states resulting from cirrhosis, tumor invasion of liver, and from the presence of tumor in the host.

B. Further efforts to establish the requirements for other amino acids will proceed after clarification of the role of l-arginine.

PROJECT REPORT FORM (cont'd)

10. NCI-720(C)
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

Drs. Jesse Greenstein, M. Winitz and J. Birnbaum in the Laboratory of Biochemistry, NCI, are investigating amino acid requirements and metabolism in rats utilizing intra-peritoneal administration. Constant consultation and exchange of information as these studies have progressed have been of mutual benefit.

The Laboratory of Biochemistry has also prepared, and made available, the purified l-amino acids utilized in this study.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS,) IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 & 16.

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. General Medicine Branch (LABORATORY OR BRANCH)

3. Nutrition and Metabolism (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-721(C) (SERIAL NO.)

6. Metabolism of Nitrogen, Minerals and Vitamins in Subjects with Malignant Disease
PROJECT TITLE

7. Donald M. Watkin, M.D. and Donald P. Tschudy, M. D.
PRINCIPAL INVESTIGATOR(S)

8. Jesse L. Steinfeld, M D ; John L. Fahey, M.D.; and Donald P. Tschudy, M.D.; in addition, Residents Montague Lane and Charles B. McCall and Clinical Associates Wroth, Roush, Mohler, Gold, Silver, Schroeder, Fritz, Landau, Levine, Forkner, Schick, Goulian, Flick and Paton
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Investigation of the over-all and intermediary metabolism of protein, fat, carbohydrate, minerals, electrolytes, vitamins, calories and water in the natural course of malignant disease and in the response of the disease to nutritional, chemotherapeutic, endocrine, surgical or radiologic therapy.

Methods employed: The metabolic balance technique is used to identify changes in tumor and host by measuring quantitative differences between the intake and output of body constituents. Quantitative measurement of water balance together with analysis of expired air (Dr. McCall) are used to estimate energy expenditure. Radioisotope techniques are utilized to measure albumin turnover and red cell survival time (Dr. Steinfeld). Electrophoretic partition of plasma proteins is used to identify abnormal plasma protein patterns and observe changes during the course of a study (Dr. Fahey). Heavy isotope (N^{15}) techniques are used to study the metabolism of urea and uric acid (Dr. Tschudy).

Patient Material:

In patients January 1, 1955	1
In patients admitted January 1, 1955 - December 31, 1955	
Initial Clinical Center Admissions	18
Second Clinical Center Admissions	4
Third Clinical Center Admission	1
In patients other than above studied during 1955 -	11
Total Deaths	3
Total Autopsies	3

NCI-721(C)
SERIAL NO.

PROJECT REPORT FORM (cont'd)

Outpatients:

Screening	8
Follow-up	6
Research	1

Total patient days attributable to patients studied on this project-3692

Major Findings: Because of the large number of different studies which were completed under the general heading of NCI-701(C), a separate report on each study is given herein.

A. Nitrogen, albumin, mineral, electrolyte and energy metabolism during hyperalimentation by intravenously administered fat emulsion in subjects with malignancy and cachexia: Donald M. Watkin

Objectives: Quantitation of effects of excessive calories supplied by fat administered intravenously on host and tumor, evaluation of caloric hyperalimentation as a therapeutic measure; exploration of the relationship between energy metabolism, protein metabolism and tumor growth.

Methods employed: a) Metabolic balance technique utilizing liquid constant diets.
b) I^{131} labelled human serum albumin turnover.
c) Open circuit measurement of respiratory quotient (R.Q.) and basal metabolic rate (B.M.R.).

Patient Material: Two men and one woman with inoperable carcinoma, one man with sarcoma, and one man with post-gastrectomy cachexia.

Major Findings: All subjects maintained weight on liquid diets during control periods. Fat emulsion intravenously increased caloric intake by 1/3, resulted in weight gains, retention of protoplasmic constituents, and an increase in total albumin and rate of albumin turnover. R.Q.'s in patients with rapidly progressing carcinoma were so low as to suggest ketone formation during control periods. All patients showed low R.Q.'s during fat emulsion infusions. Side effects from infusion of emulsions were back pain with the initial infusion, BSP retention, and a moderate anemia during the infusion. A striking finding of general interest was the ease by which cancer patients can consume extremely high caloric intakes when the combination of liquid diets and intravenous fat emulsions are used. In one patient, hyperalimentation was associated with objective increase in the rate of tumor growth.

Significance to Cancer Research: Practically, these studies show the feasibility of feeding adequate calories to cachectic patients. From a theoretic point of view, they suggest the presence of a defect in carbohydrate metabolism in patients with advancing cancer. They show that fat supplied calories can be utilized to spare protein in cancerous subjects.

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SERIAL NO.

PROJECT REPORT FORM (cont'd)

Proposed Course of Project: The abnormality in carbohydrate metabolism suggested by R.Q. studies will be actively pursued. New emulsions of fat will be tried with the ultimate goal of providing adequate nutrition completely by the parenteral route and hence eliminating cachexia and starvation as a cause of death in cancer patients. Abnormalities of energy metabolism during hyperalimentation in cancer patients will be studied.

B. Renal excretion of uric acid at various plasma uric acid levels in subjects with leukemia and other malignancies: Donald M. Watkin

Objectives: To investigate the relations among the renal tubular reabsorption of uric acid, plasma uric acid level and uric acid production.

Methods employed: Renal clearance technique.

Patient material: Two patients with leukemia, two with multiple myeloma, and one with reticulum cell sarcoma. In two, serial studies were performed.

Major findings: One subject with chronic myelocytic leukemia studied before, during, and after treatment with myeleran demonstrated a depression in uric acid clearance with no change in plasma uric acid level during therapy followed by a marked increase in uric acid clearance, a decrease in the absorption of filtered uric acid, and a decline in plasma uric acid level after completion of treatment. This was accompanied by an increase in glomerular filtration rate, renal plasma flow and TmPAH. The reciprocal relationship between the tubular transport of uric acid and PAH is an incidental observation corroborating similar findings made by NHI workers utilizing rabbit kidney slices.

Significance to Cancer Research: Uric acid is in man the end product of purine metabolism. In leukemia and certain allied malignancies plasma uric acid levels are high. This level is determined in part by renal tubular reabsorption of uric acid, in part by uric acid synthesis, and in part by the release of uric acid from tissues undergoing destruction. The renal contribution to the maintenance of a plasma level must be known before any interpretation on uric acid production or release from tissue can be made.

Proposed Course of Project: Renal function studies designed to measure glomerular filtration rate, renal plasma flow, TmPAH, uric acid clearance and renal tubular transport will be conducted in subjects with leukemia or other malignancies associated with abnormalities in uric acid plasma levels. Every effort will be made to study these subjects serially before, during and after successful chemotherapeutic, endocrine, radiologic or surgical therapy.

NCI-721(C)
SERIAL NO.

PROJECT REPORT FORM (cont'd)

In addition, the information acquired will serve as guides to more elaborate studies of the uric acid pool and uric acid synthesis carried out with the aid of heavy or radio isotopically labelled uric acid and its precursors.

C. Vitamin B_{12}: Vitamin B_{12} metabolism in normal subjects of various ages and in subjects with malignant disease; Donald M Watkin

Objectives: Qualitative and quantitative observations on the over-all metabolism of vitamin B_{12}, its levels in blood plasma, its distribution in the bodies of normal subjects and those with cancer, and its excretion by the kidney.

Methods employed: Microbiological assay for vitamin B_{12} in blood plasma and urine. Measurement of Co^{60} labelled radioactive B_{12} in blood, urine, tissue and feces.

Patient Material: To date all studies have been conducted in roughly 250 presumably healthy individuals without known malignant disease.

Major Findings: Normal values for blood levels of B_{12} in various age categories have been established. Absorbtion of vitamin B_{12} from the G.I. tract after oral ingestion has been related to dose, age of subject and the subject's gastric acidity. Urinary excretion following various intramuscular doses of B_{12} has been quantitated for presumed normals aged 20 - 100 years. Renal clearance of B_{12} in various age groups has been quantitated at low and intermediate plasma B_{12} levels. Recent studies have demonstrated the mechanism of B_{12} clearance at high plasma levels.

Significance to Cancer Research: Vitamin B_{12} plays an indispensable role in the metabolism of all foodstuffs and in hematopoiesis. It has been used with reported success in treating neuroblastomas. Plasma B_{12} levels in leukemia are extraordinarily high. B_{12} antagonists are being developed.

Proposed Course of Project: The techniques for the microbiological assay using L. Leichm nii and for the analysis of Co^{60} labelled radioactive vitamin B_{12} are being developed. Bloods will be collected from all patients with cancer admitted to the Clinical Center, assayed for B_{12}, and analyzed electrophoretically for plasma protein. In selected patients, B_{12} distribution studies using the renal clearance technique will be performed. Distribution of radioactive Co^{60} labelled B_{12} in normal and tumor tissue will be determined in surgical and autopsy specimens.

NCI-721(C)
SERIAL NO.

PROJECT REPORT FORM (cont'd)

D. Leukemia: Donald M. Watkin

Objectives: Characterization of over-all metabolism in chronic and acute leukemia and the changes induced by anti-leukemic therapy.

Methods employed: Metabolic balance technique. Discrete renal function studies.

Patient Material: In 1954 three patients with chronic and one with acute leukemia. In 1955, one patient with chronic myelocytic and one with acute lymphocytic leukemia as in patients; one patient with chronic myelocytic leukemia as out-patient.

Major Findings: Myeleran therapy in chronic leukemia results in a marked early increment of phosphorus and uric acid excretion followed by a reduction in uric acid excretion to below-control values with continued therapy. Methotrexate therapy in acute leukemia induces an increase in phosphorus and uric acid excretion. 6-Mercaptopurine therapy was associated with a temporary diarrhea, although no definite evidence of a sprue-like syndrome could be proven. Hydrocortisone in combination with 6-MP induced a remarkable increase in uric acid excretion. 6-MP over a prolonged course reduced plasma and uric acid levels to extremely low values.

Significance to Cancer Research: These studies provide quantitative means of evaluating the rate of progression of the leukemic process and the extent to which this process may be altered by antimetabolite therapy. They point toward possible mechanisms of action of antimetabolites in leukemia.

Proposed Course of Project: Selected leukemic patients who are sufficiently well to cooperate in balance and renal studies will be studied with particular emphasis on the excretion of uric acid and on calcium and phosphorus metabolism.

E. Nitrogen Utilization: A Study of Nitrogen Utilization in Human Tumor Patients by Means of N^{15} Labelled Amino Acids. (D. P. Tschudy)

Objectives: To study the effects of malignant tumors on the organism with respect to over-all rate of protein synthesis, size of nitrogen metabolic pool, rate of incorporation of nitrogen into the tumor and other aspects of nitrogen metabolism.

Methods Employed: Synthesis of isotopically labelled amino acids, isolation of compounds from blood and urine, preparation of samples for mass spectrometer.

Patient Material:

	No.	Aver. stay
Admissions:	3	5

Major Findings: Tumors contain higher levels of isotopic N than serum proteins examined at the same time. Rate of labelling of urea and ammonia is about the same in tumor and non tumor

PROJECT REPORT FORM (Cont'd)

patients. Some cancer patients may excrete less isotope than normals.

Significance to Cancer Research: Following the mathematical model of Rittenberg and San Pietro we will quantitate the effects of cancer on the protein synthesis rate and size of the nitrogen metabolic pool in patients.

Proposed Course of Project: Data is being applied to various mathematical models for calculations of both rates and rate constants for the over-all organism with respect to nitrogen metabolism. The physiological validity of these models may be tested by direct experimentation and measurement of certain rates.

PROJECT REPORT FORM (Cont'd)

10. NCI-721(C)
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

A, B, C, D and E - none

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

A, B, C, D and E - none.

No entries for items 14, 15 and 16.

PROJECT REPORT FORM

1. N. C. I. (INSTITUTE) 2. General Medicine (LABORATORY OR BRANCH)

3. Metabolism (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA) 5. NCI-722(C) (SERIAL NO.)

6. Measure of Blood Volume, Red Cell Mass and Total Circulating Albumin in Patients with Cancer.
PROJECT TITLE

7. J. L. Steinfeld
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine whether there is a consistent decrease in patients in the various stages of carcinoma growth in the red cell mass, since conflicting reports in the literature state that there is and that there is not a decrease in the red cell mass in patients with carcinomas.

To determine whether the decreased concentration of serum albumin seen so frequently in patients with cancers is a result of an absolute decrease in albumin or is the result of an increase in plasma volume.

Methods employed: Blood Volume, Red Cell Mass and Plasma Volume are determined using the isotope dilution method with I^{131} albumin or red cells labeled with sodium chromate^{51}.

Patient Material: 80 Patients Admitted for other NCI clinical research programs.

Major Findings: Red Cell Mass was consistantly decreased in 80 studies in 60 patients with advanced carcinomas. Plasma Volume - in the absence of congestive heart failure - was within normal limits.

Recalculation of the conflicting data available in the literature using the concept of "body hematocrit" and a body hematocrit: peripheral venous hematocrit ratio of 0.91 reconciles the reports so that the available published data are consistent with the present findings at the NCI.

PROJECT REPORT FORM (Cont'd)

Significance to Cancer Research: In order to adequately characterize a metabolite it is essential to know not only its concentration (previously available as hemoglobin or albumin concentrations) but also its volume of distribution. To further characterize any metabolite the rate of production and rate of metabolism also must be determined, however, this project is concerned with only the first of the above two requirements.

Proposed Course of Project: To extend these observations to include more patients with cancer in various states (extent) of disease and to make repeated observations on the same patient throughout the course of his illness.

PROJECT REPORT FORM (Cont'd)

10. NCI-722(C)
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for items 14, 15 and 16.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. _____ — SECTION OR SERVICE
4. _____ — LOCATION
5. NCI 750(C) — SERIAL NO.

6. Removal of Cancer and Other Tissues for Histologic, Biochemical and Other Studies as Required by Scientists and Other Investigators for Correlated Studies. — PROJECT TITLE

7. Dr. R. R. Smith, Dr. J. H. Waite, Dr. W. E. Schatten — PRINCIPAL INVESTIGATOR(S)

8. Dr. A. Ship, Dr. R. Miller, Dr. W. Kramer, Dr. R. Milch, Dr. L. Cramer, Dr. H. Herbsman — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The object of this project is to supply human tissue for histologic, histochemical, biochemical and biophysical studies to scientists working in various branches of the Cancer Institute who require tissue for studies. As a rule, such studies are done as cooperative projects with various investigators but on occasions specific types of tissue are required for specific studies. For example, one investigator requested that specimens of skin and its associated neoplasm be made available for keratinization studies, and various muscle biopsies and/or biopsies of liver tissue under specific conditions have been requested. These materials are obtained during operations for standard therapeutic reasons.

Methods Employed:

Methods employed are standard surgical procedures.

Patient Material:

Material for this project is mostly obtained from patients hospitalized for study in other projects. An example of such a case is a patient with an islet cell adenoma of the pancreas. This patient was admitted and studied mostly by the Endocrinology Branch but material was used in a number of correlated biochemical and histologic studies. 4 patients with extensive melanomas and/or basal cell carcinomas were admitted especially to obtain tissue for special studies by investigators in other branches. It is difficult to differentiate patients admitted

PROJECT REPORT FORM (Cont'd)

NCI-750(C)
SERIAL NO.

Patient Material: (Continued)

specifically for this project. However, 127 minor procedures were performed for other branches of the Cancer Institute, and 62 minor operative procedures were performed for other institutes on patients admitted by those institutes to obtain tissue for biopsy and/or biochemical, biophysical or histochemical studies.

Major Findings:

Presented in reports of other projects by other investigators.

Significance to Cancer Research:

This project is more or less a service function for other branches of N.C.I. and N.I.H.

Proposed Course of Project:

The number of such cases will continue at about the present level in proportion to the patient load.

11. BUDGET ACTIVITY

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

(No entries for items 12, 13, 15 & 16)

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. _____ — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-751(C) — SERIAL NO.
6. Evaluation of Radical Surgery and the Development of New Surgical Techniques as a Therapeutic Means of Palliating or Definitive Therapy of Advanced Cancer. — PROJECT TITLE
7. Dr. Robert R. Smith — PRINCIPAL INVESTIGATOR(S)
8. Dr. John H. Waite - Dr. William E. Schatten — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objectives of this project are to develop methods of obtaining increased palliation and/or definitive cure of advanced cancer. Present day therapy depends upon the complete removal of cancer or its local destruction by physical agents; i.e., surgery, radiation, or chemotherapy. Salvage by any of these methods is low (15-30%), and points to the ineffectiveness of the tools that we now have available. Examples of this type are found especially in cancers of the pelvis and in the head and neck areas. Clinical material from these two areas is readily available.

Methods employed: Patients with advanced cancer of the pelvis and/or head and neck areas are admitted for study. These patients do not have dissemination of their disease beyond the operative area. This project is in reality composed of a number of projects of specific sites but because of the relative infrequency of cancer of specific areas, studies must be arranged to take advantage of the clinical material as it becomes available. In the report of a year ago, study of paranasal sinus cancer was mentioned. Seven patients with this type of cancer were admitted for surgery and in only two were conditions right for the more radical type of surgery to be performed. This surgery consisted of resection of the floor of the anterior cranial vault including the cribriform plate, contents of the nose, the orbit, and the involved antrum and/or hard palate. Twenty-eight primary

NCI-751(C)
SERIAL NO.

Methods employed: (Continued)
carcinomas of the cervix were admitted. The radical surgery consisted of pelvic lymphadenectomy associated with the radical hysterectomy and/or pelvic exenteration.

Patient material: As noted in the previous project reports there is considerable overlap of patient material; that is, a patient is used in more than one project. There were, however, 7 patients admitted for the primar antrum study and 28 patients admitted for study of cervix cancer. The total number of hospital days for this project was 1666 with 102 out-patient followup visits.

Major findings: Of the 28 cervix cases admitted, some of which were admitted and treated in the previous year but never reported, 17 pelvic exenterations were performed. An additional 8 patients had radical hysterectomy and node dissections, and 1 patient had a radical resection of a cervical stump cancer, plus radical iliac node dissection. Complete analysis of this group of patients is not possible at this time, mainly because a sufficient length of time has not elapsed since surgery to allow adequate evaluation. It is possible at this time to note that in our hands as the surgery is performed at this hospital the operation is relatively safe. There has been one postoperative death in this group. This patient died about ten days after radical pelvic exenteration. She died as a result of intestinal obstruction complicated by peritonitis. We feel that for the type of clinical material presented at this hospital the radical surgical approach to advanced pelvic cancer gives worthwhile palliation and increased longevity. More complete analysis of this group of patients should be available within the next year.

Of the 7 paranasal sinus cancer patients, only 2 of them were suitable for the radical resection described above. Both of these patients have done well and are still free of disease after a relatively short postoperative period. Our experience with this small number has shown that the operation is safe. It does not produce objectionable deformities and is well received by the patients. In 2 of the patients admitted for this study the disease was found to extend into the middle cranial fossa and definitive surgical procedure could not be performed. In one of the 7 antral cases, the patient died with disseminated cancer throughout his lungs and mediastinum. This patient had a radical resection of the maxilla but not the anterior cranial fossa. The other patient had a modified radical antrum resection and is free of disease at the present time. The 7th patient

NCI-751(C)
SERIAL NO.

Major findings: (Continued)
has had surgery performed only a short time and is not available for evaluation.

Significance to Cancer Research: We believe that the development of effective means of palliating and/or curing advanced cancer is one of the fundamental purposes of the Cancer Institute's program. Documentation of the course of cancer of these areas as the patients continue through the course of their disease adds considerably to the total knowledge of the natural behavior of cancer. It should also be pointed out that this project provides considerable material for both excised adjacent normal tissues as well as the cancer itself for scientists to use in other projects in the Cancer Institute.

Proposed course of project: During the coming year it is proposed to continue the studies as outlined above. It is anticipated that in the future as in the past, one of the main problems with pelvic cancer which requires the excision of the urinary bladder is a means to control the urinary stream. Studies are underway at the present time in which the ureters are placed in an isolated loop of bowel. This looks promising but is still not the ideal method of maintaining adequate urinary drainage. It is hoped that additional cases of advanced but locally operable paranasal sinus cancer will be obtained in order to further study the neuro-physiological changes involved in this type of radical resection. As in the past, it should be pointed out that the neurosurgical portion of paranasal sinus study was undertaken in conjunction with neurosurgeons from the Institute of Neurological Diseases and Blindness.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Surgery Branch (LABORATORY OR BRANCH)

3. _____ (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-752(C) (SERIAL NO.)

6. Clinical Investigation in the use of Viruses in the Treatment of Human Cancer. (PROJECT TITLE)

7. Dr. R. R. Smith and Dr. R. J. Huebner (PRINCIPAL INVESTIGATOR(S))

8. Dr. W. E. Schatten, Dr. L. B. Thomas and Dr. W. P. Rowe (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives:

To develop means of treating human cervix cancer using culture fluid containing live virus, attempting to reproduce in vivo the in vitro observation of the destructive action of viruses on cells in tissue culture.

Methods Employed:

APC (adenoid-pharyngeal-conjunctival) viruses grown in 5% chick serum in Hanks-Simms solution containing HeLa cells was filtered through a sintered glass filter and tested for sterility and safety. This fluid was then injected by direct needle injection into the cervix tumor mass via the vagina, and from above directly into the mass, or by intra-arterial cannula passed up through the femoral artery into the lower abdominal aorta. It was necessary to determine the antibody levels of the serum of the patients injected to make sure that the proper virus was used. Following the injection it was necessary to obtain culture swabs from the vaginal fluid and/or biopsy tissues or cervical scrapings. Blood cultures were taken regularly. The patients were thoroughly studied for evidence of systemic manifestation of a virus disease. At the start of this investigation, it was determined that using this agent in susceptible individuals, a local necrosis could be produced without the production of any recognizable systemic disease. In a number of instances, following the use of the local chemotherapeutic agent, more definitive surgical therapy was instituted. In 8 patients radical pelvic exenteration was possible following the virus therapy. In an additional 3, radical hysterectomy and node dissection was performed. These were standard operative procedures which have been modified as described in Project 751(C).

PROJECT REPORT FORM (Cont'd)

NCI-752(C)
SERIAL NO.

Patient Material:

During the past twelve months 19 patients were admitted especially for this study, for a total of 2242 patient days. 35 out-patient visits were made by this group. As pointed out before, this patient material was available for a number of correlated biochemical and histological studies as well as the radical pelvic surgery study as described in Project 751(C).

Major Findings:

To date, 28 patients have received virus fluid for therapeutic studies of their cervix cancer. An additional 6 patients received control material consisting of the tissue culture fluid containing everything but the virus.

The findings during the past year have confirmed those given in the report last year; that is, patients whose sera does not contain antibodies to APC virus develop an area of necrosis at the site of injection; when antibodies are present such necrosis has not been observed. In 24 patients that have been more thoroughly evaluated, injection of these viruses in amounts up to 400 cc. of culture media did not produce appreciable systemic disease. On three occasions, an influenza-like disease was produced, consisting of general malaise, photophobia, and fever. These symptoms disappeared without therapy in a few days. There was no pharyngitis or conjunctivitis as observed in upper respiratory infections caused with APC viruses occurring spontaneously. Local necrosis progressed to extensive cavity formation in the vaginal pelvic portion of the cancer in 20% of the patients. There was moderate slough of the lesion in 52% of the patients, in 20% a slight to questionable necrosis was observed, and in 8% no response was observed. Injected virus was recovered from tissue biopsies and/or vaginal smears as late as 17 days after the treatment. A prompt antibody rise was observed in all cases. Extensive cyto-histologic studies of repeated biopsies and smears failed to show any specific recognizable effect that could be attributed to the virus. Increasing amounts of necrosis were observed on biopsies taken during the period of slough but no inclusion bodies or other specific viral effects were demonstrated. In no instance was there complete destruction of the cancer observed. Regrowth of the tumor was observed in the area of slough as early as 10 days.

It soon became apparent that the drug did produce local changes which we are convinced have a specific effect upon the cancer tissue. No observable effect has been noted on the other tissues of the body. It is apparent, however, that the prompt rise of antibodies prevents the continued growth of the virus in the tissue, thus limiting its effectiveness as a destructive agent. Attempts have been made to use cortisone in increasing amounts. This did not prevent the rise of antibodies. Its effect on the local lesion in conjunction with the virus used remains to be settled. It did seem, however, that the best results of the virus were obtained in patients that were closer

PROJECT REPORT FORM (Cont'd)

NCI-752(C)
SERIAL NO.

Major Findings: (Cont'd)

to the terminal portion of their disease; that is, that they were debilitated and more seriously ill. This suggests that the nonspecific defense mechanism of the body is probably a factor in the limitation of the use of this agent as it exists today.

Significance to Cancer Research:

This program helps to carry out one of the fundamental aims of the Cancer Institute, that is, of developing new and more effective methods of therapy of common malignant neoplasms. It also helps to identify and categorize the newly discovered APC viruses and helps to delineate the reaction on human tissue of the virus used in the experiment. If a method can be developed to allow the propagation of this virus in the tumor tissue, it is reasonable to expect that the continued propagation, not only locally but in the disseminated disease, might be developed into an effective means of controlling cancer.

Proposed Course of Project:

During the coming year it is hoped that this project can be pursued more vigorously. During the past year and a half it was recognized that this was a new approach and that caution was necessary to prevent misunderstanding in using human material in this type of study. We are sure now that this material is not harmful, that it does not produce aggravation of the growth of the neoplasm, and that we are doing these patients a service by studying them and providing the care that is available. It is believed that the course the project should take is as follows:

(1) As noted in the in vitro study of the effect of this virus on HeLa cells, the degree of destruction is directly proportionate to the concentration of the virus present in the fluid. It is hoped that this virus can be concentrated 100 to 1000 times so that tremendous amounts of this material can be given to the patients in a smaller volume of fluid. This is a problem in production of virus which is being studied.

(2) The serial use of APC viruses whose antigenicity is not exact and will allow the use of multiple viruses through multiple injections to produce the same type of effect.

(3) Continued studies to alter the host resistance to infections in an effort to produce a viremia which would allow contamination of all of the cancer cells and a more complete destruction.

(4) Repeat the histologic study in an effort to definitely determine the mode of action of this material.

PROJECT REPORT FORM (Cont'd)

NCI-752(C)
SERIAL NO.

11. BUDGET ACTIVITY

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

(No entries for items 12, 13, 14 and 16)

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. ____ — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-753(C) — SERIAL NO.

6. Cytologic Determination of Number of Tumor Cells and/or Recurrences Recovered or Found in an Operative Wound Following Removal of a Primary Cancer in Continuity with its Regional Lymph Drainage Area Containing Metastases. — PROJECT TITLE

7. Dr. R. R. Smith — PRINCIPAL INVESTIGATOR(S)

8. Dr. A. W. Hilberg, Dr. J. H. Waite and Dr. W. E. Schatten — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

This project is the clinical part of Project No. 754 dealing with the seeding of operative wounds by cancer cells. Its primary objective is to demonstrate the frequency and the nature of tumor cells found in the washings obtained from operative wounds from which operable cancers have been removed.

Methods Employed:

Patients with locally advanced primary cancers of the head and neck, pelvis, or other appropriate sites, with regional lymph node metastases, have been admitted for primary surgical therapy. For the most part, these cases have been those with extensive metastases, in other words, the borderline operable ones. The surgery performed is mostly the standard operative procedures accepted as treatment for the specific cancer sites. In part, these same patients provided material for Project 751(C) in which the limits of operability have been further defined by a more extensive resection with and without primary reconstruction.

Following the removal of the tumor and its metastases, the operative area is thoroughly washed with a fine saline spray. The aspirate is fixed in an alcohol-ether mixture and in the Pathology Lab is centrifuged down, the sediment being treated by laking the red cells which are present in the fluid. The remaining sediment is then smeared and studied in the usual Papanicolaou technique and/or clumped together in a block and the usual histologic sections prepared.

PROJECT REPORT FORM (Cont'd)

NCI-753(C)
SERIAL NO.

Patient Material:

25 patients were admitted specifically for this project for a total of 1737 hospital days. 100 outpatient visits were made by these patients. It should again be pointed out that nearly all these patients were used in other studies, and in some patients admitted for other projects, wound washings were taken. This was demonstrated by the fact that during the calendar year of 1955, 72 different patients had wound washings studied in the Cytodiagnostic Laboratory.

Major Findings:

Complete analysis of all 72 cases is not available at this time. The results of a study of 36 of these cases have been tabulated and was published in the December 1955 issue of the J.N.C.I.

In summary of the 36 cases, wound washings were positive in 9 instances and in an additional 5 cases the washings contained suspicious cancer cells. When the wound washings were positive, local recurrences had already developed in 2 of these patients and in an additional case in which the washings were suspicious. In 2 instances where the washings were negative, local recurrences had already occurred.

The finding of clumps of tumor cells in 25% of this small series would certainly make one suspect that this figure would represent a minimum number of cases that could be expected to demonstrate local recurrence of tumor. However, it should be pointed out that the finding of tumor cells in a wound is not necessarily assurance that a recurrence will develop. It is possible that washing of the wound would destroy the implants, and the host factor which allows the recurrence to develop could possibly control the remainder. On the other hand, the finding of negative wound washings would be no guarantee that recurrences would not develop because of the difficulty in assuring ourselves that the washings are truly negative.

The important point of this work to date has been that malignant cells in wound washings can be relatively easily identified. Most of the washings that were examined contained no isolated tumor cells but small fragments which could be readily identified. In one instance this contained a piece of cancer one-tenth of a millimeter in diameter. Cell structure could very easily be identified in this case. In all instances cells or clumps of cells seen in the wound washings were comparable when compared with the tissue biopsies of the surgical specimen. In all cases in which the washings were considered positive, the tumor cells identified corresponded in morphological characteristics with the tumor cells seen in the tissue section.

NCI-753(C)
SERIAL NO.

Significance to Cancer Research:

Demonstration of cancer cells in operative wounds again points up the deficiency in our present methods of cancer therapy. On the positive side it demonstrates a possible cause of failure of this type of therapy, and certainly suggests a means of approaching the problem from a chemotherapeutic standpoint.

Proposed Course of Project:

It is proposed to continue this study as outlined above, further delineating the frequency and the characteristics of a seeded wound. It is hoped that this series will be allowed to increase to the point where it will be a statistically significant one. It will be necessary to continue to follow the patients that have been so treated over the next 2-3 years to further determine the ratio of local recurrence and failure of therapy to the finding of tumor cells in an operative wound.

It is hoped that within this next year the results of the laboratory part of this experiment (Project 754) will have progressed to the point where chemotherapy of operative wounds can be brought to the operating room.

11. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]
REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Paper entitled "Cancer Cell Seeding of Operative Wounds"
By: Robert R. Smith and Albert W. Hilberg
Published in J.N.C.I., Vol. 16, No. 3, December 1955

(No entries for Items 12, 13 and 16)

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. ____ — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-754 — SERIAL NO.

6. Evaluation of the Nature, Cause, and Means of Preventing Seeding of Operative Wounds Using Transplantable Animal Tumors. — PROJECT TITLE

7. Dr. Robert R. Smith — PRINCIPAL INVESTIGATOR(S)

8. Mr. Richard V. Eck - Dr. John H. Waite - Dr. Ross Miller - Dr. Arthur Ship - Dr. William Kramer — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The objectives of this project have been broadened during the past year from methods of producing experimentally in animals multiple recurrent tumors in operative wounds, to the establishment of the standard experimentally seeded operative wounds which provides methods of testing the efficacy of various therapies aimed at preventing the wound seeding.

Methods employed:

As pointed out in Project No. 753(C) a frequent finding in radical surgical therapy of cancer is the presence of tumor implants in the operative wound. In trying to evaluate the effectiveness of any treatment applied to an operative wound, and in order to demonstrate the safety as well as the effectiveness of any given chemotherapeutic regime, it is believed necessary to develop in the animal a situation which is comparable to that in the human. This would allow a statistical analysis of a given form of therapy over a short period of time. In order to develop this experimental tool the V^2 carcinoma in rabbits has been utilized to produce in our hands a seeded operative wound which in practically 100% of the cases produces multiple tumor implants in the operative wound within a 2 to 3 week period. K^2 ascites tumor injected intraperitoneally produces numerous implants in the peritoneal cavity. S-91 mouse melanoma produces multiple implants in the lungs when injected intravenously or subcutaneously in that area. Mr. Richard Eck has developed a method of raising an air bubble on the back of a mouse by injecting air in a subcutaneous pocket. Tumor suspension injected into this area, following which

PROJECT REPORT FORM (Cont'd)

NCI-754
SERIAL NO.

Methods employed: (Continued)

the air is removed, produces a wound with multiple implants. With V^2 carcinoma, dissection of the axilla and removal of the pectoral muscles produces a setting very similar to that seen in the operating room in which a radical mastectomy was done.

During the past year the procedure has been perfected to the point where the three types of tumor described above have been standardized so that in our hands they produce consistent results. The three transplantable tumors provide the opportunity to test the cancer study qualities of a compound or a procedure in vitro as well as in vivo. By mixing in the test tube the compound to be tested with the inoculum, allowing it to remain in contact for a stated period of time and then applied in the air pocket or intravenously or intraperitoneally, would allow a fast screening method to determine the carcinolytic properties of a given compound.

Another factor which requires study is the safety of the use of a given compound on tissue. It is believed that any compound that would be of any value clinically, must not damage to any degree large blood vessels or adjacent nerves. Any extensive necrosis of normal tissue would contraindicate its use in such a study. To test the efficacy of this a system has been developed whereby the brachial plexus of the mouse and rabbit is exposed and the chemical under study is applied directly to the brachial plexus and observations made in this manner. An additional test for the safety of the drug under study was obtained by washing the operative wound on one side of the animal and leaving the opposite side contaminated with tumor tissue to grow untreated. In most instances these animals acted as their own control.

Major findings:

1. Using the methods outlined above it has been shown that the washing of the wound with a saline spray caused a decrease in the number of implants that grew, but in no instance was it possible to prevent the development of tumor implants by the spray washing or blotting of the wou with saline.
2. A group of animal tumor implants were tested with podophyllin resin drugs, podophyllin, and alpha peltatin. Toxicity studies showed that over 10 mg. per kilo of alpha peltatin produced severe generalized toxic manifestations and local toxicity consisting of damage to nerves was apparent with as little as 1 milliliter of 5 mg.% alpha peltatin producing nerve paralysis. More concentrated solutions of the drug produced a coagulating type of necrosis involving the entire axilla. In 16 animals in

PROJECT REPORT FORM (Cont'd)

NCI-754
SERIAL NO.

Major findings: (Continued)

which the wound was washed with alpha peltatin solution, allowing the drug to remain in the wound, the tumor continued to grow luxuriantly in all dilutions well into the range where toxic symptoms occurred. In fact, it was the observer's impression that in certain instances the alpha peltatin seemed to cause the tumor, if anything, to grow better on the treated side as compared with the controlled side.
3. Additional chemicals - NCI - 1136, 3022, 1894, podophyllotoxin, alpha peltatin and crude podophyllin resin, were all ineffective in reducing the number of lung tumors in S-91 melanoma injected intravenously. In some experiments these compounds caused an increase in the number of lung tumors up to 100%. The possibility of this being due to a stress phenomena seemed to be borne out when the same results were obtained with heat stress, cortisone or systemic formaldehyde stimulation. The possibility of using systemic podophyllin-type drugs in conjunction with local washing was tried but inconclusive results were obtained. Because of the extensive local tissue damage it was suspected that this drug in its present form has very little to offer at the clinical level at this time.
4. Formaldehyde seems to be the most effective means yet available to prevent the seeding of operative wounds. Toxicity studies in mice and in rabbits as outlined above show that in mice 1% formaldehyde left in contact with the brachial plexus for five minutes produced no symptoms. In rabbits, using a ½% formaldehyde, no damage could be demonstrated to the brachial plexus in the 11 animals tested. 1% solution left in contact with nerves for 20 minutes produced nerve damage. ½% formaldehyde solution prevented the growth of tumor implants in 3 of 5 animals tested and allowed a single implant to develop into others. In saline control animals, consistent results were obtained in growing large numbers of implants in the area.
5. 27 different chemicals have been tested, both *in vivo* and *in vitro* studies. The ones that show promise of results and possible application at the clinical level are (1) citric acid, a 1% solution which lowers the pH to 2 which increases to 2.5 in contact with tissues. A 1% solution was between 90% and 98% effective in preventing the growth of K^2 ascites tumor. At the other end of the spectrum, sodium carbonate with a pH of 11.2 dropping to 10.9 when in contact with the tissues produced no appreciable nerve injury when left in contact with the plexus for 5 minutes, and was 100% effective in the prevention of growth of the K^2 ascites tumor. (2) Ethanol, a 23% solution was also 100% effective in the *in vitro* test. It has not been tested as yet in the actual operative wound seeding.
6. Hypertonic solution, such as 10% sodium chloride, is also effective in diminishing the number of tumor implants.

PROJECT REPORT FORM (Cont'd)

NCI-754
SERIAL NO.

PROJECT DESCRIPTION (Continued)

Significance to Cancer Research:

With the development of methods to artificially create a seeded operative wound it has been shown that a standard formaldehyde solution produces results which will, if applicable to humans, greatly increase the effectiveness of cancer therapy as it exists today. The immediate applicability of this type of procedure to the clinical cancer problem as it exists offers opportunities for immediate benefits even though the ultimate answer for cancer will probably not reside in this study.

Proposed course of project:

It is proposed to continue the studies of delineating the effects of these chemicals on seeded wounds, seeking to find the ideal chemical which will prevent 100% the seeding of an operative wound and at the same time leave the wound in a condition which will allow primary healing.

It is recognized that even though therapy of an operative wound would become 100% effective in preventing the development of tumor implants, therapy of cancer would still not be 100% effective because of the dissemination of the tumor implants which occurs at the time of surgery or shortly thereafter. It seems reasonable to expect that the ideal therapy would entail a drug which could be used systemically and would be effective in preventing the implantation of lung or liver metastases, and locally to prevent the implantation of tumor cells in the tissues of the operative wound. Dr. William Kramer is investigating the use of colchicine-like drugs and Dr. Ross Miller the use of several arsenic compounds to try to fulfill these criteria.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Surgery Branch (LABORATORY OR BRANCH)

3. (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-755(C) (SERIAL NO.)

6. The Development of New Methods for Treatment of Lung Cancer Using Regional Chemotherapy: Clinical Research (PROJECT TITLE)

7. Dr. John H. Waite (PRINCIPAL INVESTIGATOR(S))

8. Dr. William Kramer (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives: The chief purpose of this project is to improve treatment of those cancers of the lung which are found to be beyond hope of surgical cure by resection.

Methods employed: The plan of attack is one of combined surgery and chemotherapy. When open thoracotomy reveals a tumor not amenable to surgical resection, catheters are implanted in the vascular channels supplying the diseased lung, and exteriorized through the chest wall. After the patient has recovered from his thoracotomy, a potentially chemotherapeutic agent such as nitrogen mustard is injected through the catheter. Serial measurements of the size of the x-ray shadow cast by the tumor are made. Changes, if observed, are compared with the changes in other lesions in untreated portions of the lung fields if other lesions exist. Although the project was conceived primarily to get a high dosage into primary lung carcinoma, a certain percentage of which have been shown to be slightly susceptible to general chemotherapy, it was also planned to study metastatic tumors when discovered at open thoracotomy and unsuitable for surgical excision.

Thus far, we have followed the policy that it would be unfair to subject patients with definitely known inoperable tumors to the risk of thoracotomy when the possibility of benefit from regional chemotherapy is as yet unproven.

NCI-755(C)
SERIAL NO.

Methods Employed: (Continued)

Therefore, we have carefully worked up every case before surgery, rejecting as unsuitable those patients with obviously non-resectable tumors. Most of these non-explorable patients are placed on a suitable study program in the General Medicine Branch while receiving palliative x-ray therapy. Lung tumors found to be resectable at open operation would of course be given the benefit of the best excisional surgery available, thus making this sub-group also unavailable for regional chemotherapy studies. The excisional surgery patients would contribute to lung cancer studies by stimulating our thoughts toward better surgical procedures, by being available for wound seeding studies under Project no. 753(C), and as subjects for early diagnosis studies.

One of the major points to be worked out is the exact placement of the catheters with regard to the blood supply to the neoplasm. Three sets of vessels, viz, the pulmonary vein, the pulmonary artery and the bronchial arteries, are available. Experiments under Project no. 756, Dog Research in Catheter Implantation, are being used to develop a technique for these different implantations.

Patient Material:

14 patients have been studied during a total of 20 admissions. 15 of these admissions were during the current calendar year.

		No.	Average Stay Days
Admissions:	Adult males	9	60
	Adult females	5	60
Outpatient:	Number of patients	20	
	Number of visits	45	

Major Findings:

Seven patients have been found to be suitable for the study in that workup confirmed the presence of lung tumor and possible curability by excisional surgery. Of these seven patients, two proved to have a bronchogenic carcinoma suitable for pneumonectomy, and hence not available for catheter implantation. No primary bronchogenic patients have yet been found in whom thoracotomy was indicated, but who were found inoperable.

In five patients metastatic tumors were found. Two of these metastases were apparently solitary and it appeared best to treat these patients by excisional surgery which was accordingly performed. This left three patients with metastatic

PROJECT REPORT FORM (Cont'd)

NCI-755(C)
SERIAL NO.

Major Findings: (Continued)
tumors available for catheter implantation, the catheter being implanted in the pulmonary artery in two cases and in the pulmonary vein in one case. Nitrogen mustard injected daily has been the chemotherapeutic agent studied so far to establish base line values. One patient, with metastatic adenocarcinoma from a colon primary showed no apparent change in the size of her tumor until the time of death from a cerebral metastasis six weeks later. The second patient, also with metastatic adenocarcinoma from a colon primary, has shown a slight regression of the treated pulmonary metastasis during the same time that a new metastasis has appeared in the contra-lateral lung and grew constantly. Perfect access to the pulmonary artery bed was maintained in this patient for the nine months that he lived after catheter implantation. Both of these patients have shown minimal bone marrow changes compared with the changes usually seen after intravenous administration of nitrogen mustard. In one patient, with metastatic cancer from the cervix uteri, the catheter was implanted in a radical of the pulmonary vein and the pulmonary vein tied off between catheter implantation site and the heart with a thought to gain access thereby to the bronchial vascular bed. On injection of nitrogen mustard into this patient, she developed an abscess of the treated portion of the lung which appeared to start first at the tumor site but later spread to the entire lobe requiring subsequent lobectomy. Seven of the 14 patients worked up have been not suitable, because of proven metastases, or because of the disease found was other than a suitable carcinoma. Three of the four far-advanced patients were found suitable for metabolic studies on Dr. Watkin's service.

Significance to Cancer Research: Continuing increase in the incidence and death rate due to lung cancer demands maximal efforts to control the disease. The primary or basic science approach involves efforts to determine the biologic nature and environmental relationships of lung cancer. While of great hope, this research has yet to produce applicable results. A second phase is seen in programs aimed at the discovery and treatment of lung cancer in its earliest stages. This attack has succeeded in curing up to 5% of all patients seen by extirpative surgery, the only curative treatment now known. The third phase of lung cancer research involves improvement of treatment of the disease when it is past its earliest stages, and the large number of well-advanced lung cancer patients now seen provides an urgent motivation for the clinical investigator to search for an approach such as described in this project.

NCI-755(C)
SERIAL NO.

9. PROJECT DESCRIPTION (Continued)

Proposed course of project: Thus far it has been shown that catheter implantation into the pulmonary vessels is a feasible procedure and that it provides accurate access to the vascular bed for a prolonged period of time. It is planned to continue the study as outlined, using patients with operable metastatic and bronchogenic carcinomas. It is hoped to get a number of bronchogenic carcinomas to test this method inasmuch as this is the type of tumor which has been shown to be slightly sensitive even to intravenous administrations of mustard. We are also considering whether it might not be justifiable to perform thoracotomy with catheter implantation even on patients with clinically inoperable tumors, inasmuch as so little of honestly therapeutic value is available to this group today.

A new method of gaining direct access to the bronchial artery bed is under investigation in the animal laboratory, Project 756, and may be available soon for clinical evaluation.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Surgery Branch (LABORATORY OR BRANCH)

3. (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-756 (SERIAL NO.)

6. Development of New Methods in Treatment of Lung Cancer using Regional Chemotherapy: Laboratory Research (PROJECT TITLE)

7. Dr. John H. Waite (PRINCIPAL INVESTIGATOR(S))

8. Dr. William Kramer - Dr. William Banfield (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives:

This has been a key project for the development of techniques which were later used in the human lung cancer project 755(C). Using the dog as an experimental animal, various methods for the catheterization of pulmonary vessels at open operation were studied, in an effort to potentiate the known effect of parenteral nitrogen mustard on bronchogenic tumors and to provide a satisfactory route for evaluation of new possible chemotherapeutic agents as they are developed.

Methods employed:

Fine polyethylene catheters were introduced at thoracotomy into a bronchial artery or into a branch of a pulmonary artery or pulmonary vein. The catheters were brought out through the chest wall and allowed to remain in situ for a long period of time. The continuing patency of the catheters was tested and possible deleterious effects on the surrounding tissue were watched for. By injecting diodrast, serial angiograms were obtained under various experimental conditions and studies on approximately 50 dogs. It was also attempted to determine whether the differential damage to normal dog lung structures could be accomplished by the injection of extremely high doses of nitrogen mustard.

Major findings:

Implantation of catheters at open thoracotomy was perfected. Catheters can be left in for periods of time as long as six months providing continued access to the pulmonary circulation. The feasibility and harmlessness

NCI-756
SERIAL NO.

Major findings: (Continued)

of the catheterization technique was demonstrated satisfactorily, enabling us to bring it to the human project. Previous experiments had shown that differential damage to the bronchial mucosa could be obtained by injection of large quantitities of mustard directly into the bronchial arteries, which vessels are known to be the direct blood supply to bronchogenic carcinoma in humans. Direct application of this technique to humans did not seem possible because of the variability of bronchial vascular supply in humans, and because of the likelihood of finding difficulty in the dissection of finer hilar structures in advanced carcinoma of the bronchus. We have continued the studies with attempts to gain access to the bronchial vascular beds.

Injection into the pulmonary artery branches failed to produce specific burning of the mucosa, although of course this does not necessarily mean that mustard so injected in humans would not cause a greater effect on the bronchogenic carcinoma which is much more sensitive than the normal tissues of dogs. In another attempt to gain access to the bronchial vascular bed the pulmonary vein to the affected lung was ligated near its entrance to the heart. Diodrast injection of these preparations seemed to visualize the bronchial vascular bed. However, nitrogen mustard injections failed to produce specific burning of the bronchial mucosa, and further studies on the collateral vessels which have developed after pulmonary vein ligation have revealed that these are actually connections between pulmonary and systemic veins which sidetrack the bronchial vascular bed. The most pronounced local effect on lung tissue as compared with a general body effect measured by bone marrow examinations, occurs immediately after ligation of the pulmonary veins. Injection of pulmonary veins with nitrogen mustard occasionally produced enough damage to cause local necrosis of lung tissue.

Investigations have now begun on utilizing the thoracic aorta itself as an access vessel. If the aorta were temporarily obstructed at the level of the diaphragm and a quantity of chemotherapeutic agent introduced into the thoracic aorta it would immediately be carried to the bronchial circulation, chest wall, mediastinal lymph nodes and other structures usually involved by locally advanced bronchogenic carcinoma. Methods of accomplishing this at open thoracotomy and by aorta catheterization in the intact animal are being studied.

Significance to Cancer Research:

Continuing increase in the incidence and death rate due to lung cancer demands maximal efforts to control the disease. The primary or basic science approach involves efforts to determine the biologic nature and environmental relationships of lung cancer. While of great hope, this research has yet to produce applicable results. A second phase is seen in programs aimed at the discovery and treatment of lung cancer in

NCI-756
SERIAL NO.

Significance to Cancer Research: (Continued)

its earliest stages. This attack has succeeded in curing up to 5% of all patients seen by extirpative surgery, the only curative treatment now known. The third phase of lung cancer research involves improvement of treatment of the disease when it is past its earliest stages, and the large number of well-advanced lung cancer patients now seen provides an urgent motivation for the laboratory investigator to search for an approach such as described in this project.

Proposed course of project:

Continued studies will be made to determine the most advantageous vascular route of drug administration in bronchogenic carcinoma. The project will provide a continuing opportunity to evaluate changes in a catheterization technique to be employed in humans. After deciding on a standard technique we would like to use that technique for toxicity studies in dogs before using new drugs in humans by the regional vascular approach.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

1. National Cancer Institute (INSTITUTE) 2. Surgery Branch (LABORATORY OR BRANCH)

3. (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-757(C) (SERIAL NO.)

6. Prevention of Operative Site Recurrence by Improvement of Biopsy Wound Closure Technique (PROJECT TITLE)

7. Dr. John H. Waite (PRINCIPAL INVESTIGATOR(S))

8. Dr. Robert R. Smith - Dr. Albert Hilberg - Dr. Horace Herbsman (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives:

The purpose of this project has been to gather some experimental data concerning the validity of biopsy wound closure techniques, to determine if any currently used techniques are satisfactory, and to develop, if necessary, improved techniques for management of biopsy wound closure.

Methods employed:

Patients with apparently primary operable carcinoma of the breast are prepared for surgery. A routine local incision is made, and the suspected tumor excised, examined and submitted for frozen section histodiagnosis. The cut surface of the excised tumor is carefully washed to procure positive cytologic material for comparison with later skin surface washings. Cases with positive frozen section are suitable for study.

The local wound is closed by one of the standard methods under study, and the skin area completely cleansed and redraped for radical mastectomy. At this point, the closure is carefully examined for gross escape of serous or bloody fluid. Any of this serous or bloody fluid which does escape is carefully aspirated for cytologic examination, and in addition, washings are taken from the margins of the closed biopsy wound or any cover which may be fixed over it for cytologic examination to determine whether cancer cell escape has occurred with the method under evaluation.

NCI-757(C)
SERIAL NO.

Methods employed: (Continued)

The present scope of the project is limited to the following closure methods:
1. Closure with a tight interlocking continuous suture.
2. Closure with a tight interlocking continuous suture followed by a plasticized film.
3. Suture closure with a sheet of rubber submitted to the surrounding skin.

Patient material:

		No.	Average Stay Days
Admissions:	Adult males	2	15
	Adult females	8	12
Outpatient:	Number of patients	10	
	Number of visits	40	

Major findings:

Five breast carcinomas and one chondrosarcoma have been carefully studied according to the methods outlined. That the opportunity exists for wound seeding to occur from the biopsy site appears probable from the following data:
1. Three of these six patients yielded cancer cells from the skin surface, using cytologic detection techniques.
2. In all six patients, oozing of serous and bloody material through the closed biopsy wound occurred. This occurred despite a tight closure, and it was noted that the bleeding usually occurred from the suture needle puncture site. It was reasoned that the blood escaping from the biopsy site might easily contain a suspension of tumor cells.

Closure of the biopsy site with a continuous tightly interlocking suture was unsuccessful in sealing the wound as was Aeroplast (an aerosolized plastic spray which dries rapidly to form a film) which was applied in all of the five breast carcinoma patients. Oozing from the closed biopsy wound occurred, lifting the dried plastic film from the skin surface in a few seconds' time.

The four patients who were found to have benign tumors on biopsy contributed to the program by yielding cytologic control specimens. 2 cc. of a dye placed within the depths of one of these biopsy wounds appeared immediately on the surface of the skin through the suture needle puncture sites, imitating, we think, the ease with which a tumor suspension could reach the skin surface.

PROJECT REPORT FORM (Cont'd)

NCI-757(C)
SERIAL NO.

9. PROJECT DESCRIPTION (Continued)

Significance to Cancer Research:

Wound recurrence following biopsy and local surgery is not uncommon. In treating one of the most common cancers, that of the breast, leading clinics admit to a 12% local recurrence rate. The two most likely factors are inadequate surgical margins and spillage of cells at the time of biopsy. Exfoliated cells have been recovered from skin surfaces by the principal investigator even after more than usually careful attempts at biopsy site closure and sealing off, using conventional techniques. A simple method which would guarantee 100% riddance of skin surface tumor cells would be of immediate value to all surgeons in treatment of many common malignancies.

Proposed course of project:

Thus far none of the techniques in current practice for biopsy site closure is even theoretically capable of preventing escape of a tumor cell suspension from the closed biopsy wound. We will evaluate other methods in common use. In the meantime we will be trying to develop a foolproof technique under the animal project no. 758. We will continue to get washings from the operative wound site to correlate positivity of skin surface washings with the radical operative wound washings, and we will continue to follow these patients clinically in order to correlate tumor recurrences in the operative site with the positivity of skin washing cytology.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

1. National Cancer Institute
INSTITUTE

2. Surgery Branch
LABORATORY OR BRANCH

3.
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-758
SERIAL NO.

6. Prevention of Operative Site Tumor Recurrence by Improvement of Biopsy Wound Closure Techniques: Laboratory Research
PROJECT TITLE

7. Dr. John H. Waite
PRINCIPAL INVESTIGATOR(S)

8. Dr. Horace Herbsman - Mr. Richard V. Eck
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The purpose of this project is to evaluate methods which have been proposed for biopsy wound closure by laboratory techniques on the experimental animal. A closure method is sought which would prevent the escape of tumor cells from the skin surface into the radical surgery wounds after preliminary biopsy. If currently used techniques are found to be unsatisfactory, new techniques will be developed.

Methods employed:

For experimental convenience, a blue dye is used to imitate the tumor cell suspension which must be retained within the biopsy wound in order to prevent radical surgical wound contamination. Using rabbits as experimental animals we have made standard biopsy wounds, closed them with continuous interlocking sutures, and injected dye into the biopsy wound cavity, to determine the quantity and pressure of fluid retained by the wound closure being tested, before leakage occurs onto the skin surface.

Thus far three methods of closure have been evaluated;
1. Closure with a continuous interlocking suture as described.
2. Closure with a continuous interlocking suture overlaid with a plastic film sprayed onto the skin surface.
3. Closure using Raney surgical skin clips.

NCI-758
SERIAL NO.

PROJECT DESCRIPTION (Continued)

Major findings:

1. After closure of the standard biopsy wound with a continuous interlocking suture only, 1-2 cc. of dye injected under 2 cm. of water pressure appeared immediately on the surface of the closed biopsy wound. The appearance of this dye was not at the incised skin margin, but always at a suture needle puncture site, the same locus from which bloody oozing was invariably noted after the clinical closure of biopsy wounds in humans. Apparently the skin defect created by a suture needle puncture should be regarded as continuous with the incision into the underlying tumor, and a likely source for tumor cell escape.

2. Sealing of the closed biopsy wound with Aeroplast (an aerosolized plastic spray which dries rapidly to form a film) permitted about three times as much dye to be injected under a slightly higher pressure. However, if only slight manipulation of the closed biopsy wound was performed, dye immediately burst from beneath this very fragile seal, easily dissecting the plastic seal off of the surface of the skin. Thus, this type of a seal, including the older collodion seal, would seem to add little protection to the biopsy site.

3. Application of Raney clips to coapt skin margins resulted in an extremely efficient closure permitting the injection of about 50 cc. of dye under about 18 cm. of water pressure, before appearance of the dye at the openings of nipples or sweat glands.

Significance to Cancer Research:

The intact skin overlying a soft part cancer must usually be incised to obtain a biopsy, rupturing the envelope of normal tissue which theoretically should be removed intact with its indwelling malignancy. The prevention of escape of malignant cells through this incision constitutes one of the oldest problems in cancer surgery. Many methods of closure of biopsy wounds have been recommended empirically. It is hoped that utilizing the testing methods described, we will be able to recommend or develop a standard method for biopsy wound closure backed by experimental data.

Proposed course of project:

The most important untried method for wound closure is a method which has been recommended using the cementing of a sheet of rubber over the closed biopsy wound. This technique will be tested, and further experiments will be conducted on the Raney clip method of closure.

10. NCI-758
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

1. National Cancer Institute
INSTITUTE

2. Surgery Branch
LABORATORY OR BRANCH

3. _____
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-759
SERIAL NO.

6. Technic of Hypophysectomy in the Guinea Pig
PROJECT TITLE

7. Dr. Lester M. Cramer
PRINCIPAL INVESTIGATOR(S)

8. Dr. Eli Nadel
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To develop and perfect a technic for hypophysectomizing the guinea pig.

Methods employed:

Guinea pig hypophyseal fossae were approached transcervically, transcranially, and transbuccally and the pituitary gland removed by suction. Animals maintained postoperatively on antibiotics, clyses, and steroid.

Major findings:

No complete hypophysectomies were accomplished, the limiting factor being the extension of anterior pituitary cells up around the stalk to the hypothalamus.

It is felt that radioactive substances, such as yttrium, implanted into the pituitary fossa will offer a better chance to perform a complete hypophysectomy. Neither investigator is authorized to use radioactive substances, and the project is presently shelved until an interested personage with the necessary radioactivity clearance can be included in the study.

NCI-759
SERIAL NO.

PROJECT DESCRIPTION (Continued)

Significance to Cancer Research:

Continued developments in the field of endocrinology demonstrate the importance of pituitary hormones upon cancer tissue. If complete removal of the gland could be easily done in guinea pigs, a very useful tool would be available to further modify this control.

Proposed course of project:

Pending the availability of radioactive yttrium, no further work is planned on this project.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

1. National Cancer Institute
INSTITUTE

2. Surgery Branch
LABORATORY OR BRANCH

3.
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NCI 760
SERIAL NO.

6. The Study of the Effects of Beta Naphthylamine and 4-Aminodiphenol on Gastrointestinal and Bladder Mucosa by Direct Contact in Dogs.
   a. Development of a Surgical Technique for In Vivo Study of Drugs in the Gastrointestinal and the Isolated Urinary Bladder.
   b. A Study of the Techniques for Handling the Genitourinary Tract after Cystectomy.
PROJECT TITLE

7. Dr. Donald Cole - Dr. William Hueper
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

A study of the carcinogenic potentialities of certain aromatic amines and azo compound yellow AB. These compounds along with yellow AB and yellow OB are involved in food dyes in common use. This is the problem of Dr. William Hueper of the Environmental Cancer Section of the National Cancer Institute that he will report in detail.

Methods employed:

An acute experiment was devised in order to study the metabolism of the dyes in an isolated segment of the gastrointestinal tract for the length of time it may be present during normal digestion. In effect, in vivo test tubes were created by isolating the gastrointestinal tract of dogs throughout its length from the stomach to the rectum with no reconstruction of the anatomy. In another group of chronic experiments, in vivo test tube bladders and segments of gastrointestinal tract, stomach to colon, were isolated with reconstruction of the anatomy for what was believed to be the best functional results. A total of 19 acute experiments were performed on the gastrointestinal tract. The dogs were prepared preoperatively so that their gastrointestinal tract was clean. The organ was satisfactorily isolated at the time of surgery, being careful to maintain the integrity of the blood supply. The drug under study was then placed into the isolated loop and specimens obtained from the isolated loop at various intervals. In the chronic experiments 10 mg. of yellow AB were fed in meat balls to the dogs. After three weeks they were sacrificed and autopsied to be examined for metabolic analysis and pathologic changes.

NCI-760
SERIAL NO.

Methods employed: (Continued)

Two dogs had pouches formed with implantation of ½ gram of yellow AB and two others had jejunal loops and colon loops prepared and the drug placed into the isolated segments in a variety of circumstances. Multiple procedures were carried out to ascertain the best method for diverting the urinary stream to obtain an isolated urinary bladder and still maintain life. The drug was placed through the dome of the empty bladder which was then closed with silk sutures. The urethral opening was then closed in a similar manner.

Major findings:

The result of the experiment is not complete as yet. The dyes were not left in place long enough to produce any histologic changes. As for the chemical degradation products formed by the compounds, definitive results are not as yet available. As in the similar instance of using humans, the placement of the ureters in the colon or on the skin produced a hydronephrosis and hydroureter with ascending urinary infection which made it impossible to maintain adequate kidney function. The placement of the carcinogenic agent in the bladder with the urinary stream diverted has not been completed as yet.

Significance to Cancer Research:

A more thorough understanding of the possible carcinogenic effect of certain dyes would possibly lead to a useful prophylactic measure in the prevention of cancer.

Proposed course of project:

See report of Dr. William Hueper regarding future course of study.

11. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]
REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

1. National Cancer Institute — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. Memorial Hospital & Sloan-Kettering Institute — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-761 — SERIAL NO.

6. Blood-Pressure Servo-Regulator — PROJECT TITLE

7. Dr. John H. Waite — PRINCIPAL INVESTIGATOR(S)

8. John Laughlin - Naomi Sager - William Peppall - William Howland — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To construct a machine for automatic regulation of blood pressure in shock due to vasodilation, and in hypotensive anesthesia.

Methods employed:

The mean arterial blood pressure is measured directly with a transducing manometer. Appropriate vasopressor (norepinephrine) or vasodepressor (arphonad) solution is administered continuously intravenously by a variable speed infusion pump. The pump speed is regulated by a signal generated by a servo-loop. The servo-loop signal measures the difference between the blood pressure measured, and the blood pressure clinically desirable for the patient being treated. The therapist thus needs only to set the control panel for the desired blood pressure, and the auto-regulated machinery administers blood pressure regulating substance as needed.

Major findings:

Construction of the blood-pressure servo-regulator apparatus has been completed. The apparatus has been tested on a series of experimental animals (dogs) in the Physiological Laboratories of the Sloan-Kettering Institute. Necessary refinements in design and construction have been made and completed. The apparatus has now been moved to the recovery room of the Memorial Hospital. The participation by the principal investigator has been on a consultative basis during the calendar year, 1955.

NCI-761
SERIAL NO.

PROJECT DESCRIPTION (Continued)

Significance to Cancer Research:

The machine, if successful, will be an aid in maintaining blood pressure in patients with neurogenic shock due to radical surgery or drug reactions. It will also provide a method for controlled lowering of blood pressure (hypotensive anesthesia) in patients undergoing radical surgery for cancer.

Proposed course of project:

The apparatus will be evaluated in controlling the blood pressure of the next six patients requiring vasopressor agent administration to maintain blood pressure at the Memorial Center.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. Sloan-Kettering Institute - Memorial Hospital, New York City
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

NO ENTRIES FOR ITEMS 13, 14, 15 & 16.

1. National Cancer Institute (INSTITUTE) 2. Surgery Branch (LABORATORY OR BRANCH)

3. _____ (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-762(C) (SERIAL NO.)

6. Blood Volume, Plasma Volume, Red Cell Mass and Total Circulating Plasma Protein and Electrolytes in Cancer Patients During Preoperative Bowel Preparation and the Period after Radical Surgery. (PROJECT TITLE)

7. Dr. Robert A. Milch - Dr. Jesse L. Steinfeld (PRINCIPAL INVESTIGATOR(S))

8. Surgical Staff, N.C.I. (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives:

Although considerable data have been presented from several laboratories concerning the metabolic responses to surgery and other forms of trauma, little attention has been directed to metabolic changes in patients undergoing various preparatory procedures during the immediate preoperative period, the operative and immediate postoperative period. This is particularly true of female patients undergoing extensive preparation of the bowel for pelvic surgery prior to contemplated exenterative procedures. The objectives of the present study are to determine the above parameters serially in patients undergoing "vigorous" as opposed to "mild" preparation of the gastrointestinal tract for both pelvic and extra-abdominal surgical procedures.

Methods employed:

Injection of known small amounts (approximately 10 microcuries) of I^{131}- labelled human serum albumin and measurement of its dilution at 15 and 30 minutes after injection in both whole blood and plasma. Coupled with serial determinations of the plasma concentration of red cells (hematocrit), plasma protein, and serum electrolytes, it will be possible to calculate the total amounts of these various substances which are circulating in the plasma and the effect of bowel preparation of them from calculated data on red cell mass, plasma volume and blood volume.

Patient material:

Patients undergoing surgery by the N.C.I. Surgery Branch will be studied. Daily EKG's and if possible, EEG's will be taken. No patients admitted during this year for this project.

NCI-762(C)
SERIAL NO.

9. PROJECT DESCRIPTION (Continued)

Major findings:

None to date.

Significance to Cancer Research:

In order to improve our methods of treating cancer, more precise information is necessary concerning the metabolic status of cancer patients coming to surgery. This project should help the surgeon prepare patients for major surgery.

Proposed course of project:

During the next year it is planned to study patients undergoing radical pelvic surgery, both during the preoperative and immediate postoperative periods. It is anticipated that approximately 24-48 patients will receive such therapy and be available for such studies.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. _____ — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI- 763 — SERIAL NO.

6. The Effect of Prednisone (Metacorten) Upon the Healing of Wounds — PROJECT TITLE

7. Dr. Lester M. Cramer — PRINCIPAL INVESTIGATOR(S)

8. _____ — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

Because of its greater anti-inflammatory action and its lesser effect upon ion metabolism, prednisone is rapidly replacing compounds E and F in a large segment of clinical medicine, especially in the management of rheumatoid arthritis, chronic pulmonary asthma, and many ocular and dermatologic conditions.

The inhibitory effect upon wound healing of compounds E and F is well documented. It becomes imperative to know if this new compound will have greater wound healing inhibitory effect as might be expected from its greater effect upon inflammation.

Methods employed:

280 C57Bl mice were divided into two groups of 140 each, and then each of these subdivided into 14 groups of 10 each. One-half of all the animals were given prednisone .25 mgm/20 gm. daily for 5 days before wounding, the other one-half served as pair-fed controls.

The wound consisted of a 2 cm. incision in the anterior wall of the distal stomach. 5-0 nylon thread was used to close the wound in linear fashion, used a continuous horizontal mattress suture for serosal coaption.

PROJECT REPORT FORM (Cont'd)

NCI-763
SERIAL NO.

Methods employed: (Continued)

Prednisone was given daily postoperatively, and all animals were weighed daily. A group from each of the prednisone and control groups was sacrificed on each day for fourteen days, and the wounds studied in two ways: (1) Strength of wound as determined by introduction of compressed air until the suture line bursts. (2) Histologic character.

Major findings:

All of the technical aspects of the work have been completed.

I. Bursting strength results.

a. Curve has been established for the healing wound in the mouse stomach which is comparable to the accepted curve for a similar wound in the rat stomach.

b. The curve increment of healing strength in the mouse treated with prednisone is of the same shape as the normal curve, but is very slightly lower. Limited studies using cortisone showed that healing strength was about the same at mid points on the curve as that found with prednisone.

II. Histology results.

Not available, slides are being processed.

Significance to Cancer Research:

The use of new steroids as a palliative agent in treatment of cancer produces serious problems in the surgical management of patients, especially if it could be shown that these drugs do inhibit wound healing.

Proposed course of project:

Until complete analysis of the material already at hand has been performed, it remains impossible to definitely state whether this drug has any effect on wound healing. It may be necessary to run a few more animals to further determine this point.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. ____ — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-764 — SERIAL NO.

6. The Transplantation of "Conditioned" Endocrine Tissue — PROJECT TITLE

7. Dr. Lester M. Cramer — PRINCIPAL INVESTIGATOR(S)

8. Dr. William Schatten - Dr. William Mohler - Dr. Jesse Steinfeld. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

It has been shown, but not conclusively, that the immune response to homografted tissue may be abrogated by modifying the antigenicity of the donor tissue.

We plan to use a tissue culture method of modifying neonatal rabbit thyroid glands, and then transplanting them to hypothyroid recipients.

Methods employed:

Thyroidectomy is performed on rabbits less than 48 hours old, and explants of these thyroids grown in media consisting of Eagle's nutrients and serum of the recipient rabbit. Thyrotropic hormone is placed in one-half of the cultures, and all of the culture have histologic section and I^{131} uptake performed on a representative sample.

After a suitable length of time in tissue culture, the explants are placed subcutaneously in the anterior abdominal wall of a rabbit which has been thyroidectomized two weeks previously. Thyrotrophic hormone is administered two days prior to and ten days after the homografting.

I^{131} uptakes will be determined over the graft bi-weekly for a three month period at which time grafts which are not functioning will be sacrificed. Functioning grafts will be left intact and I^{131} uptake determined monthly for at least six more months at which time histologic examination will be performed.

PROJECT REPORT FORM (Cont'd)

NCI-764
SERIAL NO.

Methods employed: (Continued)

It is contemplated using the transparent chamber for some of the transplants when function has been demonstrated. Controls are liver treated in the same fashion.

Major findings:

Presently we are working on technics for giving TSH in vitro, for counting I^{131} in vitro, and determining optimum timing for the conditioning. Three grafts have been in recipients for two weeks. Two of these have demonstrated no function, the third one has picked up 6% of the I^{131}, but the control transplant of liver tissue has also picked up 6% of the I^{131}.

Significance to Cancer Research:

In the treatment of cancer of endocrine glands it is often necessary to remove the entire gland. This necessitates that the patient continue taking substitution drug treatment for the remainder of life. If a method of producing a functioning homologous graft could be developed, possibly using tissue cultured grafts, considerable benefit to clinical management would be obtained.

Proposed course of project:

It is planned to continue the experiment as outlined above. If it can be shown that these grafts grow and function, one might consider the possibility of attempting the same procedure in humans. It is doubtful if during the present year, progress on this experiment will develop to the point that would allow such a study.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute — 2. Surgery Branch
INSTITUTE — LABORATORY OR BRANCH

3. _____ — 4. _____ — 5. NCI-765
SECTION OR SERVICE — LOCATION (IF OTHER THAN BETHESDA) — SERIAL NO.

6. Effect of Carcinogenic Agents using a Bronchial Pouch Preparation in the D
PROJECT TITLE

7. Dr. John H. Waite
PRINCIPAL INVESTIGATOR(S)

8. Dr. W. C. Hueper and Dr. William Banfield
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

(1) To introduce and utilize the blind pouch technique into bronchogenic carcinoma carcinogenesis study. Results may be added to current evidence concerning the etiology of human lung cancer.
(2) To provide a satisfactory large animal tumor for treatment studies.

Methods Employed:

As performed at present the pouch is fashioned from the left lower lobar bronchus. A left lower lobectomy is first performed, amputating the bronchus more distal than usual. The lower lobe bronchus is then re-amputated at a higher level, severing the cartilagenous ring and endobronchial structures, but leaving the peribronchial structures intact as a vascular pedicle. The left main bronchus is sutured as are both ends of the pouch, forming in effect a pseudocyst. The end of a fine polyethylene catheter is implanted into the lumen of this pouch, and the other end of the catheter is exteriorized through the chest wall where it is buried beneath the skin, available for injection purposes.

Suspected carcinogenic agents will be injected into the bronchial pouch via the polyethylene catheter at regular intervals. Suitable controls where the vehicle only is injected, will be included. Serial roentgenograms will be taken to detect and follow the progress of any left hilar radiodensity which may develop. The animals will be followed for four to five years, after which time they will be sacrificed. It is proposed to

NCI-765
SERIAL NO.

Methods Employed: (Continued)

study the following agents initially: 3,4 benzpyrene, tobacco tar, and internal combustion engine exhaust concentrate.

If sufficient animals and facilities are available, rates of uptake of radio-labelled samples of these compounds may be studied. Further experimer will be conducted to attempt production of a bronchial pouch or bronchostome continuous with the skin surface, for carcinogenesis studies.

Major Findings:

In a pilot group of 3 dogs that were studied for technique development only, all three dogs survived the procedure and in all three a satisfactory pouch preparation was found at sacrifice two weeks later. Pathologically, the pouch appeared to be identical to a pseudocyst lined by healthy bronchial mucosa and filled with a non-infected gelatinous material.

Significance to Cancer Research:

To date the majority of evidence purporting to bear on the genesis of human bronchogenic carcinoma is adduced from the ability of a suspected carcinogenic agent to produce a tumor when painted on the skin of a rabbit or mouse. In interpreting the results of these experiments, not only must the heterogenecity of the experimental animal with respect to humans be con sidered, but dissimilarity between epidermis and bronchial epithelium plays an important role. Exposing the experimental animal to an atmosphere laden with the suspected carcinogenic agent has almost always been unsuccessful in producing tumors. This has lead to the conclusion by some investigators that the agents are not respiratory carcinogens and by others that the animals respiratory defense (the cough, the dilution of the carcinogenic agent in respiratory secretions, and the action of cilia) are so efficient as to make exposure to the carcinogenic agent negligible using this technique There is much logic to support the second hypothesis, and a good method to provide contact with a strong concentration of the suspected agent over a pro longed period of time and against which contact the animal may not have an effective defensive mechanism might be of some value in settling this point.

We feel that a study on the uptake of radioactive benzpyrine from our pouch preparation alone might yield sufficient data to justify the project.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

(No Entries for Items 12, 13, 15 & 16.)

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Surgery Branch (LABORATORY OR BRANCH)

3. _____ (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-766(C) (SERIAL NO.)

6. Combination Therapy of Esophageal Carcinoma. (PROJECT TITLE)

7. Dr. John H. Waite - Dr. J. Robert Andrews (PRINCIPAL INVESTIGATOR(S))

8. Dr. Andrew G. Morrow (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives:

This project will attempt to develop a satisfactory method for treatment of squamous carcinoma of the esophagus. Improvement of operability, resectability, and cure rate, will be sought by a program of preoperative rotational supervoltage radiation. A byproduct of the study will be the availability of the recently irradiated esophagus for intensive pathologic study to evaluate the effects of rotational supervoltage on this tumor. By use of tube feedings during the entire period of preoperative radiation an attempt will be made to improve caloric intake and reduce the incidence of hemorrhage and perforation during the course of therapy.

Methods employed:

(1) Complete workup to determine the extent of tumor. Patients with metastatic carcinoma or with regional invasion of a vital structure, such as heart or lung, will not be suitable.
(2) Insert plastic Levin tube at esophagoscopy to provide maintenance and improvement of nutrition. Consider insertion of a nuchal esophagostomy tube if patient develops hypopharyngeal irritation due to the indwelling Levin tube.
(3) Radiation plan of study by Dr. Andrews as coinvestigator. In general, he plans to give external two million volt therapy by rotation technique, beginning with low doses and increasing gradually to a total of 6,000 to 7,000 "r" axial dose over a period of six to seven weeks. If difficulty in achieving a satisfactory vertical width of port is encountered, the possibility of delivering supplemental gamma rays from an available intra-luminal source will be considered. The presence of the indwelling Levin tube would be ideal for this technique.

NCI-766(C).
SERIAL NO.

Methods employed: (Continued)

4. Surgery (with Dr. Morrow) about four weeks after completion of radiation. If resectable, total esophagectomy with dissection of the regional nodes will be performed with immediate or delayed reconstruction of gastrointestinal continuity.

Evaluation criteria will include (1) changes in tumor size by serial roentgenography during radiation, (2) extent of radiation reaction and tumor fixation at the time of surgery, (3) extent of tumor and lymph node metastases by pathological examination of surgical specimen, and (4) clinical course of patient after treatment.

Patient Material:

Patients studied during the calendar year 1955 are reported by Dr. R. R. Smith under Project No. 751(C), "Evaluation of Radical Surgery and Development of New Surgical Techniques as a Therapeutic Method of Palliating or Definitive Therapy of Advanced Cancer." This included the initial 3 patients studied, for an average of 41 hospital days and one outpatient visit each.

Major findings:

Of the 3 patients studied, 1 patient was unsuitable for the program because of extreme senile debility, and 2 patients were suitable. These patients both satisfactorily completed a course of rotational 2-million volt therapy, and came to surgery one month after completion of therapy. Maintenance of alimentation by tube feedings was very successful in both patients and in itself may prove to be a significant contribution to the treatment of this lesion. Of the 2 patients brought to surgery, one proved incurable by virtue of abdominal metastases found at the time of preliminary laparotomy.

An esophagectomy was performed in one patient. Resection of the lesion was not hindered in this one instance by preliminary radiation, on the contrary it is felt that the preliminary radiation probably improved the resectability of the lesion which was in the region of the arch of the aorta. This patient expired postoperatively due to a culmination of temporary hemorrhagic shock during surgery, poor cardiac action, and pulmonary edema postoperatively. An operative mortality of about 15% is expected for this type of procedure but will be acceptable because of the certain early fatality in esophageal carcinoma treated by current methods.

NCI-766(C)
SERIAL NO.

PROJECT DESCRIPTION (Continued)

Significance to Cancer Research:

Carcinoma of the esophagus is the fifth highest in frequency among internal cancers in adult males. This frequency and the poor results available from present methods of treatment make it an ideal subject for cancer research. Adequate surgery and adequate radiation each fails in its own way to control more than 2% of the entire group of patients presenting themselves with esophageal carcinoma. The chief advantage of supervoltage rotational therapy x-ray is appearing to be better immediate palliation, although the incidence of eventual recurrence and death probably will not be much lower. Combination of surgery and supervoltage rotational therapy is being tried at several centers, utilizing surgery as the primary treatment and roentgen therapy as a "mop up" treatment. For the reasons already enumerated, the principal investigator feels that the initial use of radiation may provide superior results.

Proposed course of project:

See "Methods employed" above. The availability of material will make this a slowly developing project.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. _____ — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-767(C) — SERIAL NO.
6. Physiologic and Environmental Factors Associated with Hypothermia During Extensive Surgical Procedures. — PROJECT TITLE
7. Dr. Horace Herbsman — PRINCIPAL INVESTIGATOR(S)
8. Dr. Robert B. Smith and Dr. Clarence Hebert — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: It has been shown that hypothermia may be induced in patients with resultant physiologic changes advantageous to the performance of certain major surgical procedures. However, spontaneous hypothermia has recently been noted to occur during extensive surgical procedures in the Clinical Center operating rooms. Associated with this sudden decrease in body temperature has been a concomitant decrease in the clinically detectable blood pressure, falsely suggesting to the operating team that blood replacement has been inadequate. The objective of this study is to further evaluate this problem of spontaneous hypothermia, investigating such contributing factors as the patient's age, weight, body build, state of hydration, disease being treated, previous treatments, premedication, anesthetic agents employed, operating room temperature and humidity, degree of surgical draping, and the surgical procedure being performed. The advantages and disadvantages of this spontaneous hypothermia in relation to the surgical procedure will also be evaluated. If found advantageous, methods of uniformly producing hypothermia will be investigated. If found disadvantageous, methods of preventing the spontaneous occurrence of hypothermia will be studied.

9. PROJECT DESCRIPTION (Continued)

Methods employed:

Temperatures of the patient from various body surfaces will be recorded simultaneously with a multi-channel thermocouple which will also record the prevailing room temperature. Various physiologic determinations on the patient will be obtained using standard methods.

Patient Material:

Material for this project will be obtained during operations on patients admitted for other projects.

Major Findings:

None. Not started yet.

Significance to Cancer Research:

The information to be derived from this study will be used to help render major surgical procedures for cancer more successful.

Proposed Course of Project:

During the coming year many of the patients undergoing major surgery for cancer will be studied during their surgical procedures. Constant body temperatures and other physiologic data will be determined to be correlated with the procedure being done, the room temperature and humidity, and the other contributing factors.

10. 767(C)
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. NONE
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT.

13. NONE
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH.

15. NONE
PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

16. NONE
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

1. National Cancer Institute
INSTITUTE

2. Surgery Branch
LABORATORY OR BRANCH

3. _____
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-763
SERIAL NO.

6. Study of the Role of the Properdin System in Natural Immunity
PROJECT TITLE

7. Dr. William E. Schatten
PRINCIPAL INVESTIGATOR(S)

8. Dr. Lester M. Cramer - Dr. Horace Herbsman - Dr. Robert R. Smith
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To evaluate the effect of altering the serum properdin level in an attempt to abrogate the immune mechanism that plays a role in the neutralization of viruses and in the prevention of successful homo- and heterotransplantation of tumor tissue.

Methods employed:

One study that would indicate whether or not zymosan, a carbohydrate that combines with the serum globulin, properdin, is effective in altering host resistance would be to ascertain whether or not zymosan is effective in the heterotransplantation of tumor tissue. If zymosan were effective, it would strongly indicate that properdin plays a role in the natural resistance of host animals, since zymosan has a specific action on properdin. This study will be carried out by administering zymosan intravenously to rats in different doses at different intervals of time prior to and after inoculation of rats with tumors. The S-91 melanoma of mice will be used for heterotransplantation in this study of the effects of altering properdin levels in rats. If it is proved that zymosan is effective in successful heterotransplantation, then transplantation of tumors from patients to rats will be studied. Tumors will be transplanted into groups of rats that have been pre-treated with zymosan and with zymosan and cortisone. Tumors that survive transplantation and grow will be periodically transplanted to other rats, some pre-treated, and others not treated. This will be an attempt to adapt a transplantable tumor strain. Tumor growth will be measured directly following subcutaneous inoculation and b weight of the tumor following intraperitoneal inoculation.

NCI-768
SERIAL NO.

Methods employed: (Continued)

If heterotransplantation of tumors can be effected successfully, the oncolytic effects of viruses on tumors will be studied by injection of viruses into the tumors. Following virus injection, subcutaneous tumors will be measured and biopsies taken periodically for histological studies. The effects of different viruses on a tumor, as well as the role of zymosan, cortisone, and other agents in enhancing virus effects will be studied. Also, this would provide a method of increasing the virulence of a virus by passing the virus thru a patient's tumor to "train" it against the tumor before adminstering the virus to a patient.

Major findings:

This project is in its preliminary phase. However, it is believed that there was a definite difference in the size of heterotransplanted tumors in rats that were treated with zymosan prior to tumor inoculation when compared to rats that were not treated.

Significance to Cancer Research:

If human tumors could be heterotransplanted into other animals successfully, this would provide a method for studying individual tumors of patients in laboratory animals. This would be a method of testing many chemotherapeutic agents in laboratory animals. As mentioned above, this would also provide a method for the evaluation of the oncolytic effects of viruses on tumors and perhaps this would be a method of conditioning a virus to become more virulent with regards to its effect on a particular tumor. If zymosan were effective in altering host resistance so that a tumor could be transplanted, it might also be used to alter the immune mechanism that prevents continued propagation of a virus following its administration. This antibody response following virus administration is one of the major problems that now presents itself in the virus treatment of carcinoma.

Proposed course of project:

Heterotransplantation of tumors will be performed in a sufficient number of rats that are pre-treated with zymosan and with zymosan and cortisone to determine the effectiveness of this treatment in heterotransplantation. If zymosan does promote successful heterotransplantation, then further studies will be carried out as outlined above.

10. NCI-768
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

1. National Cancer Institute — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. ____ — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-770 — SERIAL NO.

6. Study of the Effect of Growth Hormone on Cartilage Transplants and of the Survival and Growth of These Transplants — PROJECT TITLE

7. Dr. William E. Schatten — PRINCIPAL INVESTIGATOR(S)

8. Dr. Lester W. Cramer - Dr. Horace Herbsman - Dr. Delbert M. Bergenstal — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To develop a method of stimulation of growth of cartilage transplants and to study the survival and growth of homotransplants and heterotransplants of cartilage.

Methods employed:

Hypophysectomized rats are used in this study because they are sensitive to small amounts of growth hormone and because non-treated hypophysectomized animals are better controls than normal rats in this experiment. Three groups of rats are being studied. Autografts of xiphoid and costal cartilages are transplanted in one group, homografts in another, and heterografts from rabbits in the third group. The cartilage grafts are transplanted into the axilla and abdominal wall. Rats in each group are sacrificed at weekly intervals. On the second and third days prior to sacrifice, S-35 is injected intraperitoneally. After the animals are sacrificed the cartilage grafts and the animal's own costal and tibial cartilages are removed for study. The primary method of studying activity of the cartilage is by determining the S-35 uptake of the cartilage as expressed in counts per 100 mg. of dry cartilage per minute. The transplants are compared with animal cartilage that was not transplanted and also with the transplants of a control animal that did not receive growth hormone. The growth of cartilage transplants is also evaluated by means of comparisons of measurements and wet weights of the cartilages taken at the time of transplantation and at the time of sacrifice.

NCI-770
SERIAL NO.

9. PROJECT DESCRIPTION (Continued)

Major findings:

The project is in the preliminary phase. It is believed that the data that has been accumulated up until the present time shows that autografts of cartilage can be stimulated by growth hormone. Homografts and heterografts of cartilage can be stimulated to a lesser extent by growth hormone. In addition, heterografts of cartilage in animals treated with growth hormone do not take up as much S-35 as heterografts in non-treated hypophysectomized animals. This finding is, of course, of great interest and is being more fully investigated.

Significance to Cancer Research:

It is generally agreed that homologous cartilage grafts survive transplantation for varying periods of time. The exact reasons that cartilage grafts are not affected by an immune response as are almost all other tissues, is not known. It is also not known how well homografts of cartilage do survive. It is difficult to perform many studies that might give this answer because of the low metabolic activity of cartilage. It is believed that the uptake of S-35 by a cartilage graft will be a critical test of metabolic survival. It has been shown that, after dialysis of cartilage for 48 hours following removal of a specimen from an animal that has received S-35, 96% of the sulfur is bound in an organic compound. Sulphate is taken up by cartilage cells where it is probably attached to the mucopolysaccharide molecule and thereafter appears in the ground substance. It is hoped that this study will indicate some of the reasons that homografts of cartilage survive transplantation.

It is also the purpose of this experiment to determine if cartilage grafts, including autografts, homografts and heterografts, can be stimulated by growth hormone following transplantation. If this could be achieved, it would have clinical application in various reconstructive procedures in which cartilage grafts are used.

Proposed course of project:

Three groups of hypophysectomized rats will be used for studying autografts, homografts, and heterografts of cartilage. There will be 40 rats in each group, 20 serving as controls, and the other 20 receiving growth hormone following transplantation. Following transplantation of all 40 rats, 10 rats, 5 controls and 5 treated, will be sacrificed each week for 4 weeks. S-35 will be given to the rats prior to sacrifice as outlined above. Growth of the transplants will be studied as outlined above. If autografts can be stimulated to grow by the use of growth hormone in hypophysectomized rats, normal rats and other animal species will then be used in an attempt to see if growth hormone will stimulate the growth of cartilage transplants in those animals. Also, cartilage

NCI-770
SERIAL NO.

Proposed course of project: (Continued)

transplants will be performed and period of time allowed to lapse prior to administration of growth hormone. This will be done in a separate group of animals.

The fact that heterografts of cartilage have been found to take up less S-35 in hypophysectomized animals that receive growth hormone than in hypophysectomized animals that are not treated indicates that hypophysectomized animals may not be able to react to foreign tissue with an immune response. If this finding that was present in the first few experiments that were done is corroborated by our further experiments it would be indicated to heterotransplant tumors to hypophysectomized rats in an attempt to see if the tumors would not be affected by host resistance and would therefore progress at their own rate of growth. If this were true, then such tumors could be passed one or more times in hypophysectomized rats and the tumors might become adapted to the new hosts. They might then be transplanted to normal rats and the tumor developed as a transplantable tumor.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

1. National Cancer Institute (INSTITUTE) 2. Surgery Branch (LABORATORY OR BRANCH)

3. (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-771 (SERIAL NO.)

6. Experimental Reconstruction of the Extra-hepatic Biliary System (PROJECT TITLE)

7. Dr. William E. Schatten (PRINCIPAL INVESTIGATOR(S))

8. Dr. Lester M. Cramer - Dr. Horace Herbsman - Dr. Robert R. Smith (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives:

To develop a successful method of repair of common bile duct defects.

Methods employed:

Adult mongrel dogs are selected as experimental animals. A cholecystectomy and ligation of the common bile duct is performed in order to closely simulate conditions encountered clinically. Three days following this operation, a second operation is performed. At that time, a split-thickness graft of skin is taken from the abdominal wall. The common duct region is exposed and a portion of common duct proximal and distal to the area of previous ligation is excised in order to create a defect that is at least 2½ cm. in length. The skin graft is then used to form a tube over a rubber T-tube, and the T-tube is then introduced into both ends of the common duct and an accurate anastomosis between the common duct and skin graft is performed. After the graft is sutured in place, a tab of omentum is placed in the region of the graft. The end of the T-tube is tied off, brought out through a stab wound in the right upper quadrant, and then placed in a subcutaneous pocket in the musculature of the right upper quadrant of the abdomen. The skin graft is constructed into a tube in the direction of Langer's lines in one-half of the dogs and is constructed so that the long axis runs perpendicular to Langer's lines in the remainder of the dogs. The T-tube will be allowed to remain in place for three months in one-half of the dogs and for six months in the remainder. The dogs will be observed for three to six months following removal of the T-tubes in order to observe them for evidences of bile duct stricture formation. The dogs will then be sacrificed and gross and histological studies of the common ducts will be performed at the time of autopsy.

NCI-771
SERIAL NO.

9. PROJECT DESCRIPTION (Continued)

Major findings:

At the present time the above procedure has been carried out successfully in 14 dogs. The procedure has been performed in three other dogs and these dogs have died at varying intervals postoperatively due to leakage of bile in the region of the graft. Technical points have been learned from the loss of these three dogs and none of the last nine dogs has been lost because of bile leakage.

Significance to Cancer Research:

The repair of common bile duct defects is one of the most difficult problems in surgery today. This is evidenced by the multitude of experimental and operative procedures that have been employed. There is general agreement that it is advantageous to preserve the sphincter of Oddi in reconstruction of the biliary tract. For this reason it would be more preferable to repair common bile ducts by means of a successful transplantation of tissue to bridge existing defects than to effect repair by means of Roux-en-Y procedures that are advocated today. The proposed method of reconstruction of the common bile duct will allow preservation of the spincter of Oddi and therefore will be of value as a new surgical technique. If successful, this method will be recommended to surgeons for clinical application.

Proposed course of project:

This method of common bile duct reconstruction will be performed in a number of dogs so that a total of 20 will have survived the procedure and can then be observed for evidences of stricture formation over the proposed period of time.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

NO ENTRIES FOR ITEMS 12, 13, 14, 15 & 16.

PROJECT REPORT FORM

1. N. C. I. (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-8000 (SERIAL NO.)

6. Endocrine Aspects of the Progression and Therapy of Cancer of the Breast in Women and Men (PROJECT TITLE)

7. Roy Hertz, Delbert M. Bergenstal, and M. C. Li (PRINCIPAL INVESTIGATOR(S))

8. James A. Pittman, Harold Altman (M. G. Sherer and A. Breslow up to 7/1/55) (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION -

Objectives:

1. To extend and intensify detailed clinical observation of the natural course of cancer of the breast in men and women in order to better appraise the effectiveness of various methods of therapy and learn more concerning the etiology and pathogenesis of the disease.

2. To improve upon existing forms of hormonal therapy for palliation of advanced breast cancer and to elucidate the mechanisms involved.

Methods employed:

1. The acceptance of complete clinical responsibility for the further management of referred patients with proven diagnosis of cancer of the breast and the application to their problems of all available modes of therapy.

2. The exploration of new modes of hormonal therapy for palliation.

3. The detailed analysis of endocrine and metabolic factors operating in relation to the genesis and course of the disease in these patients up to the time of their demise.

4. Careful appraisal of pathological specimens obtained at biopsy or autopsy in collaboration with Pathology staff.

Patient material:

		No.	Average Stay Days
Admissions:	Adult females	48	43
Outpatient:	Number of patients	44	
	Number of visits	272	

PROJECT REPORT FORM (Cont'd)

NCI-800(C)
SERIAL NO.

Major findings - 12/1/54-12/1/55:

1. The number of cases of breast cancer which have come under our observation now equals 218. These patients have been studied with varying degrees of intensity but they all have contributed to the general clinical background and teaching functions of the group. This activity continues to provide constant liaison with local physicians and medical institutions for additional patient referral to the Clinical Center.

2. The necessary clinical precautions to be taken in the administration of massive parenteral estrogen therapy in patients with breast cancer and now rather completely determined, thus making this form of therapy an entirely feasible procedure in this and other clinics. In the face of the highly variable clinical conditions present in patients with advanced breast cancer, it seems increasingly unfeasible to attempt to determine definitively whether this form of therapy presents any distinct clinical advantages over more conservative forms of estrogenization. Nevertheless, our own clinical impression at this time is that in certain selected cases massive parenteral estrogen administration presents certain advantages for prompt and intensive hormonal therapy.

3. Additional data on estrogen withdrawal bleeding in elderly patients with advanced breast cancer indicate an incidence of approximately 15 percent. These observations confirm the marked difference in endometrial response in senile women from the uniformly positive response seen in women under 40 years of age. This difference in response suggests that an additional factor other than estrogen is essential for the activation of the human endometrium.

4. The failure to encounter a single case of endometrial carcinoma among all of our breast cancer patients treated with prolonged and intensive estrogen confirms the low level of carcinogenicity which estrogens have for the endometrium in patients over 40.

5. Initial studies on the use of massive intravenous androgen therapy have proven discouraging. Local and systemic tolerance is not good and we have failed to detect any material blood level of circulating androgen by bioassay even after as much as 750 mgm. testosterone propionate given intravenously. Moreover, the mechanical problem of rendering such androgen solutions free of particulate material has thus far proven insurmountable. Accordingly, our clinical efforts in the development of massive androgen therapy in breast cancer have been momentarily suspended.

6. Techniques for complete balance studies of breast cancer patients have been developed. These patients can now be followed with respect to balance of sodium, potassium, calcium, nitrogen,

PROJECT REPORT FORM (Cont'd)

NCI-800(C)
SERIAL NO.

Major findings (cont'd.)

phosphorous and creatinine.

These techniques have thus far permitted a complete characterization of the metabolic action of the newer corticoids, suggesting a greater field of usefulness for these compounds in the practical palliation of breast cancer patients. One of the more immediately demonstrable effects is a rapid correction of hypercalcemia, a frequently fatal complication in patients with breast cancer with extensive osseous metastases.

7. The aforementioned balance techniques have provided more reliable quantitative data on the B-complex balance in patients undergoing protein catabolic effects of exogenous corticoids. Although excretion of biotin, riboflavin, pyridoxin and folacin is unaltered during corticoid-induced catabolism there is a distinct and reproduceable loss of pantothenate. Thus, the participation of this dietary trace factor in the steroid catabolic effect is suggested. In addition, these data extend earlier observations indicating a retention of pantothenate during androgen induced anabolic states.

8. Cytological studies of the vaginal smears of patients treated with estrogen, androgen, corticoids and progesterone have been continued. No evidence of metaplastic changes have been noted.

9. A new steroid, 17 ethyl 19 nor testosterone which is known to be highly anabolic has been partially evaluated for its relative androgenicity in a series of six breast cancer patients. The compound thus far seems to present no marked advantage over other androgens available.

10. A study of the effects of potent pituitary follicle-stimulating hormone preparations on the human ovary has been conducted in six patients just prior to surgical ovariectomy for palliation of advanced breast cancer. Marked follicular stimulation was observed in 4 cases and no response in 2. No detectable rise in serum or urinary estrogen accompanied these effects and no detectable estrogen was found in the ovarian vein blood obtained at the time of ovariectomy. Thus the trophic response of the human ovary is not accompanied by the readily detectable changes in blood and urinary steroid level seen when the human adrenal is activated by ACTH.

11. Attempts to modify adrenal cortical function in breast cancer patients by Amphenone administration have led to a more complete appreciation of the clinical toxicity of this compound and its consequent limitations as an inhibitor of adrenal cortical function in man. Nevertheless, this information has proven useful in application of this agent to other endocrinological problems to be described under project 803.

PROJECT REPORT FORM (Cont'd)

NCI-800(C)
SERIAL NO.

Significance to cancer research:

The direct pertinence of the foregoing studies to the grave problem of advanced or persistent breast cancer is too obvious to require discussion.

Proposed course of project:

These comprehensive clinical, endocrinological and metabolic studies in breast cancer patients are to be extended essentially along the lines already described above. A new departure will be the evaluation of the place of hypophysectomy in the management of breast cancer, a study to be carried out in cooperation with the neurosurgical staff of N.I.N.D.B. In addition, studies of the effect of radiothyroidectomy on the clinical course of breast cancer will be undertaken.

11. BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

NO ENTRIES FOR ITEMS 12 & 13

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. The effect of meticorten and meticortelone on thyroid function, by M. G. Sherer and B. N. Siefring (J. Clin. Endocrinol. & Metab.) In press.

2. Amphenone: Toxicity and effects on adrenal and thyroid function in man, by R. Hertz, J. Pittman and M. Graff (J. Clin. Endocrinol. & Metab.) In press.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

1. Dr. D. M. Bergenstal was invited to attend the International Rheumatology Congress, Rio de Janeiro, Brazil, August 1955.

2. Dr. R. Hertz was invited to participate in the meetings of the Societe d'Endocrinologie d'Haiti, Port-au-Prince, Haiti, Dec. 1955 (unable to attend).

PROJECT REPORT FORM (Cont'd.)

NCI-800(C)
SERIAL NO.

16. HONORS AND AWARDS (Cont'd.)

3. Dr. R. Hertz is invited to preside over forthcoming session of the Endocrine Society, June 1956.

4. Dr. R. Hertz was reappointed Chairman of Endocrinology Panel and Member of Executive Committee on Growth, National Research Council.

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) NCI-301(C) (SERIAL NO.)

6. Endocrine Aspects of Cancer of the Prostate in Man
PROJECT TITLE

7. Roy Hertz, Delbert M. Bergenstal, M. C. Li, and Lois F. Hallman
PRINCIPAL INVESTIGATOR(S)

8. James A. Pittman and Harold Altman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION -

Objectives: 1. To improve non-surgical therapy of cancer of the prostate
2. To increase understanding of endocrine factors involved in genesis and course of prostate cancer.
3. To gain basic knowledge of the metabolic action of certain steroids in man.

Methods employed: Detailed clinical and metabolic observations of patients with advanced prostatic carcinoma who are no longer amenable to accepted forms of clinical management.

Patient material:

	No.	Average Stay Days
Admissions: Adult males	4	51
Outpatient: Number of patients	2	
Number of visits	4	

Major findings: 1. The administration of chorionic gonadotropin to 3 patients with prostatic carcinoma just prior to orchiectomy has shown that such gonadal activation even in the senile man will aggravate the disease as manifested by exacerbation of pain and elevation of acid phosphatase. The expected rise in urinary excretion of biologically active estrogen was not detected. The spermatic vein blood obtained at the time of the orchiectomy also contained no detectable androgen or estrogen. Thus, although it is apparent on histological and clinical grounds that the senile human testis can be activated by chorionic gonadotropin, this trophic action on the testis is not reflected in detectable increments in steroidogenesis as is the case in the activation of the human adrenal by ACTH.

2. Further studies of the mechanism of the elevation of serum acid phosphatase in prostatic cancer patients have yielded no new data of value. Thus we have failed in our attempts to transmit this activity to human fibroblasts in tissue culture. This was attempted

PROJECT REPORT FORM (Cont'd)

NCI-801(C)
SERIAL NO.

Major findings (cont'd.)

on the theory that this enzymatic activity may possibly be associated with a virus-like agent whose titre rises in advanced prostate cancer patients. Initial impressions that this elevation of enzymatic activity represents the failure of an inhibitory agent in normal serum have failed of confirmation.

Significance to cancer research: The high frequency of prostatic carcinoma in man and initial inroads upon it by hormonal therapy indicate the pertinence of this study to cancer research.

Proposed course of project: Additional studies of the clinical and endocrinological factors involved in the extension and growth of advanced prostatic cancer will be undertaken. Major emphasis will be placed upon the evaluation of several atypical estrogenic compounds and corticoidal steroids in the therapy of prostatic carcinoma.

11. BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

NO ENTRIES FOR ITEMS 12, 13, 15 & 16.

PROJECT REPORT FORM

1. N. C. I. (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-802(C (SERIAL NO.)

6. Preoperative Hormonal Therapy of Cancer of Cervix
PROJECT TITLE

7. Roy Hertz, John H. Waite, and Robert Smith
PRINCIPAL INVESTIGATOR(S)

8. James A. Pittman and Harold Altman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To determine the value of steroid therapy as a palliative measure in advanced cancer of the cervix.

Methods employed:

Patients who are considered amenable to definitive surgical therapy are pre-treated for 4 to 6 weeks with massive doses of various steroid hormones singly and in combination and then are operated upon. Their progress is evaluated by frequent pelvic examination, serial biopsies and smears and by histopathological study of the surgical specimens removed. Pyelograms and barium enemata are also employed to evaluate changes in the pelvic viscera. The amount of vaginal bleeding is checked from day to day. The patient's general clinical status with respect to weight, appetite, blood status, and pain are also closely observed.

Patient material:

	No.	Average Stay Days
Admissions: Adult females	3	10
Outpatient: Number of patients	3	
Number of visits	12	

Major findings:

No extension of our findings beyond last year's report has been made due to the justified preocupation of our surgical staff with the virus approach.

PROJECT REPORT FORM (Cont'd.)

NCI-802(C)
SERIAL NO.

Significance to cancer research:

Palliation of advanced cervical carcinomas urgently needed in view of the low over-all 5 year survival rate of patients with this disease (40-50%).

Proposed course of project:

The further course of these studies will depend upon the interests of the surgical staff which is at the moment justifiably concentrated on the virus approach to cervical cancer.

11. BUDGET ACTIVITY:

RESEARCH	☐	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957:

Surgery Branch, NCI - Dr. R. Smith and staff.

NO ENTRIES FOR ITEMS 13, 15 & 16.

PROJECT REPORT FORM

1. N. C. I. (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-803(C) (SERIAL NO.)

6. The Nature of Hormone-Producing Tumors of Pituitary, Adrenal, Ovary, Testis, Pancreas, Parathyroid and Chorion; Abnormalities of Somatic Development and Growth (PROJECT TITLE)

7. Roy Hertz, D. M. Bergenstal, M. C. Li, Sally B. Fand and M. M. Graff (PRINCIPAL INVESTIGATOR(S))

8. James A. Pittman, Harold Altman, V. W. Tullner and Donald Spencer (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives:

1. To learn more about the basic metabolic phenomena involved in the process whereby neoplasms in hormone-producing organs produce highly excessive amounts of hormone, resulting in such syndromes as Cushing's Disease, Acromegaly, Hyperparathyroidism, Hyperadrenalism, and similar endocrinopathies.

2. To maintain in tissue culture hormone-producing tissues and to ascertain the factors involved in quantitative hormone production in vitro.

3. To assess the capacity of new drugs to alter the course and hormonal activity of hormone-producing tumors.

Methods employed:

Hormone producing tumors from patients with one of the above-named syndromes are obtained at surgery and carried in tissue culture. The supernatant fluid from the cultures are tested for hormonal activity by appropriate bioassay methods and the quantity recovered compared with the content of a piece of tissue exactly equivalent to that originally placed in culture.

Patients presenting such syndromes are completely characterized endocrinologically and metabolically before and after therapy in order to ascertain the relative effects of various forms of therapy.

PROJECT REPORT FORM (Cont'd).

NCI-803(C)
SERIAL NO.

Patient material:

		No.	Average Stay Days
Admissions:	Adult males	6	11
	Adult females	16	33
	Children males	4	11
	Children females	4	9
Outpatient:	Number of patients	72	
	Number of visits	289	

Major findings:

1. Two additional patients with proven chorio carcinoma have been shown to lack the histidinuria seen in normal pregnancy.

2. A patient with proven choriocarcinoma has exhibited a marked suppression of urinary gonadotropin secretion while on amethopterin therapy. This indicates a specific interference with the normal metabolism of this hormone-producing tumor.

3. Attempts to transplant human hormone-producing tumor tissue to cortisone-treated rats have failed in the case of 1 islet cell tumor, 2 adrenal adenomata, 1 parathyroid adenoma and 1 simple follicular cyst.

4. An attempt to grow one islet cell adenoma in tissue culture has failed and one additional islet cell tumor is surviving in tissue culture 2 weeks after explantation. Its further course and possible in vitro production of insulin remain to be evaluated.

5. Two adrenal carcinoma patients have been studied directly by us and two in collaboration with outside investigators at other hospitals. All 4 have shown a decisive drop in urinary corticoid excretion under Amphenone administration. This demonstrates that Amphenone will markedly depress the activity of this hormone-producing tumor. The duration of these studies was too limited by clinical circumstances to permit any evaluation of the effect of Amphenone on the course of the malignancy.

6. In one patient with advanced metastatic adenocarcinoma of undetermined origin the administration of Germanin (Bayer 205) has led to an extensive necrosis of the adrenal cortex similar to that observed experimentally and in prior clinical studies in patients with pemphigus. This patient also showed clinical and biochemical evidence of adrenal insufficiency

7. Endocrinological analysis has permitted the differentiation of constitutional sexual precocity from that due to hormone-producing hyperplasia or tumor in 5 additional children.

PROJECT REPORT FORM (Cont'd.)

NCI-803(C)
SERIAL NO.

Major findings (cont'd.)

8. Cytological methods for the chromosomal determination of the true somatic sex of patients with adrenal hyperplasia or abnormal gonadal development has facilitated the endocrinological evaluation of 10 problem cases.

9. Further observations in acromegaly have been seriously limited by paucity of case material. Nevertheless, in 2 instances appropriate specimens for growth hormone studies have been obtained.

10. Complete endocrinological study of a patient with Lawrence-Moon-Biedl syndrome and of another with Sjorgen's syndrome revealed no distinct endocrinopathic pattern in these cases.

11. A new progestational compound, 19-nor ethisterone previously found by us to be 5 times as active as oral progesterone in rabbits and monkeys has been assayed in 5 patients for oral progestational potency. Endometrial biopsy showed that this compound has the same enhanced potency in the human as was shown experimentally. It is now available in amounts sufficient to permit its trial in the therapy of breast cancer.

Significance to cancer research:

Further understanding of the conditions permitting the excessive hormonal production of tumors of endocrine origin is basic to our understanding of the metabolism of neoplastic tissue generally. In addition, further analysis of abnormalities in somatic growth and development should provide data pertinent to the problems of tissue growth generally.

Proposed course of project:

Further elaboration of these studies with major emphasis on biochemical and pharmacological control of hormone-producing tumors and anomalous tissue growth is projected.

11. BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957:

Dr. Earle's Tissue Culture Unit collaborates in this program.

PROJECT REPORT FORM (Cont'd.)

NCI-803(C)
SERIAL NO.

NO ENTRY FOR ITEM 13.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Green, Stanley, Evans, J. M. and Hertz, R., Acyclic menstrual bleeding associated with erythrocytosis, J. Clin. Endocrinol. & Metab. 15: 199, 1955.

2. Hertz, R., Tullner, W. W., Schricker, J. A., Dhyse, F. G., and Hallman, L. F., Studies on Amphenone and related compounds, Recent Progress in Hormone Research, Vol. 11, pp.119-147, 1955.

3. Hertz, R., Waite, J. H., and Thomas, L., The progestational effectiveness of 19-nor-ethisterone by oral route in women, J. Clin. Endocrinol. & Metab. (In press).

4. Thorn, G. W., Renold, A. E., Goldfien, A., Nelson, D. H., Reddy, W. J., and Hertz, R., Inhibition of corticosteroid secretion by Amphenone in a patient with adrenal cortical carcinoma, New Eng. J. Med. (In press).

5. Hertz, R., Pittman, J. A., and Graff, M. M., Amphenone: Toxicity and effects on adrenal and thyroid function in man, J. Clin. Endocrinol. & Metab. (In press).

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Translation of paper entitled "Studies on Amphenone and Related Compounds" into Spanish and published in "Revista Argentina de Endocrinologia y Metabolismo, Buenos Aires, Argentina, 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Endocrinology Branch — LABORATORY OR BRANCH
3. Endocrinology Service — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA
5. NCI-804(C) — SERIAL NO.

6. Biotin and the Avidin-Biotin Complex in Relation to Normal and Neoplastic Tissue Growth — PROJECT TITLE

7. Roy Hertz, Frederick G. Dhyse and William W. Tullner — PRINCIPAL INVESTIGATOR(S)

8. Dr. Lloyd Law — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION A) Objectives: 1. To ascertain the role of biotin, a dietary trace factor, in the growth of normal tissue and in the development of neoplasms in animals and man.

2. To learn the normal metabolism of biotin in man and to produce biotin deficiency in cancer patients administering low biotin diets plus concentrates of avidin prepared from egg-white.

3. To develop methods for the cheap and practical preparation of large amounts of avidin for therapeutic trial in selected cancer cases.

B) Methods: 1. Standard methods of protein fractionation of egg white are employed to prepare potent avidin concentrates which are assayed for growth suppressing effect on a biotin requiring yeast organism.

2. Biotin deficiency is produced in mice by feeding egg white and avidin concentrates and the effect of this deficiency on such standardized tumors as transplantable leukemia is determined in collaboration with Dr. Lloyd Law.

3. Patients are carried on diets of known and constant biotin content and the urinary biotin excretion is followed microbiologically. The effects of egg white feeding and avidin feeding are observed upon the patient's clinical nutritional status and upon the urinary excretion of biotin.

C) Major Findings: 1. An excellent, economical method of preparing potent concentrates of avidin has been developed and a stock pile of this material for clinical and experimental use has been prepared.

A large industrial laboratory with ready access to abundant supplies of fresh egg-white has adapted this method to mass scale and is now providing our first adequate supplies of avidin for extended clinical trial.

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. _____ (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA) 5. NCI-805C (SERIAL NO.)

6. Effect of Steroid Therapy on Metabolism of B-Complex Factors in Cancer Patients (PROJECT TITLE)

7. Roy Hertz and Frederick G. Dhyse (PRINCIPAL INVESTIGATOR(S))

8. Phillip Rayford (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION -

Objectives: To determine the effect of protein anabolic action of steroid therapy in cancer patients upon the metabolism of B-complex factors consumed in the diet.

To correlate the foregoing with specific therapeutic effects obtained.

Methods employed: Patients are maintained on a fixed diet and their dietary intake determined by a balance procedure. Continuous urine collection provides specimens for determining urinary excretion of nitrogen, biotin, riboflavin, niacin, folocin, and pantothenate (See project 800C).

Patient material:

	No.	Average Stay Days
Admissions: Adult females	2	90
Outpatients: None		

Proposed course of project: It is proposed to develop and extend these studies as a new project largely by continuing studies already in progress. Observations would be made in suitable patients with androgen-induced protein-anabolism and in cortisone-induced protein-catabolic states. In this manner the relationship of B-complex balance to nitrogen balance may be determined with a view to clarifying the mechanism of action of these steroids on protein metabolism generally.

NCI-805C
SERIAL NO.

PROJECT REPORT FORM (Cont'd)

Proposed course of project: (cont'd.)

The paired tray technique has been replaced by full metabolic study. This more exact technique will provide necessary control data regarding the differences between androgen-induced nitrogen retention and simple retention following increased intake.

11. BUDGET ACTIVITY:

RESEARCH	☐	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957:

Dr. Donald Watkin, General Medicine Branch, is collaborating in completing the studies on patients on the complete balance regime.

NO ENTRIES FOR ITEMS 13, 15 & 16.

PROJECT REPORT FORM

1. N. C. I. (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-806(C) (SERIAL NO.)

6. Endocrine Characterization of Females with Cancer of the Larynx
PROJECT TITLE

7. Roy Hertz, Morris M. Graff and Lois F. Hallman
PRINCIPAL INVESTIGATOR(S)

8. James Pittman and Harold Altman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: 1. To determine the endocrinological characteristics of the relatively rare female patient with cancer of the larynx in order to assess the role of endocrine factors in the pathogenesis of this disease.

Methods employed: Women with proven cancer of the larynx will be hospitalized and a complete endocrine evaluation made by: 1) recording a complete pubertal, menstrual, and reproductive history and any other pertinent endocrine facts regarding the patient and her family; 2) complete physical appraisal of endocrine make-up with emphasis on obesity, masculinity, size of clitoris, hirsutism, voice pitch, acne, baldness, vaginal smear and other endocrine factors; 3) determination of urinary corticoids, estrogens, ketosteroids, and gonadotropins; also glucose tolerance, B.M.R., protein bound iodine, eosinophile response test and other measures of endocrine functions.

These data would be collected in order to determine whether there is any evidence of gonadal or adrenal androgenization of the female who gets cancer of the larynx as compared with normal females of the same age group.

Major findings: Preliminary superficial clinical observation of 2 females with cancer of larynx has revealed no striking difference.

Significance to Cancer research: Literature review indicates that the sex ratio for cancer of the larynx is variously estimated as between 16 to 1 and 200 to 1. In any case it is clear that females are relatively rare among patients with laryngeal cancer.

PROJECT REPORT FORM (Cont'd.)

NCI-306(C)
SERIAL NO.

Significance to cancer research (cont'd.)

The larynx is a secondary sex organ in that its pubertal differentiation is hormonally dependent. Moreover, androgen administration readily alters the larynx in the human female but no case of cancer of the larynx in the human female has yet been reported following androgen administration.

Proposed course of project: This project is deferred pending additional clinical facilities for the admission of appropriate patients.

11. BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

NO ENTRIES FOR ITEMS 12, 13, 15 & 16.

PROJECT REPORT FORM

1. N.C.I. (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-807(C) (SERIAL NO.)

6. Methods of Steroid Analysis in Urine and Blood from Cancer Patients
PROJECT TITLE

7. Morris M. Graff
PRINCIPAL INVESTIGATOR(S)

8. Silas Jackson, Foster Burnett and Willie Logan
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION -

Objectives: To develop and improve the chemical methodology for determination of urinary corticoids and ketosteroids and blood corticoids, estrogens, and androgens.

Methods employed: Classical methods of reagent and enzymatic hydrolysis, chromatographic adsorption and elution, paper chromatography, and spectrophotometric determination of specifically developed chromogens. Chemical methods to be correlated with equivalent bioassays available under Project 806.

Major findings: Methods for urinary ketosteroids and corticoids have been developed and perfected and are now adaptable for clinical studies.

Blood corticoid methods have been investigated and an improved method devised which is now available for clinical research studies involving assessment of adrenal corticoid function.

Progress in the development of a clinically useful method for estrogen determination in body fluids has been very limited. However, certain technical difficulties have been overcome.

Significance to cancer research: These methods are basic to the endocrinological study of patients with cancer of the breast, prostate, cervix, and larynx.

Proposed course of project: The further extension of methodological investigation is proposed especially for blood estrogen and urinary estrogen by fluorometric methods. Extended investigation of the use of the Anthrone reagent in steroid analysis is planned.

Methods for the fractionation of alpha and beta ketosteroids and for pregnandiol determination are to be developed in relation to further endocrine characterization of patients

PROJECT REPORT FORM (Cont'd).

NCI-807(C)
SERIAL NO.

Proposed course of project (cont'd.)

with tumors of adrenal, testis, or ovary.

11.

BUDGET ACTIVITY:

RESEARCH	☐	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

NO ENTRIES FOR ITEMS 12, 13, 14, & 16.

PROJECT REPORT FORM

1. N. C. I. (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-808C (SERIAL NO.)

6. Endocrine Factors Governing Hormone-Induced Tissue Growth and Tumor Formation in Animals; Bioassay of Blood and Urine from Cancer Patients (PROJECT TITLE)

7. Roy Hertz, William W. Tullner, Donald Spencer, D. M. Bergenstal (PRINCIPAL INVESTIGATOR(S))

8. Sally B. Fand and Morris M. Graff (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives:

1. To gain basic information concerning endocrinological and nutritional factors governing hormonally conditioned growth and involution in organs such as the uterus, breast and prostate which are the frequent seat of hormone-sensitive neoplasms.

2. To develop and improve bioassay methods for the quantitation of hormones in the blood and urine of patients with cancer who are either receiving various forms of hormonal therapy or who present unusual hormonal patterns.

3. To determine the biological properties of steroid and related compounds in terms of their potential usefulness in cancer therapy.

Methods employed:

Standard methods of biological study are employed. These include: 1) the maintenance of animals of various species on specified hormonal or nutritional regimens; 2) daily observations of physical and behavioral changes in such animals; 3) complete autopsy of test animals with detailed weighing and chemical analysis and histochemical study of specific organs; 4) surgical alteration of the endocrine status of animals by gonadectomy, adrenalectomy, hypophysectomy, thyroidectomy, parabiosis, hysterectomy and other operative procedures.

Major findings:

These are detailed in the publications listed under Item 15. They may be briefly enumerated as follows:

PROJECT REPORT FORM (Cont'd.)

NCI-808(C)
SERIAL NO.

Major findings (cont'd.)

1. The biological properties of Amphenone in relation to adrenal cortical function have been extensively studied. Those findings were presented before the Laurentian Hormone Conference. Conclusive evidence of interference with corticoid synthesis by Amphenone in the dog was presented. These data are derived from quantitative corticoid determination in the adrenal-vein blood of the hypophysectomized dog given variable doses of ACTH both with and without Amphenone. A marked suppression in response of the adrenal to exogenous corticoid is readily demonstrated.

A series of analogues of Amphenone have been synthesized and studied. These include alpha, beta, dimethyl-4 4'stilbenediamine, the dimethylamino derivative of Amphenone, the oxine derivative of Amphenone, and the butanone derivative of Amphenone. These compounds share the biological properties of Amphenone and further differential studies are being made with respect to relative toxicity and central nervous system depressant effect. Twenty-five related compounds have been found to be inert, indicating a relatively high degree of specificity for these interesting endocrinological effects.

Extended work with these and related substances, such as DDD and Germanin, is projected with a view to deriving a non-toxic, specific inhibitor of adrenal-cortical function.

Chromatographic analysis of the steroid content of the adrenal vein blood of the Amphenone-treated animals is also to be carried out.

2. Further studies on potent anti-gonadotrophic sera have led to some degree of purification of the active inhibitory material. These efforts are to be extended by means of specific immunochemical adsorption and elution techniques now being developed.

3. Additional steroids have been screened for possible dissociation of pituitary depressant action from other biological effects. The studies on the biological properties of allopregnane, 21-ol-3, 20 dione and Reichstein's compound S have been completed and reported. Assays of the new corticoids, metacortandrosin and metacortandrolone have indicated no material dissociation of pituitary depressant action and enhanced corticoid action.

4. Extension of studies on the local catabolic function involved in the involution of hormone sensitive organs following gonadectomy substantiate the complete quantitative and chronological independence of this process from general catabolic effects on the body as a whole

5. Substantial progress has been made on a sensitive and highly specific assay for luteinizing hormone. A normally cycling female

PROJECT REPORT FORM (Cont'd.)

NCI-808(C)
SERIAL NO.

Major findings.(cont'd.)

rat is followed by vaginal smears until she is found to be in the pre-ovulatory or pre-estrus phase which is characterized by the appearance of very large numbers of large rounded, nucleated epithelial cells in the vaginal smears. Our previous studies had shown that hypophysectomy at this point will abort the succeeding ovulation. Since this ovulation depends entirely on luteinizing hormone, a sensitive and specific assay is based on the restoration of ovulation by injection of such hormone preparations. It is hoped that this will permit us to assay for this hormone in the blood and urine of cancer patients under steroid regimens.

6. A strain of guinea pigs has been identified which has a large number of hypogonadal males. These animals have normal mating behavior but have complete aspermia and extremely atrophic testes. A complete endocrine, behavioral, and anatomical study of these animals is being undertaken in collaboration with Dr. W. C. Young, Prof. of Anatomy at the University of Kansas.

7. The hormonal factors involved in the post-natal differentiation of the ovary in the rat and rabbit have been investigated. A post-natal period of 10 days in the rat and of 10 weeks in the rabbit has been characterized as a period during which the ovary is completely insensitive to pituitary gonadotropin stimulation. During this period the ovary undergoes the first delineation of normal follicular structure, the final step being that of antrum formation. It is only such antrum-containing follicles that respond to gonadotropin administration. Moreover, the ovary of a new-born rat grafted to its mother's kidney will undergo normal post-natal differentiation even when the mother had been completely hypophysectomized. Further studies on the possible role of the hypothalamus in this process are under way.

8. The overlap between progestational and corticoid action of certain steroids is well exemplified by the fact that desoxycorticosterone is about 10% as active as progesterone for progestational effect. We now find that whereas, hydrocortisone lacks progestational activity, the introduction of the fluorene atom in the 9 position imparts not only increased corticoid potency but also renders the compound progestationally active at about 5% the activity of progesterone itself in both the monkey and rabbit. Similarly the fluorination of 11 beta hydroxyprogesterone imparts progestational activity to this compound at a level equal to 10 percent the activity of progesterone. These observations indicate that fluorination has implications not only for corticoid action but also for gonadal steroid properties. Moreover, the availability of compounds having combined corticoid and progestational action provides an opportunity

PROJECT REPORT FORM (Cont'd.)

NCI-308(C)
SERIAL NO.

Major findings (cont'd.)

for study of a compound which will proliferate the uterine epithelial structures at the same time that stromal resistance is depressed. Long-term studies are in progress to determine what the final result of such tissue changes may lead to in the way of architectural derangement possibly leading ultimately to malignancy.

9. We have previously reported that a five-fold enhancement of progestational activity in the rabbit is effected by demethylation of progesterone at the 19 position, yielding 19-nor-progesterone. Similarly, 19 nor-ethisterone was shown to have the same increased progestational action in the rabbit when orally administered. These studies have now been extended to the monkey, employing the inhibition of estrogen-withdrawal bleeding and the production of a secretory endometrium. Both compounds show comparably enhanced effectiveness in the monkey. These studies have led to similar observations in the human (see project 803, item 9 C11). Thus the quantitative ratios are similar in rabbit, monkey, and human.

10. Recent studies on the effect of hypophysectomy on breast cancer have implicated the pituitary growth hormone as a direct trophic influence on breast cancer. It becomes imperative to be able to measure the level of growth hormone in human blood or urine. The rat's tibial cartilage and costal cartilage have been shown to respond to administered growth hormone by increased uptake of radioactive sulfur. Preliminary studies suggest that this may provide a sufficiently quantitative and sensitive index of the action of growth hormone to permit its assay at much smaller levels than current histological methods permit.

11. Detailed histochemical analysis of the reaction of hormone sensitive tissues and tumors to altered hormonal states have been initiated. Examples are: (a) the relationship of initial fat accumulation to the post-natal differentiation of the ovary; (b) the relationship of carbonic-anhydase to the progestational response of the rabbit and monkey uterus; (c) the histochemical characterization of the lipoidal substance present in the adrenal of the Amphenone-treated rat, and (d) the relationship between the functional state of the human pituitary and specific enzymatic content as demonstrated histochemically.

12. The role of dietary trace factors in hormonal response has been studied in particular relationship to the activation of the adrenal gland of the hypophysectomized dog by exogenous ACTH. It has been demonstrated that the pantothenate, riboflavin, biotin, folacin, pyridoxine and ascorbic acid content of the adrenal gland remains unchanged when the gland is at complete rest as manifested by low corticoid content of the adrenal vein effluent or after

PROJECT REPORT FORM (Cont'd.)

NCI-808(C)
SERIAL NO.

Major findings (cont'd.)

maximum activation by exogenous ACTH leading to enormous elevation in corticoid content of the adrenal vein blood. Moreover, the adrenal vein blood in either state shows no change in its content of these trace factors despite the very marked rise in corticoid content. Thus it appears that these trace factors are not quantitatively critical for this type of immediate hormonal response to ACTH.

Proposed course of project:

It is expected to continue and extend the foregoing studies along the lines already indicated.

11.

BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

NO ENTRIES FOR ITEMS 12 & 13

15.

PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. The effect of 17-alpha-hydroxy-11-desoxycorticosterone on estrogen-stimulated chick oviduct growth, by W. W. Tullner and R. Hertz. (Endocrinology)(In press).

2. Progestational activity of the halogenated corticosteroids and related compounds in the rabbit and monkey, by R. Hertz, and W. W. Tullner (Proc. Soc. Exper, Biol. & Med.) (In press).

3. Amphenone inhibition of adrenal corticosteroid output in the hypophysectomized dog, by W. W. Tullner, M. M. Graff and R. Hertz (Endocrinology).(In press).

4. High progestational activity of nor-ethisterone and norprogesterone in the monkey, by R. Hertz and W. W. Tullner. (Endocrinology). (In press).

PROJECT REPORT FORM (Cont'd.)

NCI-308(C)
SERIAL NO.

15. Publications (Cont'd.)

5. Vitamin content of dog adrenals and adrenal vein blood before and after ACTH, by R. Hertz, W. W. Tullner, M. Graff and J. A. Schricker. (Proc. Soc. Exper. Biol. & Med.) (In press).

6. Studies on Amphenone and related compounds, by R. Hertz, W. W. Tullner, J. A. Schricker, F. G. Dhyse, and L. F. Hallman. (Recent Progress in Hormone Research, Vol. 11, pp. 119-147, 1955).

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Mr. William W. Tullner was admitted to the Graduate Council of George Washington University as a candidate for the degree of Doctor of Philosophy in Physiology to be conferred in June 1956.

PROJECT REPORT FORM

1. N. C. I. (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-809(C) (SERIAL NO.)

6. The Preparation of Crude Natural Extracts of Hormonal Activity from Ovary, Testis, Adrenal and Pituitary and their Biological and Clinical Characterization. (PROJECT TITLE)

7. To be recruited. (PRINCIPAL INVESTIGATOR(S))

8. (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives: To prepare for biological and clinical assay crude preparations of hormonally active tissues in order to determine their effect upon tumors in hormone-sensitive organs. We know the crystalline steroids now so widely used represent only partial elements of the over-all activity of the various endocrine organs and that even optimum mixtures of them do not reproduce the characteristic effect of the total internal secretion of the endocrine gland itself. Such effects may be more profound and more desirable than those obtained by the separate synthetic crystalline factors.

Methods employed: 1. Standard chemical methods of total lipid and protein extraction of freshly prepared tissues to be collected at the slaughter house and processed immediately.

2. The application of bioassay techniques to such preparations to determine their endocrine effectiveness and their toxicity.

3. Clinical trial of non-toxic, active materials in suitable patients with endocrine deficiency or cancer of breast, prostate or cervix.

NO ENTRIES FOR ITEMS 11, 12, 13, 15 & 16.

N.B. This project has not been activated due to failure to recruit properly qualified professional personnel.

. PROJECT REPORT FORM

1. N. C. I. — INSTITUTE
2. Endocrinology Branch — LABORATORY OR BRANCH
3. Endocrinology Service — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-810(C) — SERIAL NO.

6. Studies in Thyroid Function — PROJECT TITLE

7. M. G. Sherif — PRINCIPAL INVESTIGATOR(S)

8. Harold Altman, Betty N. Siefring and Paul Marks — OTHER INVESTIGATORS

. PROJECT DESCRIPTION - A) The hepatic clearance of plasma protein bound iodine in man.

Objective: To determine, in man, the role of the liver in the excretion, conjugation, and utilization of thyroid hormone. Results obtained are to be compared with experimental information available from similar work done in animals (rat, mouse, cat, dog).

Methods employed: Hepatic vein catheterization in man; serum or plasma protein bound iodine determinations, hepatic blood flow measurements employing the bromsulfaphthalein technique.

Major findings: The major findings of this project during the past year have been the demonstration of a significantly lower plasma protein bound iodine concentration in blood from the hepatic veins of humans as compared to the concentration of plasma protein bound iodine in the peripheral veins or femoral artery blood of the same subject. This enables one to calculate the amount of protein bound iodine removed by the liver and gives a measure of the entero-hepatic circulation of thyroxine; the first such information available in man.

Proposed course: See notation attached to page 1.

B) The effects of various steroids on thyroid function and the metabolic fate of thyroxine.

The effect of estrogens, androgens and corticoids on thyroid function and the metabolic fate of thyroxine.

Methods employed: Radioiodine 24 hour tracer uptakes; thyroid gland hormone secretion rates; paper chromatography of protein bound radioiodine compounds, serum protein bound iodine determinations; disappearance rate measurements of blood radiothyroxine; serum protein thyroxine binding capacities.

PROJECT REPORT FORM (Cont'd.)

NCI-810(C)
SERIAL NO.

PROJECT DESCRIPTION (Cont'd.)

Major findings: a) Estrogens cause a significant rise in serum protein bound iodine without a concomitant rise in 24 hour tracer radioiodine uptake of the thyroid gland; b) secretion rate: in 2 patients, so far, one has had no change in secretion rate with estrogen administration; one has had slowing of secretion rate with estrogen administration; c) testosterone causes a marked fall in serum protein bound iodine and 24 hour radioiodine tracer uptake by the thyroid gland; d) Metacortandrocin and Metacortandrolone, corticoids similar to hydrocortisone, cause a marked fall in uptake and serum protein bound iodine; e) paper partition chromatography has failed to reveal any qualitative change in the iodinated compounds released by the thyroid under the influence of the steroids mentioned above.

Proposed course: It is proposed, during the next calendar year to extend these studies to a greater number of cases and to employ radioactive iodine labelled thyroxine for tissue utilization studies during control and steroid administration periods in the same patient.

C) Extra thyroidal effects of thyroid stimulating hormone; TSH effect on thyroxine to triiodo-thyronine conversion:

Methods employed: Measurements of serum or plasma protein bound iodine, rate of radiothyroxine utilization in vivo by scintillation gamma counting of blood protein fractions in sequential fashion.

Major findings: One case studied so far over 2 week period. No change in thyroxine utilization rate was found.

Proposed course: Extension of present study to several patients including radiothyroxine utilization studies.

D) In vitro conversion of thyroxine to triiodo-thyronine by surviving tissue slices and homogenates, and the factors influencing this interconversion.

Objectives: To confirm and extend the work of Albright and Larsen who demonstrated in vitro conversion of thyroxine to triiodothyronine by rat kidney slices. To isolate the enzyme systems responsible for the deiodination involved and to ascertain the factors influencing this enzyme's action.

Methods employed: Surviving tissue slices, tissue cultures and homogenates incubated with radioiodine labelled l-thyroxine. The products of these incubations are then analyzed by paper partition chromatography.

PROJECT REPORT FORM (Cont'd.)

NCI-810(C)
SERIAL NO.

PROJECT DESCRIPTION (Cont'd.)

Major findings: No conversion to triiodo-thyronine by mouse liver tissue cultures; slight appearance of triiodo thyronine after incubation with monkey surviving kidney slices and homogenates in phosphate buffer.

Proposed course: This project was discontinued due to separation from the Service of the principal investigator. No additional progress is reported at this time.

Significance to cancer research: Further elucidation of our knowledge of the mechanism of action of the steroid hormones on peripheral tissues may be expected to help explain some of the favorable and unfavorable effects on cancer of the breast, prostate and uterus.

11. BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

12. COOPERATING UNITS, OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957:

Dr. Paul Marks, NIAMD cooperated in this project.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The effect of meticorten and meticortelone on thyroid function, by M. G. Sherer and B. N. Siefring, J. Clin. Endocrinol. & Metab. (In press).

NO ENTRIES FOR ITEMS 13 and 16.

PROJECT REPORT FORM

1. N. C. I. (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-811(C) (SERIAL NO.)

6. Studies on Thyroid Adenomata and Related Problems in Pituitary-Thyroid-Hypothalamic Relationships (PROJECT TITLE)

7. Monte A. Greer (PRINCIPAL INVESTIGATOR(S))

8. Leslie J. De Groot, Eleanor Siperstein, Howard Erwin and Robert O. Scow (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION - Correlation of histologic morphology and metabolism of simple goiter with its response to thyroid therapy

Objectives: To determine whether there is any correlation between the microscopic appearance and metabolic activity of various types of simple goiter with their diminution in size when desiccated thyroid therapy is administered.

Methods employed: Studies of the metabolic activity of simple goiter are made with I-131 tracer studies. A wedge biopsy is then made of the gland and 3 weeks later administration of 3 grains daily of desiccated thyroid begun. At the end of a 4-6 month period a final biopsy is performed. In the case of single nodules, the entire nodule is removed at the end of the therapy period.

Patient material:

		No.	Average Stay Days
Admissions:	Adult males	4	15
	Adult females	7	29
Outpatient:	Number of patients	15	
	Number of visits	77	

Major findings: During the past year, only 4 such patients have been studied. These included cases of simple goiter, chronic thyroiditis, and single thyroid adenomas. All goiters thus far studied have experienced considerable diminution in size.

Additional findings of note are indicated by the titles of publications listed below under Item 15.

Proposed course: A continuation of the study as outlined above is planned for the coming year.

PROJECT REPORT FORM (Cont'd.)

NCI-311(C)
SERIAL NO.

PROJECT DESCRIPTION (CONT'D.)

B) Isolation and identification of precursor of naturally occurring antithyroid compound, 1-5 vinylthiooxazolidone.

Objectives: To isolate, crystallize, and identify the structure of the precursor of the only known naturally occurring antithyroid substance, venylthiooxazolidone. The precursor may be of a type that is more widespread among various plant foods than is now believed.

Methods employed: Brassica seed extracts are fractionated by various procedures, including column chromatography, after first heat denaturing the thioglycosidase which specifically hydrolyzes the precursor. Assay of the fractions is greatly facilitated by following the UV abortion spectrum. The precursor has a specific peak absorption at 2270 A°. Crystallization and identification of the pure compound are by standard chemical and microchemical procedures.

Major findings: During the past year, the precursor has been purified and crystallized. It has been found to be a thioglycoside having a molecular weight of about 1000, m.p. 130°C. Both sodium, potassium, and SO4 -- have been identified in the molecule. The glycone has been tentatively identified as a fructose polymer.

Proposed course: It is planned to continue with degradation experiments to work out the structure of the compound and to determine by animals and human assay whether any biologic system not containing the specific thioglycosidase found in Brassica members is capable of liberating thiooxazolidone from it.

C) Stimulation of the central nervous system of rats by radiofrequency transmission.

Objectives: To devise apparatus capable of stimulating the central nervous system, particularly the hypothalamus, of rats. The animals are to be completely unrestrained and without any external connections to the pulse generating equipment. It is hoped to produce hyperfunction of the pituitary by stimulation of those areas which, when destroyed by electrolytic lesions, result in decreased hypophysial function. In this way, considerable information may be gained by neuroendocrine interrelationships.

Methods employed: A technique has been devised for fixing radio receivers to the skull of rats, with stimulating electrodes buried according to calculation in various parts of the hypothalamus. A ratio sending and receiving apparatus has been devised to carry variable pulses to the hypothalamic electrodes. The receiver in the rat is entirely subcutaneous. It is possible to stimulate 36 or more rats at

PROJECT REPORT FORM (CONT'D).

NCI-811(C)
SERIAL NO.

PROJECT DESCRIPTION (Cont'd.)

one time and with variable intensities and frequencies.

Changes in hypophysial function are measured by gross and cytologic changes in the pituitary and target endocrine glands and by noting alterations in the functional activity of the target glands.

Concomitantly observations are made of the behavioral changes produced in the animals by stimulation.

Major findings: During the past year, the major emphasis has been on developing equipment and techniques to a satisfactory level. It has been discovered that stimulation of the hypothalamic area lateral and just posterior to the paraventricular nuclei will induce marked drinking behavior in the rats. Stimulation slightly posterior to this area will induce marked eating behavior.

Proposed course: The major emphasis during the coming year will be devoted to working out the "bugs" in the stimulation system and develop it to the point where stimulation can be continued for several weeks without any appreciable loss of response in the stimulated animal.

D) Study of the structure and function of heterotopic compared with normal pituitaries.

Objectives: To determine the alterations in structure and function produced in the pituitary gland by transplanting it to an abnormal site and to determine what factors are involved in the changes thus produced.

Methods employed: Pituitaries from new born mice are transplanted into the eye or kidney of genetically homogeneous mature mice. The hosts are hypophysectomized one week later. Three weeks following hypophysectomy the function of the heterotopic pituitaries is measured by following changes in body growth and changes in the metabolism and structure of the target endocrine glands. Pituitary cytology is studied by the various advanced techniques now available.

Major findings: 1. Heterotopic pituitaries are able to maintain thyroidal I-131 metabolism at a level approximately equal to that in intact animals. They are not able to maintain thyroid weight or the weight of other target endocrine organs significantly above that of hypophysectomized animals. Raising or lowering the level of circulating thyroxin will cause marked changes in the secretion of the pituitary hormone controlling thyroidal I-131 metabolism in these animals without having any significant effect on thyroid weight.

PROJECT REPORT FORM (Cont'd.)

NCI-811(C)
SERIAL NO.

PROJECT DESCRIPTION (Cont'd.)

2. Glycogen has been demonstrated in the pituitary for the first time. This is present particularly in the younger glands and in highest concentration in the pars tuberalis.

3. The differences in cytology of heterotopic compared to in situ pituitaries has been compared for various ages. One of the most striking differences is the marked diminution in chromophiles in the heterotopic glands. There is also a marked hypertrophy of the pars intermedia comparable to that seen following hypothalamic lesions.

Proposed course: The major effort during the coming year will be devoted to a study of whether function of the heterotopic pituitaries can be increased by transplanting various parts of the central nervous system, particularly the hypothalamus, in direct connection with the pituitary transplants. A system has already been devised for making transplants of considerable size to the kidney of adult mice.

It will also be determined whether the heterotopic pituitaries secrete the substance necessary for ovarian and uterine response to chorionic gonadotropin. A further study will involve whether they can concentrate isotopically labelled triiodothyronine to as marked an extent as do normal glands.

E) The effect of hypothalamic lesions on pituitary function.

Objectives: To determine what effect electrolytic lesions in various parts of the hypothalamus have upon the regulation and secretion of the various hormones produced by the pituitary gland.

Methods employed: Electrolytic lesions are made in the hypothalamus of the rats by means of a specially designed stereotaxic instrument. Following a period of 1-3 weeks for recovery, studies are made of the ability of the pituitary to secrete the various trophic hormones. These determinations are made by studying the alterations of structure and function produced in the target endocrine glands by various stimuli. For example, thyrotrophin secretion is studied by the change produced in thyroid size and I-131 metabolism by chronic administration of propylthiouracil.

Major findings: During the past year it has been found that the location of the hypothalamic area controlling thyrotropin secretion in the rat lies in the midline between the paraventricular nuclei and the median eminence. Destruction of all or part of this area will markedly interfere with thyrotrophin secretion.

PROJECT REPORT FORM (Cont'd.)

NCI-811(C)
SERIAL NO.

PROJECT DESCRIPTION (Cont'd.)

It has also been found that although lesions in the above area will prevent the goitrogenic response to Amphenone administration, they will not prevent the usual concomitant adrenal hypertrophy. This provides further evidence that the hypothalamic areas controlling the various pituitary functions are discrete.

Proposed course: It is planned to make a study of the location of the hypothalamic area responsible for maintaining normal growth of the pars intermedia of the hypophysis. Material from over 500 rats with hypothalamic lesions has already been assembled and the remaining requirement is to determine how far from the hypophysial stalk it is necessary to place lesions in order to avoid the gross and bizarre hypertrophy of the pars intermedia which occurs with lesions close to the stalk.

It is also planned to investigate the area controlling ACTH secretion more thoroughly and to see if it is possible to place lesions that will interfere with ACTH secretion as much as previous lesions have been interfered with thyrotropin secretion.

F) Study of the mechanism of action of stable iodine in thyrotoxicosis.

Objectives: To determine the mechanism of action of stable iodine in ameliorating thyrotoxicosis and, secondarily, to gain some insight into the pathogenesis of thyrotoxicosis.

Methods employed: Thyrotoxic and euthyroid subjects are given a 100 microcurie tracer dose of I-131. Twenty-four hours later the administration of 30-60 mg. tapazole every 8 hours is begun to prevent reaccumulation in the thyroid of I-131 broken down from endogenously labelled thyroxin. In vivo counting over the thyroid is made by means of an externally placed scintillation counter. The thyroidal secretion rate can thus be determined by plotting on semilog paper.

When the rate has been established, 300 mg. per day of NaI are added to the regimen. When the maximum effect from iodine has been obtained, 10-100 units of thyrotrophin are given daily for a period of several days.

In the case of the control euthyroid subjects, thyrotrophin was frequently given before iodine administration was begun.

Major findings: It has been found that iodine interferes with the release of hormone from the thyroid gland in thyrotoxic subjects but has little, if any, effect in euthyroid subjects. It has also been found that iodine does not interfere with the stimulation of euthyroid glands by exogenous thyrotropin.

PROJECT REPORT FORM (Cont'd.)

NCI-311(C)
SERIAL NO.

PROJECT DESCRIPTION (Cont'd.)

These results suggest that iodine acts in hyperthyroidism by inhibiting the release of thyrotrophin from the pituitary. Therefore, hyperthyroidism must be a suprathyroidal disturbance and not solely a disease of the thyroid gland.

Proposed course: The project is terminated due to the separation of Dr. Monte A. Greer.

Significance to cancer research: Studies of thyroid adenomata will have a direct bearing on the problem of the pathogenesis of thyroid cancer. In addition, further knowledge of the pituitary-thyroid interrelationship will provide potential means of controlling abnormal thyroid growth.

11.

BUDGET ACTIVITY:

RESEARCH	☐	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

12.

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957:

Dr. Robert O. Scow, NIAMD, collaborated.

NO ENTRY FOR ITEM 13.

15.

PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Demonstration of thyroidal response to exogenous thyrotropin in rats with anterior hypothalamic lesions, by M. A. Greer Endocrinology (In press).

2. Observations on the morphology and histochemistry of the mouse pituitary implanted in the anterior eye chamber, by E. R. Siperstein and M. A. Greer, J. Nat. Cancer Inst. (In preparation).

3. Effect on the thyroid gland of experimental alteration of the level of circulating thyroxine in mice with heterotopic pituitaries, by R. O. Scow and M. A. Greer, Endocrinology: 56: 590, 1955.

PROJECT REPORT FORM (Cont'd.)

NCI-S11(C)
SERIAL NO.

15. PUBLICATIONS (Cont'd.)

4. Histochemical demonstration of glycogen in the mouse pituitary, by Eleanor R. Siperstein, Proc. Soc. Exper. Biol. & Med. 88: 296, 1955.

5. Suggestive evidence of a primary"drinking center" in hypothalamus of the rat, by M. A. Greer, Proc. Soc. Exper. Biol. & Med. 89:59, 1955.

6. A modified Krieg stereotaxic instrument for producing intracranial lesions in the rat, by Monte A. Greer, Proc. Soc. Exper. Biol. & Med. 89: 480, 1955.

NO ENTRY FOR ITEM 16.

PROJECT REPORT FORM

1. National Cancer Institute 2. Pathologic Anatomy Branch
INSTITUTE LABORATORY OR BRANCH

850

3. Surgical Pathology & Post-Mortem Service 4. _____ 5. NCI-~~852~~
SECTION OR SERVICE LOCATION SERIAL NO.

6. No specific research projects in this Branch. However, the diagnostic service is designed to aid the investigative efforts of the N.I.H. staff responsible for patient care. In many instances, investigative study of patholcgical tissues is of great importance to clinical research.
PROJECT TITLE

7.
PRINCIPAL INVESTIGATOR(S)

Dr. Harold L. Stewart, Chief, Pathologic Anatomy Branch
* Dr. Louis B. Thomas, Head, Surgical Pathology & Post-Mortem Service
* Dr. Albert W. Hilberg, Head, Cytodiagnosis Service
* Dr. John H. Edgcomb, Pathologic Anatomy Branch
* Dr. Alan S. Rabson, Resident, Pathologic Anatomy Branch
* Dr. Richard L. Swarm, Laboratory of Pathology, N.C.I.
\+ Dr. Clyde Dawe " " "
\+ Dr. Eli Nadel " " "
\+ Dr. Joseph Leighton " " "
\+ Dr. William Banfield " " "
\+ Dr. C.Harold Steffee " " "
\+ Dr. Edwin Lerner, Laboratory of Pathology and Histochemistry, N.I.A.M.D.
\+ Dr. Leon Sokoloff " " " "
\+ Dr. Harold Stanley, Oral Pathologist, N.I.D.R.

* - These pathologists spend full time in the service and diagnostic functions of the Pathologic Anatomy Department, Clinical Center (Pathologic Anatomy Branch, N.C.I.)

\+ - These associate pathologists spend only part time in the activities of the Pathologic Anatomy Department, principally in the performance of postmortem examinations.

8.
OTHER INVESTIGATORS

PROJECT REPORT FORM (Cont'd)

9. PROJECT DESCRIPTION:

No specific research projects. The services and diagnostic functions of the Department during 1955 have included:

a. 148 autopsy examinations.
The average number of postmortems performed by each pathologist was about 15; the range was from 21 to 8 with the smaller number being performed by those who were here less than one year.

b. 1778 surgical pathology accessions.
Most of these were worked up by Doctors Thomas, Edgcomb, Swarm, Rabson, Sokoloff and Stanley.

The objectives of the Surgical Pathology and Post-Mortem Service in the Clinical Center are twofold: first, to furnish a diagnostic service in autopsy and surgically removed tissues, and second, to aid from a morphological standpoint the various clinical research problems which are under study in the Clinical Center.

The methods used are those standard methods which are used in the description of organs and tissues, the fixing and sectioning of this material and the preparation of histological slides. A wide variety of special staining procedures are used in the Histopathology Laboratory of the Department. The appended chart shows the December monthly report of the material studied in this Department and includes in the right hand column totals for the accessions during the calendar year of surgical specimens and autopsies performed. Note that the autopsy rate has doubled that of 1954 and the number of surgical specimens has increased by over 50 per cent.

In addition to the diagnostic services, there are several other functions of the Department which have continued and expanded during the past year. At present there are fifty scientists in the various laboratories at N.I.H. who have requested particular types of tissue from surgical and postmortem specimens examined in this Department. On many occasions it is possible to furnish these investigators with fresh human material.

The staff of the Department continues to take active part at numerous Clinical Center staff meetings, and in addition, routinely conducts four Departmental meetings weekly. These are the Brain Cutting Conference on Monday, the Autopsy Conference on Tuesday, the Joint Pathology Staff Conference on Wednesday and the Surgical Pathology Conference on Friday.

REPORT OF PATHOLOGIC ANATOMY DEPARTMENT

Annual 1955

INSTITUTE AND TYPE OF PATIENT

Service & Type of Activity	NCI		NHI		NIAMD		NMI		NIMH		NINDB		NIDR		TOTAL		REF		TOTAL	
	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU
Postmortem-Pathologic Anatomy Service																				
No.of autopsies performed	94		26		4		13				9				148					
Blocks of tissue	6509		1575		142		728				706				9,660					
Slides	14015		3502		502		1747				1467				21,233					
Slides actually stained	8372		2262		376		1042				899				12,951					
Surgical Pathology																				
Tissue Specimens	1064	14	127		117	2	137	19	1		24	1	84	1	1,554	37		187	1554	224
Slides H-E Staining	4055	29	374		488	6	440	7	1		147	1	116	1	5,621	44		469	5621	513
Frozen Sections	104		14		6		7				6				137				137	
Special Procedures	703	2	215		252	2	223	25			38		9	2	1,435	31		114	1435	145

PROJECT REPORT FORM (Cont'd)

10. NCI 852 (850)
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	/_/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. None
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 14, 15, or 16.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Pathologic Anatomy Branch — LABORATORY OR BRANCH
3. Cytodiagnosis Service — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI 851 — SERIAL NO.

6. Exfoliative cytology applied to diagnostic and research problems in the Clinical Center, N.I.H. — PROJECT TITLE

7. Albert W. Hilberg — PRINCIPAL INVESTIGATOR(S)

8. None — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Project: Application of Exfoliative Cytology to Human Diagnostic and Research Problems.

Objectives: To provide the service of exfoliative cytology for the diagnosis of malignant tumors of various sites in the body, notably the cervix uteri, lungs, stomach, and body cavities. Evaluation of effects of various types of therapy for cancer such as surgery, virus therapy, and endocrine therapy. To evaluate effects of hormones in non-cancer patients studied for endocrine balances. Further objectives involve attempts to better evaluate the cytological criteria of malignancy.

Methods Employed: Routine Papanicolaou staining and careful cytological screening procedures together with concurrent tissue and clinical evaluation. Additional methods involve special fixation preparation and staining techniques as developed in histochemical procedures and as needed for the precise handling of specimens of various kinds.

Patient Material: See appended chart.

Major Findings: The correlation of recurrent cancer at the site of surgical procedure and the presence of malignant cells in washings from this site has been made. Several previously undetected cancers of the cervix were found by the methods of exfoliative cytology. The presence or absence of estrogenic activity in prepubertal girls or in cases of amenorrhea was determined in many clinical cases. Evaluation of menstrual cycles. Argyrophil secretion patterns indicating estrogenic activity of a notable degree past the menopause was noted in cases of carcinoma of the cervix uteri and evaluation of this continues.

PROJECT REPORT FORM (Cont'd)

PROJECT DESCRIPTION (Cont'd):

Significance to Cancer Research: This project is a continuing demonstration of the value of exfoliative cytology as a diagnostic tool for the clinician in the attempt to detect early cancer.

The surgical wound washing continues to show distinct and valuable correlation of residual tumor cells and tumor recurrence in surgical sites.

Exfoliative cytology provides an excellent adjunct to tissue evaluation of the effects of various forms of therapy on tumors when it is not possible or practical to remove tissue for biopsy or in conjunction with biopsy study.

Proposed Course of Project: The continuation of diagnostic service to all Institutes in the Clinical Center. Continued collaboration in evaluation of various clinical research projects such as virus therapy of cancer, hormone balance, and wound washings. Further development and refinement of special preparation, fixation and staining techniques as applied to cytologic material. Expansion of activities is planned to meet increased demands for diagnostic service and to further assistance in clinical research.

PROJECT REPORT FORM (Cont'd)

10. NCI 851
SERIAL NO.

11. _____
BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. None to my knowledge
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

13. None to my knowledge
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

14. NCI 851
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Cancer Cell Seeding of Operative Wounds, Smith, Robert R. and Hilberg, Albert W., J. of Nat. Cancer Inst., Dec. 1955.

A New Inexpensive Marking Device for Slides, Del Vecchio, P.R., Ziegler, E., and Hilberg, A.W., J. of Nat. Cancer Inst. (In Press)

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

YEARLY REPORT

Cytodiagnosis Laboratory — January 1 to December 31, 1955

Vaginal & cervical specimens	NCI		NHI		NIAMD		NIMH		NMI		NINDB		NIDR		TOTAL		REF	
	In	FU	In	FU	In	FU	In	FU	In	FU	In	FU	In	FU	In	FU	In	FU
Accessions	395	219	27	5	15	2	2		17	12	10	2	5	4	1147	244	676	
Smears	1103	606	82	14	35	4	6		47	32	38	8	13	14	2728	678	1404	
Prostatic specimens																		
Accessions	5				4	2			1			1			11	3	1	
Smears	14				17	9			1			5			34	14	2	
Gastric specimens																		
Accessions	14				6										20			
Smears	54				42										96			
Bronchial Washings																		
Accessions	103	7	6		5				14	1	1				131	8	2	
Smears	400	21	30		18				32	3	2				487	24	3	
Miscellaneous Fluids																		
Accessions	166	12	8		8				12	1	4	16	1		209	29	10	
Smears	2067	98	102		98				96	5	37	28	2		2466	131	64	
TOTAL																		
Accessions	683	238	41	5	38	4	2		44	14	15	19	6	4	1518	284	689	
Smears	3638	725	214	14	211	13	6		175	40	79	41	15	14	5811	847	1473	

PROJECT REPORT

1. National Cancer Institute
INSTITUTE

2. Laboratory of Physiology
LABORATORY OR BRANCH

3. Cancer Physiology Section
SECTION OR SERVICE

4.
LOCATION (if other than Bethesda)

5. 900
SERIAL NO.

6. The "appetite factor" in Walker 256
PROJECT TITLE

7. Julius White
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To continue the studies of the growth-stimulating factor present in Walker carcinoma 256.

Methods Employed: Fractionation of the Walker 256 with the purpose of isolating the growth factor.

Major Findings:

1. Verification of the previous observations of Dr. G. E. Mider that supplementing the basal diet of animals bearing large transplantable tumors at the point where the animals lose weight and food intake, results in a resumption of food intake and growth increase.

2. Extraction of lyophilized tumor with alcohol-ether does not remove the factor.

3. The protein fraction of the tumor tissue does not contain the appetite factor (preliminary observation).

4. Pair feeding experiments, comparing growth rates of young rats ingesting a 20% casein and a 20% tumor (source of nitrogen) diet, show identical growth curves.

5. Animals ingesting the 20% tumor diet ad libitum show an approximately 25 per cent higher growth increment than do litter mate animals ingesting the 20% casein diet ad libitum.

6. Animals ingesting the 20% casein diet excrete the same total nitrogen as do the pair fed 20% tumor diet.

7. Urea nitrogen excretion of the tumor fed animals is approximately half of that excreted by the casein fed animals.

8. Allantoin excretion from rats ingesting the casein diet is approximately half of that excreted by the rats ingesting the tumor diet.

9. Animals fed the 20% casein diet and bearing Walker 256 transplanted tumors show emaciation and die sooner than do litter mate rats ingesting the tumor diet.

10. Animals ingesting 20% tumor diet and bearing a Walker 256 transplanted tumor grow massive tumors (50 per cent of total tumor and body weight) without loss of appetite or loss in weight.

Significance to Cancer Research: There is, undoubtedly, a factor present in Walker carcinoma 256 which stimulates growth, spares nitrogen and has a high "food efficiency" value.

Proposed course of project: To continue in search of the active principle in tumor tissue which is responsible for this "appetite factor."

10. 900
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW AND APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

NO ENTRIES FOR ITEMS 14, 15 and 16.

PROJECT REPORT

1. National Cancer Institute — 2. Laboratory of Physiology
INSTITUTE — LABORATORY OR BRANCH

3. Cancer Physiology Section — 4. — 5. 901
SECTION OR SERVICE — LOCATION (if other than Bethesda) — SERIAL NO.

6. Probable Sources of Available Nitrogen for Tumor Growth
PROJECT TITLE

7. Julius White
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the availability of nitrogen for the rapidly growing transplanted Walker carcinoma 256.

Methods Employed: Young, growing, as well as adult, rats were placed on a 20% casein diet of known composition. The Walker carcinoma 256 was implanted subcutaneously and, at the same time, N^{15} glycine was incorporated into the diet. At various intervals the animals were sacrificed and the total nitrogen balance was determined.

Major Findings:

1. The uptake of N^{15} glycine by the transplanted tumor was equivalent to that taken up by the host. This occurred whether the isotope was fed for 7 days or 14 days and followed by a 7-day period on the basal diet or sacrificed immediately after the cessation of isotope feeding.

2. In all cases the tumor reached a maximum size of 8 grams. It appears that tumors in their early stages of growth can utilize available nitrogen at the same rate as the host.

Significance to Cancer Research: To obtain a better understanding of the tumor-host relationship. At what stage of tumor growth does the metabolic insult appear in the host? Is the nitrogen present in tumor tissue available under some circumstances for growth of the host? Are the "building blocks" used by the tumor similar to those used by the host?

Proposed course of project: To make a similar study of nitrogen utilization in tumor-bearing animals in which the tumor has reached a growth increment where demand of energy exceeds energy intake. To make serial killings and determine if a transition takes place, where it takes place, and the nature of such an event.

10. 901
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/_/
REVIEW AND APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None.

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

14. 901
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

None.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Elected to membership "Who's Who" in Chemistry.

PROJECT REPORT

1. National Cancer Institute
INSTITUTE

2. Laboratory of Physiology
LABORATORY OR BRANCH

3. Cancer Physiology Section
SECTION OR SERVICE

4.
LOCATION (if other than Bethesda)

5. 902
SERIAL NO.

6. The Effects of X-irradiation on the Nucleic and Protein Synthesis of the Small Bowel Epithelium of the Rat as Related to Cell Division
PROJECT TITLE

7. Julius White
PRINCIPAL INVESTIGATOR(S)

8. Dr. R. Bland Williams, Naval Medical Research Institute;
Dr. James C. Reid and Jane N. Toal.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To correlate changes in nucleic acid and protein synthesis with histopathological changes in the small bowel epithelium produced by x-irradiation.

Methods Employed: Rats are exposed to total body x-irradiation of varying concentration (from threshold to lethal dose) after receiving a known quantity of N^{15} labeled glycine. Histopathological changes in the small bowel with respect to mitotic arrest, acute cell destruction, prolongation of interphase, overshooting in recovery and incomplete recovery are made. Changes in protein and nucleic acid synthesis are determined to parallel the histopathological studies.

Major Findings: It was previously demonstrated that with an arrest in cell division there was always associated an arrest in DNA synthesis but no depression in protein synthesis. This occurred if the x-irradiation exposure was 450 to 2,000 r and the synthesis studied 0-5 hours after exposure. But if examined 68-73 hours after exposure (450 r) there is a threefold increase in protein synthesis and an eightfold increase in DNA synthesis. This increased synthesis has been associated with the cells which were in interphase and are now dividing.

In the present series, the total dose of x-irradiation was fractionated in which the tissue was allowed to recover before repeating the initial dose and to determine the effect of keeping the total dose and total time constant, but varying the size of the individual doses. Divided doses were more effective than a single dose in enhancing recovery (prolongation of interphase) or preventing destruction of crypts. Protein and DNA synthesis paralled these histopathological findings. Exposure to 600 r followed by a similar dose 96 hours later repeated the delay in division, but when given 12 hours after the initial irradiation, there results a severe destruction of large numbers of crypts, with a marked arrest in DNA synthesis. Nine hundred r were given over a 12-hour period or 225 r every four hours; as 450 r followed 12 hours later by 450 r; and as 700 r followed 12 hours later by 200 r, respectively. The degree of recovery of DNA synthesis was dependent upon the size of the individual doses. DNA synthesis (recovery) was greatest following 4 doses of 225 r and least after 700 r followed 12 hours later by 200 r.

Significance to Cancer Research: To get a better understanding of the histopathological and chemical changes which take place in rapidly proliferating tissue (epithelial lining of the small bowel) following x-irradiation, which may be useful in tumor therapy by x-irradiation and/or chemotherapeutic agents.

Proposed course of project: To round off the program reported above and to present the data obtained for publication.

10. 902
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW AND APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR ITEMS 14, 15 and 16.

PROJECT REPORT

1. National Cancer Institute
INSTITUTE

2. Laboratory of Physiology
LABORATORY OR BRANCH

3. Cancer Physiology Section
SECTION OR SERVICE

4.
LOCATION (if other than Bethesda)

5. 903
SERIAL NO.

6. To determine factor(s) which produces cirrhosis of the liver in rats ingesting a low protein diet and exposed to 450-500 r total body x-irradiation.
PROJECT TITLE

7. Julius White
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine whether transmethylating agents or lipotropic substances, when added to the low casein diet, will have any effect on the production of cirrhosis of the liver produced by whole body x-irradiation.

Methods Employed: Rats were fed one of four diets differing from each other only in the amount of choline, methionine or both. Four diets were employed: (a) 6% casein diet, (b) 6% casein supplemented with 0.5% methionine, (c) 0.5% methionine and 0.5% choline, and (d) 0.5% choline. All animals received 450-500 r total body x-irradiation.

Major Findings: As reported in the last annual report, an incidence of approximately 50 per cent of cirrhosis of the liver was observed in the animals in group (a). No cirrhosis of the liver was observed in the other three groups of animals. Since methionine and choline are both intimately involved in transmethylation, it may be that the utilization of the low concentration of methyl donors present in the diet of animals in group (a) is influenced by exposure to x-irradiation and ample quantities of methionine or choline counteracts the irradiation effect.

Significance to Cancer Research: In no case was carcinoma of the liver produced. Yet, the fact that cirrhosis of the liver appeared in 50 per cent of the animals on the unsupplemented diet suggests that the nutritional state of the animal can influence the effect of total body x-irradiation on the liver and, perhaps, other tissues.

Proposed course of project: None.

10. 903
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW AND APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH.

None.

14. 903
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Julius White, Charles C. Congdon, Philip W. David, and Mona S. Ally: Cirrhosis of the liver of rats following total body x-irradiation. J. Nat. Cancer Inst. 15:1155-1163, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

PROJECT REPORT

1. National Cancer Institute (INSTITUTE) 2. Laboratory of Physiology (LABORATORY OR BRANCH)

3. Office of the Chief (SECTION OR SERVICE) 4. Department of Biology, Princeton University, Princeton, N. J. (LOCATION (if other than Bethesda)) 5. 904 (SERIAL NO.)

6. Study of Biological Effects of Ultraviolet Irradiation.
PROJECT TITLE

7. Harold F. Blum
PRINCIPAL INVESTIGATOR(S)

8. Drs. Elmer G. Butler, J. J. Chang, R. C. Mawe, S. E. Schmidt, and A. K. Parpart, of Princeton University; and Dr. J. W. Green of Rutgers University
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study the biological effects of ultraviolet irradiation with the ultimate aim of better understanding of the carcinogenic action of that agent. These studies aim to combine an experimental and theoretical approach.

Methods Employed: The approach is a combined theoretical and experimental one. Using data available from our earlier work on the induction of cancer by ultraviolet light, a mathematical model has been constructed which explains certain aspects. Realizing that any such model can only have meaning if related to real biological phenomena, experimental studies of various effects of ultraviolet radiation on living systems have been undertaken. Our model would indicate that the mechanism of cell division and its control are of particular importance in this problem, and hence the effect of ultraviolet radiation on this phenomenon has been a principal preoccupation. Our studies have extended into related fields as this has seemed indicated.

Major Findings:

I. Studies of regeneration after ultraviolet radiation in the larvae of the urodele amphibian. (In collaboration with Professor Elmer G. Butler, with the assistance of J. J. Chang, R. C. Mawe and S. E. Schmidt.) We have been concerned with questions of dosage, and relationships to photorecovery, as a necessary matter of background, but our most interesting study has been a rather extensive comparison of regeneration of irradiated and normal limbs after amputation. A rather complex situation is discovered by these experiments: The rate of regeneration is reduced in the irradiated limb; the degree of reduction varies with the time at which the amputation is performed, and does not follow the course of regression. Photorecovery is observed, illumination for two days after the irradiation greatly increasing the regenerative capacity toward normal, and reducing the development of abnormalities to a negligible number. There seems to be no close correlation between regression of the limb, regeneration of the limb or the incidence of abnormalities in the regenerate; our experiments clearly separating these different aspects, apparently for the first time. A manuscript describing these experiments has been prepared for publication in the Journal of Cellular and Comparative Physiology and will be submitted for approval in the near future.

II. Effects of ultraviolet radiation on mouse skin. (Begun in collaboration with Dr. R. C. Mawe with the assistance of Mrs. F. Constance.) In these studies it is planned to follow the changes in mouse skin resulting from ultraviolet radiation, with particular attention to the course of hyperplasia and its regression. Analysis of our earlier studies on carcinogenesis by ultraviolet radiation indicate that these events may have important bearing on that process. A method for reproducible, quantitative dosing of mouse ears has been worked out and some histological studies have been made. More precise histological methods are needed, and these are at present being worked out by Mr. Vincent Gregg, technician to Professor Butler.

III. Studies of hemolysis by ultraviolet radiation. The question of lysis by this agent was reopened by our studies on cytolysis of Arbacia eggs, carried out at the Marine Biological Laboratory, Woods Hole, in the summer of 1952. (Blum, Cook and Loos, J. Gen. Physiol. 37: 313, 1954.) Subsequently, John S. Cook undertook studies of ultraviolet hemolysis as his thesis for the Ph. D. degree, under my supervision. In an extensive paper, soon to be published in the J.C.C.P., Dr. Cook has shown two new things of particular interest:

(1) that hemolysis by this agent is a second order process, and (2) that ultraviolet radiation of short wavelengths (0.1860μ to 0.2500μ) is much the most effective part of the mercury arc spectrum for producing the hemolysis. Dr. Cook is now in Bern, Switzerland, on a Public Health Fellowship, studying effects of ultraviolet radiation on nerve with Professor Alexander von Muralt.

Dr. Cook and I began together a study of the O_2 dependence for ultraviolet hemolysis, which was suggested by his studies, and had just turned up some unexpected things when he left. I am just resuming these studies, after the construction of more adequate apparatus. It does not seem wise to report our results to date, because they need repetition and expansion, but at the present moment they seem rather fundamental, and may perhaps bear on the (to my mind) confused picture of O_2 dependence in effects of ionizing radiations.

IV. Studies of photosensitized erythrocytes. (In collaboration with Professor J. W. Green, Rutgers University, and Professor A. K. Parpart, Princeton University.) Studies on permeability of erythrocytes photosensitized with rose bengal and exposed to visible light, have shown that this treatment results in increased permeability to K^+ and Na^+ ions, but does not alter the metabolism of glucose by these cells. These studies are being continued. An abstract is being published in the Journal of Cellular and Comparative Physiology.

V. Polyspermia in the Arbacia egg. An exploratory study involving quantitative determinations of polyspermia in the Arbacia egg was carried out with Dr. Murray Eden at the Marine Biological Laboratory, Woods Hole, in the summer of 1954. The analysis of the extensive data we obtained has only now been completed in Dr. Eden's laboratory. It is hoped to prepare this material for publication in the near future.

Plans for work at the Marine Biological Laboratory, Woods Hole, during the past summer, were upset by my contracting whooping cough at a crucial time. This prevented any extensive experimental work, so the summer was spent chiefly in reading, writing, and working up with Professor Butler the results of our investigations on amphibia, carried out at Princeton during the spring.

Significance to Cancer Research: To throw light on the relationship of ultraviolet exposure to carcinogenesis.

Proposed course of project: Experiments designed to explore further the effects of ultraviolet radiation on regeneration are now under way.

Resumption of study of the O_2 dependence for ultraviolet hemolysis using more adequate apparatus.

Continuation of studies of photosensitized erythrocytes.

The synthetic approach to the problem of carcinogenesis by ultraviolet radiation will continue.

10. 904
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW AND APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

Department of Biology, Princeton University.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

15.

PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Butler, E. G., and Blum, H. F.: Regenerative growth in the urodele forelimb following ultraviolet radiation. J. Nat. Cancer Inst. 15: 877-889, 1955.

Blum, H. F.: Sunburn. In Radiation Biology (Hollaender, A., ed.). New York, McGraw-Hill, 1955, Chapter 15, pp. 487-528.

Blum, H. F.: Ultraviolet Radiation and Cancer. In Radiation Biology (Hollaender, A., ed.). New York, McGraw-Hill, 1955, Chapter 14, pp. 529-559.

Blum, H. F.: Sunlight as an environmental factor in cancer of the skin. Military Medicine 117: 202-208, 1955.

Blum, H. F.: Time's Arrow and Evolution. 2nd Edition. Princeton University Press, 1955, Princeton.

Blum, H. F.: Perspectives in Evolution. American Scientist 43: 595-610, 1955.

Papers describing work carried out under my supervision:

Zimskin, P. D., and Schisgall, R. M.: Photorecovery from ultraviolet-induced pigmentation changes in anuran larvae. J. Cell. Comp. Physiol. 45: 167-176, 1955.

16.

HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

1. I gave the initiation lecture to the Sigma Xi chapter of the University of Vermont, Burlington, on April 26. The title was Perspectives in Evolution.

2. I took part in a symposium on Biogenesis together with H. C. Urey, Philip Abelson, Sidney Fox and George Wald, as part of the Centennial Celebration of the Polytechnical Institute of Brooklyn on May 6. My position in the symposium was that of summarizer.

3. I presented a paper in a symposium at the Dedication of the Armed Forces Institute of Pathology, Walter Reed Medical Center, Washington, D. C., on May 27. The title was Sunlight as an Environmental Factor in Cancer of the Skin.

PROJECT REPORT

1. National Cancer Institute — INSTITUTE
2. Laboratory of Physiology — LABORATORY OR BRANCH
3. Cancer Physiology Section — SECTION OR SERVICE
4. — LOCATION (if other than Bethesda)
5. 905 — SERIAL NO
6. Iodide Concentrating Mechanism in the Thyroid Gland — PROJECT TITLE
7. S. H. Wollman — PRINCIPAL INVESTIGATOR(S)
8. Dr. R. O. Scow of NIAMD — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To learn about the mechanism by which the thyroid gland maintains a concentration of radioiodide elevated above that in the blood and about the way the thyroid radioiodide and iodide concentrations influence the uptake of radioiodine.

Methods Employed: Kinetic studies will be made of the ratio of radioiodide concentrations in the thyroid gland and serum and factors which control it.

Major Findings: A study was made of the inhibition of the iodide concentrating mechanism by thiocyanate. The dependence of the ratio of the radioiodide concentrations in thyroid gland and serum on the concentrations of iodide and thiocyanate in the same animal was examined. The observed data can be fit quantitatively by two qualitatively different models, an adsorption model and an active transport model. In these models iodide complexes with a substance in the thyroid, one type of site is involved with no interaction between sites; thiocyanate also complexes with the same sites and competes with iodide for the sites. The affinity of the site for iodide is twice as great as for thiocyanate. The affinity for thyroid sites is more than an order of magnitude greater than that for serum albumin sites. With the

observed constants, the adsorption model requires thiocyanate be concentrated. That it is not concentrated in rat and sheep thyroids suggests that the iodide concentrating mechanism is not an adsorption but an active transport process.

Significance to Cancer Research: Transplantable tumors of the thyroid gland in mice appear to have a marked impairment in or loss of the iodide concentrating mechanism. This loss appears directly responsible for the loss of ability of certain lines of thyroid tumors to accumulate radioiodine. The above experimental results are directed largely to provide an understanding of the iodide concentrating mechanism and the factors controlling it.

Proposed course of project: Further kinetic studies on the iodide concentrating mechanism will be undertaken. In addition, a more direct attack will be made on the problem of determining the factors which most seriously limit the accumulation of radioiodine.

10. 905
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW AND APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

NIAMD, Dr. R. O. Scow

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project may complement research in the Clinical Endocrinology Branch, NIAMD.

15.

PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

S. H. Wollman and R. O. Scow: Effect of various goitrogens and of dose of propylthiouracil on the ratio of radioiodide concentrations in the thyroid gland and serum in mice. Endocrinology 56: 448-454, 1955.

S. H. Wollman and R. O. Scow: Effect of propylthiouracil on the ratio of the radioiodide concentrations in thyroid gland and serum in normal and hypophysectomized rats. Endocrinology 56: 445-447, 1955.

16.

HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Elected to the American Physiological Society.

PROJECT REPORT

1. National Cancer Institute — INSTITUTE
2. Laboratory of Physiology — LABORATORY OR BRANCH
3. Cancer Physiology Section — SECTION OR SERVICE
4. — LOCATION (if other than Bethesda)
5. 906 — SERIAL NO

6. Transplantable Thyroid Tumors: Production and I^{131} Metabolism. — PROJECT TITLE

7. S. H. Wollman — PRINCIPAL INVESTIGATOR(S)

8. Dr. R. O. Scow of NIAMD — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

(a) To produce transplantable thyroid tumors in the rat and the mouse.
(b) To compare iodine metabolism in thyroid tumors and normal thyroid.

Methods Employed:

(a) Thyroid tumors are being produced by chronic feeding of thiouracil.
(b) Iodine metabolism was studied with the use of radioactive iodine.

Major Findings:

(a) We now have transplantable thyroid tumors in both mice and rats. These tumors will grow in hosts fed goitrogen free diets.
(b) No new results.

Significance to Cancer Research: Tumor material will now be available for radioiodine studies in two experimental species.

Proposed course of project: Studies of the radioiodine metabolism of thyroid tumors in the rat will be undertaken this year.

10. 906
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW AND APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

Dr. R. O. Scow, NIAMD

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project may resemble research in the Laboratory of Biochemistry, NCI.

14. 906
SERIAL NO.

15. _____ THIS PROJECT DURING

Place following wording on front

of thyroid lobes,
Growth and I^{131}
7, 1955.

ACTIVITIES

OF HEALTH

STITUTE

G TO THIS PROJECT

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

10. 906
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW AND APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

Dr. R. O. Scow, NIAMD

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project may resemble research in the Laboratory of Biochemistry, NCI.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

S. H. Wollman and R. O. Scow: Comparison of thyroid lobes, autotransplanted and in situ, in the rat: Growth and I^{131} uptake. J. Nat. Cancer Inst. 15: 943-947, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

None

PROJECT REPORT

1. National Cancer Institute — INSTITUTE
2. Laboratory of Physiology — LABORATORY OR BRANCH
3. Cancer Physiology Section — SECTION OR SERVICE
4. — LOCATION (if other than Bethesda)
5. 907 — SERIAL NO.

6. Autoradiographic Localization of I^{131} in Normal and Neoplastic Thyroid Tissue — PROJECT TITLE

7. S. H. Wollman — PRINCIPAL INVESTIGATOR(S)

8. Mr. I. Wodinsky, Laboratory of Chemical Pharmacology, NCI — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

(a) To locate the sites of high concentration of radioiodide in the thyroid gland.

(b) To locate the site at which the binding of radioiodine occurs in the thyroid gland, and to determine changes in the localization of the I^{131} after binding.

Methods Employed: High resolution autoradiography.

Major Findings: No new results this year.

Significance to Cancer Research: Localization of I^{131} by autoradiographic techniques is important in understanding of mechanism of uptake of I^{131} by thyroid gland and thyroid tumors.

Proposed course of project: To be continued.

10. 907
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]
REVIEW AND APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

Laboratory of Chemical Pharmacology, NCI.

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

S. H. Wollman and I. Wodinsky: Localization of protein-bound I^{131} in the thyroid gland of the mouse. Endocrinology 56: 9-20, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

None

PROJECT REPORT

1. National Cancer Institute — INSTITUTE
2. Laboratory of Physiology — LABORATORY OR BRANCH
3. Cancer Physiology Section — SECTION OR SERVICE
4. — LOCATION (if other than Bethesda)
5. 908 — SERIAL NO.
6. Theoretical Analysis of Carcinogenesis Based on the Mutation Hypothesis — PROJECT TITLE
7. S. H. Wollman — PRINCIPAL INVESTIGATOR(S)
8. — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Objectives: To aid in the test of a possible mechanism of carcinogenesis.

Methods Employed: A simple quantitative model was designed. Predictions of the model were compared with dose-response data in experimental carcinogenesis.

Major Findings: A model was designed which avoided assumptions about the growth rates of tumors, and instead involved total incidence of tumors of a particular type. Tests of the analysis do not appear possible at present because of the absence of suitable data. The suitability of most data is impaired by complications which are a consequence of the methods of administering carcinogens, by difficulties in making estimates of the number of tumors produced, and by dose-dependent lethal effects and dose-dependent indirect effects of carcinogens.

Significance to Cancer Research: Emphasizes need for further experiments avoiding interpretive difficulties recognized in the present analysis of available data.

Proposed course of project: This project has been completed.

10. 908
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW AND APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Wollman, S. H.: Comments on the analysis of dose-response data in experimental carcinogenesis. J. Nat. Cancer Inst. 16: 195-204, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

None

PROJECT REPORT

1. National Cancer Institute
INSTITUTE

2. Laboratory of Physiology
LABORATORY OR BRANCH

3. Cancer Physiology Section
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. 909
SERIAL NO

6. Metabolic Fate of the Histidine Side Chain
PROJECT TITLE

7. James C. Reid
PRINCIPAL INVESTIGATOR(S)

8. Florence K. Millar
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The purpose of this project is to acquire more information on the process by which histidine is used for the synthesis of nucleic acid.

Methods Employed: Radioactive carbon is being used as a tracer to study the problem.

Major Findings: Administration of DL-histidine labeled in the side chain with radioactive carbon leads to the formation of labeled aspartic acid, glutamic acid, alanine and threonine. In addition, nucleotide fractions derived from ribonucleic acid are highly radioactive. Before these results can be confirmed and interpreted it is necessary to resolve the histidine so that the L-form alone can be given. A resolution technique has been worked out.

Significance to Cancer Research: The significance of these findings to cancer research is that they add to our knowledge of the metabolism of protein and nucleic acid. Both of these substances are important for the growth of cancer.

Proposed course of the project: During the coming year the labeled DL-histidine will be resolved and the L-form will be used to confirm and extend the findings made to date. In particular, chemical degradation of labeled metabolites will be carried out to gain information on the probable mechanisms of the transformations.

10. 909
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES, OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 and 16.

PROJECT REPORT

1. National Cancer Institute
INSTITUTE

2. Laboratory of Physiology
LABORATORY OR BRANCH

3. Cancer Physiology Section
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. 910
SERIAL NO.

6. Tumor Autoradiography.
PROJECT TITLE

7. James C. Reid
PRINCIPAL INVESTIGATOR(S)

8. Julius White
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: It is desired to obtain information on the circulation pattern in tumors and the stability of tumor protein.

Methods Employed: Animals bearing tumors are dosed with radioactive amino acids and, after various periods of time, the tumors are removed and sliced. The slices are placed in contact with photographic film. After development, the film bears an image which shows the distribution of radioactivity through the slice.

Major Findings: When radioactive glycine is administered to rats bearing the Walker 256 tumor, isotope is found in the tumor tissue within one hour but cannot be demonstrated in 10 minutes by the methods employed. Radioactivity is found in substantial amount as late as 11 days after dosage. Necrotic areas present in the tumor when the glycine is administered do not become labeled. Conversely, if an area becomes labeled and subsequently becomes necrotic the fixed isotope is not lost. The inertness of the protein metabolism of necrotic tissue is thus established. It is also desired to obtain information on the possibility of gradients within the viable tumor tissue. This is the major aspect of the investigation and bears not only on the circulation pattern but on the question of the degree to which tumor protein is in labile equilibrium with host protein. No certain conclusions can be drawn on this point yet.

Significance to Cancer Research: The question of the relationship between the nutrition of a tumor and that of its host is an important one in understanding the uncontrolled growth of tumors. These experiments represent another mode of approach to this question. The information gained is also of interest in connection with the question of how necrosis affects the over-all metabolism of a tumor. This problem is a troublesome one for investigation of tumor chemistry.

Proposed course of project: The next step in the investigation will be to check the results obtained with glycine by repeating the experiments with labeled lysine. Glycine may not be the most suitable amino acid for this work because it undergoes extensive biochemical side reaction in addition to protein formation. It is then planned to extend the experiments to other tumors and, if it seems necessary, to other amino acids.

10. 910
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW AND APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 and 16.

PROJECT REPORT

1. National Cancer Institute — INSTITUTE
2. Laboratory of Physiology — LABORATORY OR BRANCH
3. Energy Metabolism Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. 911 — SERIAL NO.
6. Energy Metabolism Project — PROJECT TITLE
7. A. W. Pratt W. C. White and F. K. Putney — PRINCIPAL INVESTIGATOR(S)
8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The overall objective of this program remains the continuous, accurate measure of the energy metabolism of small experimental animals under different experimental conditions for prolonged experimental periods.

As the program progresses, certain immediate or relatively short term objectives may be examined in relation to accomplishment. The immediate objective of this reporting period relates to the performance of the total procedures and specifically the instrumentation, in the continuous routine study of tumor-bearing animals.

Methods Employed: Not applicable.

Major Findings:

a) Instrumentation

At the end of the last reporting period, it was considered that the short term studies conducted on the energy exchanges of rodent experimental animals indicated the need of further instrumentation on the recording-measuring equipment if continuous, accurate measures were to be accomplished. To this end, modified computer techniques were adapted to the instrument in the form of an analog to digital converter measuring and recording system. The new device (teleducer-telecomputer) was essentially a multi-channel system which would allow continuous measure and recording of all the desired parameters, i.e. O_2 output, CO_2 output,

radiation chamber, cage and room temperature comparisons, etc. In addition, we are afforded the advantage of being able to convert the total system to a fully automatic processing of the total data should such be indicated. It is apparent that the addition of this new component solved the problem of continuous, accurate measure of low value d.c. potentials over prolonged experimental periods while affording a complete printed tabulation of all data. The new instrument substantially reduced the number of technician man-hours needed for overall data handling.

As with any electromechanical device of this complexity, the expected electronic difficulties presented themselves once the instrument was in continuous operation. While they led to extensive "down-time" in the early application, the development of "by-pass circuitry" by our group has made the instrument into a dependable, accurate component in our application.

In summary, it would appear that the study of four individual animals for an average continuous experimental period of 2.5 months each indicate that the new instruments will accurately and continuously measure the energy exchanges of the rodent experimental animal. In addition, three animals have been studied in the Hays instrument. While this instrument has performed satisfactorily, it is apparent that only day to day monitoring can be achieved with this instrument.

b) Animal Findings

The animals under study were Osborne-Mendel males of an inbred strain. The dietary regime was 20% casein with added vitamin supplements and inorganic salts. The experimental period consisted for each animal for approximately 70 to 80 days. The experimental period was divided into a control period, induction period and a tumor period. During the control period observations were made on the normal animal and the energy intake; energy expenditure was determined in the usual fashion. When it was certain that the control period was adequate in length of time to reflect the metabolic behavior of the animal, a Walker 256 transplant was administered by sterile trocar and the observations on the animal continued. The induction period spanned the time between transplant and the point when the tumor was first palpable in the animal. After the tumor was palpable the tumor period was initiated and ended when the animal was sacrificed. Maximum growth of the tumor was very carefully regulated in that no animal was allowed to grow a tumor larger than 18% of the total host-tumor weight.

The general findings which are discussed below reflect the average behavior of tumor-bearing hosts studied under the conditions mentioned above. Following tumor transplant the oxygen consumption as expressed in liters per day abruptly rises to a level significantly above that

observed in the control period, reaching a maximum on the third or fourth day. The maximum is followed by a decrease which, at the time of appearance of tumor, is at a new level intermediate between the highest level observed in the control period and the highest level observed in the induction period. Carbon dioxide production roughly parallels the oxygen consumption curve. In the tumor period, the oxygen consumption level gradually, but steadily, rises as the tumor contributes a greater and greater percentage to the total host-tumor weight. It has been our general observation that the carbon dioxide production in the tumor period tends to decrease during the tumor growth period with the net result of a fall in R. Q. and a significant fall in the non-protein R. Q. It is not, at the moment, possible to interpret this observation in the sense of a real finding. It is not known whether the fall in non-protein R. Q. represents solely an increased metabolic usage of fat or whether, in addition to the increased usage of fat, there is a superimposed retention of carbon dioxide. With the fall in the value of the non-protein R. Q., the energy equivalent of calories per liter of oxygen consumed falls correspondingly, with the net result that the increase in oxygen is not a true reflection of the observed increase in energy expenditure as a function of tumor growth. We have always observed a significant slope in the increase of oxygen consumption throughout the induction and tumor periods. However, only in the induction period does an abrupt rise in energy expenditure coincide with the abrupt rise in oxygen utilization. In the tumor period, the fall in carbon dioxide produced to affect a more gradual or gentle slope in energy expenditure.

Energy intake: The energy intake of the animal during the control period is always substantially in excess of the energy expenditure and the net change in body weight can be grossly correlated with the energy retained. In the induction period the difference between the energy intake and the energy expenditure is substantially reduced, but the energy intake remains in excess of the expenditure. In animals growing a tumor, which at maximum is equivalent to approximately 15% of body weight, it has been noted that the energy intake gradually reduces and this effect can be seen almost from the beginning of the tumor period.

Energy expenditure: Comparison of the energy expenditure of the animal in the induction period to the control period shows that, following transplant of the tumor, an abrupt and significant increase in energy expenditure occurs. The maximum of this expenditure is observed on the third or fourth day following the transplant of the tumor. The energy expenditure then declines, reaching levels at the beginning of the tumor phase which is intermediate between the highest value observed for the control period and the highest value observed during the induction period. As the animal experiences progressive tumor growth, the level of energy expenditure gradually increases. As the energy intake decreases and the energy expenditure increases, the animal may achieve a condition of isocaloric balance. It has been our observation that the contribution of protein to the energy expenditure is roughly constant throughout

the control period, induction period and for most of the tumor period. As the non-protein energy contribution to the energy expended becomes equal to, or larger than, the non-protein energy intake, the slope of the protein energy expended begins to gradually increase. From our observations, it is clear that the excess energy expenditure observed in the induction period is entirely contributed to by non-protein materials. In addition, the increased expenditure during the tumor period appears to be primarily non-protein in nature. The net result being that isocaloric balance between the non-protein energy intake and the non-protein energy expenditure is achieved very early after gross tumor growth is apparent. Use of protein for energy needs of the animal is spared until a negative balance in the non-protein energy occurs. It has been our observation that the weight change of the tumor-bearing animal progressively rises as long as the protein balance is positive, even in the face of an isocaloric balance or perhaps a slightly negative balance of the non-protein energy. Presumably, this is due to the retention of new protein body weight in the form of tumor, and, in addition, an extensive hydration of the animal body as the tumor process progresses.

Certain gross correlations may be made by comparing the energy expenditure with other parameters. Weight change in the non-tumor periods reflects the retention of protein and non-protein energy in addition to water. The weight change during the tumor period is primarily dependent upon the protein energy balance and the degree of hydration. A high degree of protein sparing occurs in the sense that a significant amount of protein will be retained and deposited as tumor, while the non-protein energy contributes almost entirely to the energy demands of the animal. The energy intake does not maintain pace with the increase in energy expenditure observed in the induction and tumor periods. On the contrary, the anorexia substantially reduces the animal's energy intake.

Significance to Cancer Research: Study of the effects on the energy metabolism of a host animal, following tumor implant and during subsequent tumor growth, promises to yield significant information bearing on host-tumor relationships. The development of these new techniques for these studies should allow an accumulation of whole animal data which, together with the advances in intermediate metabolism, should increase our understanding of the pathological physiology of the tumor process.

Proposed course of project: We believe we have been successful in accomplishing an accurate measure of energy expenditure and energy intake and, thus, the energy retained. Our next immediate objective will be the distribution of retained energy into "fat-free body weight" and "fat body weight" for correlation with observed weight gain.

In addition, comparative studies on the body composition of the observed animals at the time of sacrifice are being undertaken.

The need of a direct calorimetric measure of the heat output of our experimental animals during these prolonged experimental periods is necessary if we are to compare and interpret the animal's metabolic behavior. To that end we are beginning pilot construction on a direct calorimeter which will utilize the adiabatic principle.

10. 911
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 and 16.

PROJECT REPORT

1. National Cancer Institute — INSTITUTE
2. Laboratory of Physiology — LABORATORY OR BRANCH
3. Energy Metabolism Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. 912 — SERIAL NO.
6. Mass Spectrometer Program — PROJECT TITLE
7. A. W. Pratt and Bernard E. Burr — PRINCIPAL INVESTIGATOR(S)
8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The instrument is in continuous routine use for calibration and monitoring of the energy metabolism instruments. Secondly, studies on diffusion rates of gases obtained from biological sources continues. This study is particularly concerned with the deviations that exist in the diffusion rate of these gases from that predicted by Graham's Law. Thirdly, pilot experimentation has been tried out relating the rate and kind of gases which are released from pyrolyzed proteins.

Methods Employed: Mass spectrometric techniques.

Major Findings: The primary use of the mass spectrometer continues as a basic reference instrument for gas analysis of our energy metabolism instruments. The mass spectrometer allows frequent and accurate standardization of our analytical respirometers and is primarily responsible for the present accuracy and the continuous performance of our new analytical respirometers. To completely define the accuracy of our energy metabolism measure, it is necessary that an extensive performance catalog of the mass spectrometer exist. Compilation of these data will occupy most of the mass spectrometer time for an extended period.

Studies on diffusion rate of gases is progressing very slowly. The immediate problem at hand deals with the deviations that are observed in the diffusion rate of these gases from Graham's Law. We are confronted here with fundamental problems in gas behavior and while we have made considerable effort in relation to this problem we have been unable to resolve the questions related to the fundamental behavior.

The studies from the pyrolysis products of protein are much too incomplete to be discussed at this moment. It is apparent that the study of the diffusion rates and the study of pyrolysis products are long-termed physical chemical problems which we feel will extend the use of the mass spectrometer but which at the same time will be resolved slowly.

Significance to Cancer Research: It is hoped by an approach which is of a basic physical nature we can utilize the considerable potential of the mass spectrometer in understanding biological function and biological composition of important proteins. The proposed course of the project will be to continue the pilot experimentation until such time as positive findings direct the development of the program.

Proposed course of project: It is apparent that the study of the diffusion rates and the study of pyrolysis products are long-termed physical chemical problems which we feel will extend the use of the mass spectrometer but which at the same time will be resolved slowly.

10. 912
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 and 16.

PROJECT REPORT

1. National Cancer Institute — INSTITUTE
2. Laboratory of Physiology — LABORATORY OR BRANCH
3. Energy Metabolism Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. 913 — SERIAL NO.

6. Determination of the radiochemical mechanism of L-malate decomposition in oxygenated, aqueous solutions. — PROJECT TITLE

7. A. W. Pratt and Frances K. Putney — PRINCIPAL INVESTIGATOR(S)

8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the radiochemical mechanism of L-malate decomposition in oxygenated, aqueous solutions.

Methods Employed: Chemical analysis by fluorometry, chromatography, and absorption spectroscopy.

Major Findings: It has been reported that the level of oxygen tension in the ambient air and, thus, in the blood of an experimental animal exerts a significant effect on the per cent survival ratio and survival time of irradiated animals. One approach to the understanding of this system is to study the radiochemical decomposition of important biological compounds in oxygenated, aqueous solution. Studies of x-irradiated malate solutions revealed that 65% of the decomposed malate was converted to oxalacetate and the remainder to α-keto, β-hydroxy succinate in the presence of oxygen. Neither of the compounds are formed in non-oxygenated solutions. The oxygen effect in the radical reaction is apparent from these observations. The full data including the probable radiochemical mechanisms of these reactions, which is now to be submitted for publication, clearly indicates the importance of molecular oxygen in determining decomposition reactions following exposure to ionizing radiations.

Significance to Cancer Research: To aid in the understanding of the biological effect of ionizing radiation.

Proposed course of project: Completed.

10. 913
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW AND APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH.

None

NO ENTRIES FOR ITEMS 14, 15 and 16.

PROJECT REPORT

1. National Cancer Institute
INSTITUTE

2. Laboratory of Physiology
LABORATORY OR BRANCH

3. Physical Biology Section
SECTION OR SERVICE

4.
LOCATION (if other than Bethesda)

5. 914
SERIAL NO.

6. In Vivo Measurement of Tumor pH With the Capillary Glass Electrode.
PROJECT TITLE

7. H. Kahler
PRINCIPAL INVESTIGATOR(S)

8. B. Haines
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The object of this project is to determine the in vivo pH of tumors under different experimental conditions.

Methods Employed: Following anesthesia, three fine-tipped capillary electrodes were inserted into each tumor in selected sites, one electrode was inserted into normal leg muscle, and the pH was recorded by automatic equipment over a period of 18 hours. Fine thermocouples were also inserted into selected sites such as skin, tumor, muscle and rectum, and the temperatures were recorded on another instrument throughout the experiment. After an initial period, an injection of one of numerous sugars was made.

Major Findings: The acid reaction of tumors following glucose administration seems to be quite general; all 14 rat tumors studied show this effect.

In a given tumor, the pH varies with location within the tumor.

Significance to Cancer Research: The pH of a tissue is one of its basic properties and where this pH deviates from the normal tissue value the detailed study of these changes is essential to an understanding of tumor chemical processes.

Proposed course of project: The variation of pH with position in a tumor suggests it may be a function of the proximity to blood vessels in addition to the proximity to the surrounding normal tissue. This remains to be seen.

An analysis of tumor temperatures for information on energy characteristics of tumors.

10. 914
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/_/
REVIEW AND APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

14. 914
SERIAL NO.

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

M. Eden, B. Haines and H. Kahler: The pH of rat tumors measured *in vivo*. J. Nat. Cancer Inst. 16: 541-556, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

None

PROJECT REPORT

1. National Cancer Institute — INSTITUTE
2. Laboratory of Physiology — LABORATORY OR BRANCH
3. Physical Biology Section — SECTION OR SERVICE
4. — LOCATION (if other than Bethesda)
5. 915 — SERIAL NO.

6. Physical Characterization of Cellular Bodies: Ultracentrifugal Study of Desoxyribonucleic Acid (DNA) in Bivalent Ion Solutions.
PROJECT TITLE

7. H. Kahler
PRINCIPAL INVESTIGATOR(S)

8. J. Shack and B. J. Lloyd
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To compare the sedimentation characteristics of DNA in bivalent and monovalent ion solutions.

Methods Employed: Solutions of different DNA preparations from normal and tumor tissue were centrifuged in various monovalent and bivalent ion solvents. The optical photographs were analyzed for sedimentation velocities and boundary spreading.

Major Findings: DNA has a higher sedimentation velocity and greater boundary spreading in bivalent ion solutions than in monovalent ion solutions.

Significance to Cancer Research: The properties of DNA are of significance to genetics and, hence, to cancer. It is important to know not only the properties in monovalent solutions but also in solutions containing calcium and magnesium.

Proposed course of project: Continuation of DNA study, utilizing light-scattering, ultracentrifugation, electrophoresis, and electron microscopy.

Continuation of virus part of project by studying morphology of tumor-inducing viruses sliced with a microtome, and sectioned viral tissue.

10. 915
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]
REVIEW AND APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

None

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE : (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

None

15. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

B. J. Lloyd and H. Kahler: Electron microscopy of the virus of rabbit fibroma. J. Nat. Cancer Inst. 15: 991-999, 1955.

16. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

None

PROJECT REPORT

1. National Cancer Institute — INSTITUTE
2. Laboratory of Physiology — LABORATORY OR BRANCH
3. Physical Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. 916 — SERIAL NO
6. Physical Chemical Characterization and Comparison of Nucleic Acids and Nucleoproteins of Normal and Neoplastic Tissues. — PROJECT TITLE
7. Joseph Shack — PRINCIPAL INVESTIGATOR(S)
8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to isolate in a native form the nucleic acids and nucleoproteins of various normal and malignant tissues, to develop and apply appropriate methods of fractionating and characterizing them as chemical entities, and to discover what differences, if any, there are between normal and malignant tissues with respect to these components.

Methods Employed: During the last calendar year modified deoxyribonucleates (DNA) were prepared from the DNA of calf thymus and strain A mouse lymphoma L#1 by exposure to heat, acid, alkali and low salt concentrations. The ultraviolet absorption and viscosity behavior of both the original and the modified DNA were investigated over a range of temperatures, pH values and salt concentrations.

Major Findings: The ultraviolet absorption of native DNA is the same over a wide range of temperatures and salt concentrations. The modified DNA's all show marked variation over the same range of conditions. These findings suggest that the native nucleate has a high degree of structural rigidity which is lost on even mild denaturation. The results afford some new simple criteria for recognition of the native state of DNA.

Significance to Cancer Research: According to current theories nucleic acids play a role in genetic processes, in bacterial transformations, in protein synthesis, in virus reproduction and in the action of radiation and of some carcinogens and chemotherapeutic substances. The importance of acquiring knowledge of the nature of the nucleic acids and nucleoproteins

of malignant tissues and of differences that may exist between normal and malignant cells is indicated by these facts.

Proposed course of project: Recent evidence shows that the total nucleic acid of a cell consists of a large number of different chemical individuals. Elucidation of differences among tissues must be concerned with study of the separated fractions as well as with the total nucleic acid. It is planned to carry out such fractionations on the nucleic acids and nucleoproteins of certain normal and malignant tissues. During the past year exploration and evaluation of some of the methods to be used in this work (chromatography, fractional precipitation and extraction) have been carried out. It is also planned to carry out such fractionations on the nucleic acids of the tissues of normal and tumor bearing animals that have received isotopic tracers in order to gain information regarding the metabolic activity of the individual fractions or components as well as of the total nucleic acid.

10. 916
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	TECHNICAL ASSISTANCE	[ ]

12. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE , OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None

13. IF THIS PROJECT RESEMBELS, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 and 16.

PROJECT REPORT

1. National Cancer Institute — INSTITUTE
2. Laboratory of Physiology — LABORATORY OR BRANCH
3. Physical Biology Section — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. Z17 — SERIAL NO

6. Assay, Characterization and Comparison of the Deoxyribonucleases of Malignant and Normal Tissues and of Body Fluids. — PROJECT TITLE

7. Joseph Shack — PRINCIPAL INVESTIGATOR(S)

8. _____ — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to determine the nature, quantity and properties of the deoxyribonucleases of malignant and normal tissues and of body fluids of the host, particularly of those tissues whose nucleic acids are under investigation.

Methods Employed: Extracts of tissues and preparations of body fluids suitable for enzymatic assay are made. Enzymatic action on pure nucleic acids or nucleoproteins is measured at a variety of conditions with respect to pH and type and concentration of ions present by following changes in such properties as viscosity, pH, ultraviolet absorption or solubility.

Major Findings: In studies of mouse tissues deoxyribonuclease action has been found at conditions where the previously described deoxyribonucleases I and II are inactive. The results indicate the existence of additional enzymatic components.

Significance to Cancer Research: The information gained in this project is necessary to the development of procedures for isolation in native form of the nucleic acids and nucleoproteins of the various normal and malignant tissues under study. It is also possible that the amount and nature of the deoxyribonucleases present in tissues and body fluids may be related to the presence and the rate of growth of tumors present in the host.

Proposed course of project: During the next calendar year it is planned to investigate further the new enzymatic factor and to continue studies of the enzymes present in normal and tumor tissues and body fluids.

10. 917
SERIAL NO.

11.
BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

12.
COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

None

13.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR ITEMS 14, 15 and 16.