THE HARVEY SOCIETY

THE HARVEY LECTURES

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BY

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SERIES X

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PREFACE

THE Harvey Society has reached the tenth year of its existence. It is therefore hardly necessary to review again the objects of the Society, or to offer an excuse for presenting to the public the present volume of lectures.

To the speakers whose lectures comprise this volume we take this opportunity to express our appreciation of their unselfish services.

We wish to indicate our obligations to the following journals in which certain of the lectures appearing in this volume have already been published: To the Archives of Internal Medicine for allowing the republication of the lectures by Professor Gay, Professor Loevenhart, Professor Zinsser, and Professor Joslin; and to the American Journal of the Medical Sciences, and the Journal of the American Medical Association, in which have appeared, respectively, the lectures by Professor Fordyce and Professor Mendel. The paper by Professor Lewis has been published as a part of a volume of lectures by this author.

November, 1915. ROBERT A. LAMBERT, *Editor.*

THE HARVEY SOCIETY

A SOCIETY FOR THE DIFFUSION OF KNOWLEDGE OF THE MEDICAL SCIENCES

CONSTITUTION

I.

This Society shall be named the Harvey Society.

II.

The object of this Society shall be the diffusion of scientific knowledge in selected chapters in anatomy, physiology, pathology, bacteriology, pharmacology, and physiological and pathological chemistry, through the medium of public lectures by men who are workers in the subjects presented.

III.

The members of the Society shall constitute three classes: Active, Associate, and Honorary members. Active members shall be laboratory workers in the medical or biological sciences residing in the City of New York. Associate members shall be such other persons as are in sympathy with the objects of the Society. Honorary members shall be those who have delivered lectures before the Society and who are neither active nor associate members. Associate and honorary members shall not be eligible to office, nor shall they be entitled to a vote.

Members shall be elected by ballot. They shall be nominated to the Executive Committee and the names of the nominees shall accompany the notice of the meeting at which the vote for their election will be taken.

IV.

The management of the Society shall be vested in an executive committee, to consist of a President, a Vice-President, a Secretary, a Treasurer, and three other members, these officers to be elected by ballot at each annual meeting of the Society to serve one year.

V.

The Annual meeting of the Society shall be held soon after the concluding lecture of the course given during the year, at a time and place to be determined by the Executive Committee. Special meetings may be held at such times and places as the Executive Committee may determine. At all the meetings *ten* members shall constitute a quorum.

VI.

Changes in the Constitution may be made at any meeting of the Society by a majority vote of those present after previous notification of the members in writing.

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EXPERIMENTAL STUDIES ON METHODS OF PROTECTIVE IMMUNIZATION AGAINST TYPHOID FEVER*

PROFESSOR FREDERICK P. GAY
University of California

PROGRESS in combating bacterial diseases either by preventive inoculation or by vaccine therapy might seem to lie largely in the application of recognized and fairly successful methods in new diseases. I venture to suggest, however, that great progress is possible in the present methods that have been regarded as more or less efficient in such diseases as anthrax, cholera, plague, and typhoid fever. The strikingly favorable results of protective immunization against typhoid fever have only recently been fully recognized and emphasized, and are therefore fresh in the minds of us all. Particularly brilliant have been the results attained in this country, and you were so fortunate last year in this Society as to hear the latest report of them presented by Major Russell, who is to a great extent responsible for their general acceptance. I hope that nothing I may say will in the least dampen your enthusiastic support of the current methods of antityphoid inoculation; at the same time I venture to suggest that they may be still further perfected.

Let me outline to you the reasons which have led me and my associates to undertake, during the past two years, rather extensive experimental studies of the methods of typhoid vaccination. I may further suggest that similar studies are indicated in connection with other bacterial diseases; I have only to mention the first, and in many respects the most perfect, instance of antibacterial prophylaxis, that of vaccination against anthrax in cattle. You doubtless know that, removed from the careful laboratory conditions that marked Pasteur's triumphant experiments, the method is often popularly claimed to be a failure; the living attenuated culture of *B. anthracis* dispensed by com-

* Delivered October 10, 1914.

mercial firms may either fail to protect, or, on the other hand, cause a high mortality among the inoculated animals.

Typhoid fever is now the human disease of recognized bacterial origin that is most efficiently prevented by previous administration of the etiologic agent. And yet the relatively good results that have been attained should, it would seem, only serve to stimulate us to strive for even better results. That further advance is needed is evident for the following reasons:

First: We are not certain what preparation of the typhoid bacillus is best for immunizing purposes. Over twenty different typhoid vaccines have been suggested, and almost every one of them is still being advocated as the best. In addition to this number of preparations suggested, wide variations exist as to the size and number of the doses, the intervals of inoculation, and the like. The fact is that the development of the present methods, apart from the animal experiments on which they were based, has been largely empirical. A return to the experimental study of antityphoid immunization is therefore advocated as the only means of determining the best vaccine preparation and the best methods of administering it.

Second: The methods now employed protect only relatively well even for short periods of time. The protection afforded has been estimated as from two to six times as great as that enjoyed by the unvaccinated individual under similar circumstances. I am fully aware of the exceptionally good results that have occurred in the United States Army and in certain French garrison towns, but the identical methods employed in these instances have, in other groups of cases, been far less protective.

Third: As compared with the enduring immunity that follows recovery from typhoid fever, the protection afforded by artificial immunization is very short, at most of some two years' duration.

Fourth and correlatively: We have at present no sure method of estimating the actual duration of protection in the individual. When does the preventive treatment wear off? When should I be re-vaccinated?

Fifth: The usual methods of antityphoid inoculation, although advisable under any circumstances, are frequently, perhaps in the majority of cases, attended with more or less unpleasant symptoms, which it might be possible to avoid without detriment to the protection afforded.

HISTORICAL REVIEW

Beumer and Peiper [1] were undoubtedly the first fully to appreciate the possibility of an active immunization against infection with the typhoid bacillus. In 1887 they were able to prove that mice that have recovered from a non-fatal infection with living typhoid bacilli are frequently protected against subsequent, larger, and usually fatal doses of the same organism. In their most successful experiment they found that the best results were obtained by the gradual increase in dosage on successive inoculations, and they further suggest that it may be possible to immunize by means of sterilized cultures, which, as had already been shown, contain the toxic principle of the typhoid bacillus. They raise the question as to whether it might not be possible to immunize human beings by means of gradually increasing amounts of such killed cultures. In the following year Chantemesse and Widal,[2] following the work of Salmon and Smith [3] on hog cholera, and of Roux and Chamberlain [4] on malignant œdema, found that they could protect mice against infection with living typhoid bacilli by means of sterilized cultures of the organism.

The practical application of these experimental results in animals to the prevention of typhoid fever in human beings did not come until eight years later, following the discovery of the lysins by Pfeiffer. It was A. E. Wright,[5] who, in a preliminary publication in 1896 followed by a fuller account by Wright and Semple [6] in the beginning of 1897, first outlined a method of immunizing human beings against typhoid fever. The method as outlined is notable on account not only of the essential facts involved, but also of the orderly and systematic method by which the problem was approached. Wright grew the culture of the typhoid bacilli in bouillon for two or three weeks and

then killed them by heating to 63° C. (145.4° F.) for an hour, and preserved them with 0.5 per cent. phenol (carbolic acid). These vaccines were then tested for sterility and their toxicity carefully standardized by determining the minimal lethal dose for guinea pigs; the dose chosen for injection in human beings was measured by this toxicity for animals. Wright further introduced a method of counting the number of the bacteria in the preparation employed by comparing their number in a given dilution when mixed with a suspension of red blood-cells, the number of which could be accurately determined. The dose of bacteria usually employed was from 750 to 1,000 million.

In the same year (1896) Pfeiffer and Kolle [7] described their method of immunizing and their method of estimating the protection that is produced in human beings by the agglutinins and the bactericidal potency of the serum. They employed agar cultures of an avirulent strain of the typhoid bacillus suspended in salt solution and killed by heating to 56° C. (132.8° F.). An amount of this suspension, corresponding to one-tenth of an agar culture or something like 2 mg., was usually given on the initial injection, which was, as a rule, the only one. It is frankly admitted that the symptoms produced by this amount of culture were severe, and the method has since been modified in several ways to avoid these symptoms without essentially changing the principle involved.

So much for the first two communications on typhoid immunization in human beings. They form the groundwork on which subsequent methods of vaccination against typhoid fever have been built. The various methods that have since been advocated are numerous. Metchnikoff and Besredka [8] estimate that at least twenty different methods of vaccination have been described and advocated. Friedberger,[9] in his systematic review on typhoid immunization, enumerates twelve recognized methods. Paladino Blandini [10] has actually attempted to test the comparative immunizing value of seventeen preparations. It is not our purpose to describe all these methods in detail, and one who wishes further information on them may consult the systematic description of Friedberger [9] or of Fornet [11] in regard to them. It will

be well, however, to outline the most important of these methods as evidence of the scope that the investigation has taken in perfecting this type of immunization, and as indicating the tendency which would seem to be leading to its gradual perfection.

PREPARATIONS OF THE TYPHOID BACILLUS THAT HAVE BEEN USED AS VACCINES

A. *Killed Cultures of the Typhoid Bacillus.*—We have already mentioned that the first two preparations, those of Wright and of Pfeiffer and Kolle, consist essentially in killed cultures, the one, a bouillon culture and the other a suspension of an agar culture. These two original methods have been followed by many modifications. Thus Loeffler [12] took advantage of the fact that ferments, when dried, resist heating to a considerable degree without deterioration, and, regarding the antigenic property of the bacillus as ferment-like, dried suspended agar cultures of the micro-organism and then heated them to from 120° to 150° C. (248° to 302° F.). These dried cultures were then pulverized and used in weighed amounts for immunizing animals. He states that such a culture has lost little of its property to produce antibodies. Friedberger and Moreschi [13] use a similar dried and heated culture, and administer it intravenously in very small doses; for example, an amount corresponding to 1/4,000 œse in immunizing human beings. It should be noted at this point that the method currently employed of determining the immunizing value of these preparations lies in estimation of the antibodies (agglutinins, lysins, etc.) produced. As we shall later have cause to consider, these estimations offer an indication rather of the reaction of the animal body than a sure means of determining the degree of protection that has actually been afforded.

As we shall see in a moment, the use of living instead of dead cultures has been warmly advocated by certain observers, and their assertions have apparently convinced several who are not quite willing to adopt such preparations owing to their possible danger, although they endeavor to approach them as far as possible without actually using living micro-organisms.

It is apparently now the consensus of opinion that bacterial cultures are most antigenic when employed as nearly as possible in their living, unaltered condition, and that heat in particular tends to alter or destroy essential, characteristic, antigenic properties. Several methods have been advocated for avoiding or obviating so far as possible the destructive influence of heat, and at the same time killing the bacteria. Leishmann [14] advocates killing the typhoid bacillus at 53° C. (127.4° F.) instead of 56° or 60° C. (132.8° or 140° F.). Vincent [15] while fully recognizing the superior value of living cultures, regards their use as dangerous, and therefore kills the vaccines that he employs by means of ether. As will be later mentioned, we have used alcohol for the combined purposes of killing the typhoid bacilli and accelerating their flocculation and drying. Levy and Bruch [16] killed their preparations by shaking the micro-organisms in a medium containing galactose, and find that organisms prepared in this way immunize guinea pigs as well as the living cultures, and that these two preparations are far superior to killed cultures in corresponding amounts. Fornet [11] regards the unpleasant effects that are produced by the heated vaccine as due not only to the heating itself but also to the presence of a large amount of albumin in the culture medium. He therefore grows his micro-organism in a medium containing only a small amount of peptone and kills them by heating to 55° C. (131° F.) for fifty-five minutes. Courmont and Rochaix [17] killed their preparations by heating to 53° C. (127.4° F.), and further modified the usual method by administering the antigen through the rectum. Nicolle, Connor and Conseil [18] heat their bacteria to 55° C. (131° F.) for forty-five minutes, and then to 52° C. (125.6° F.) for thirty minutes more, and inject intravenously. Wassermann [19] insists that the antibodies produced by typhoid bacilli heated to 53° C. (127.4° F.) are not markedly better than when they are heated to 56° C. (132.8° F.). Renaud [20] has advocated the use of ultra-violet rays to kill the bacteria.

B. *Extracts of Bacteria.*—In addition to killed cultures of bacteria, numerous extracts and preparations derived from the bacteria have been advocated for the purpose of immunizing

against typhoid fever. Hahn [21] has recommended the extract obtained from masses of bacteria by means of the Buchner press. McFadyen and Roland [22] have utilized liquid air as a means of killing bacteria and obtained from them an extractive substance. Neisser and Shiga [23] have utilized free receptors obtained by autolysis of bacteria at body temperature in salt solution. Wassermann [24] has suggested a similar method with the autolysis produced by distilled water. He subsequently dries the extract obtained in this way and uses it as a vaccine powder (*Impfpulver*). Brieger and Mayer [25] have used a watery, filtered extract of shaken bacteria. Bergell and Meyer [26] have used an extract of dried bacteria obtained by treating them with dilute hydrochloric acid.

Various so-called soluble toxins of the typhoid bacillus have also been suggested for immunizing purposes by Chantemesse,[27] by Werner,[28] and by Rodet, LaGriffoul and Wahby.[29] The extract of bacteria obtained by the method of Jez [30] has also been suggested.

C. *Living Cultures of the Typhoid Bacillus.*—Living cultures of the typhoid bacillus, usually more or less modified in their pathogenicity, have been warmly advocated by certain observers as producing the best immunizing preparations in a manner similar to the methods that have been employed in dealing with other diseases, notably in cholera (Strong and Kolle). Castellani [31] uses an avirulent strain of the typhoid bacillus in the form of recent bouillon cultures which are then partially killed by heating to 50° C. (122° F.) for one hour. Such a modified culture produces rather severe local and general symptoms, but when given twice would, to judge from Castellani's results, produce a most satisfactory degree of immunity, which apparently has lasted in a number of cases on which he reports for at least four years. He suggests, as an alternative, that the first injection may consist of a killed culture followed by a living culture on the second inoculation. In addition to the superior immunizing properties of the living culture, it is also pointed out by Fornet [11] that the killed cultures in a given dose give more reaction because the split products of proteins, which are recog-

nized to be toxic, are liberated by heat. Living cultures have also been employed by Pescarolo and Quadrone.[32] The form of living cultures which has been advocated by Besredka will be considered under the next heading. As has already been mentioned, living cultures are generally admitted to be of superior immunizing value by many who are not willing to adopt them, owing to the real or fancied dangers coincident with their use, and this has led to an attempt to approach the condition of living bacteria without actually employing them. (Compare Vincent,[15] Levy and Bruch,[16] and particularly Metchnikoff and Besredka, to be considered presently.)

D. *Sensitized Cultures of the Typhoid Bacillus.*—The method of active immunization by means of sensitized vaccines, that is to say, by cultures that have been first treated with an immune serum and then killed, was introduced by Besredka [33] in 1902. This method is not infrequently referred to as sero-vaccination, but it differs from the method properly called sero-vaccination suggested by Leclainche [34] in swine erysipelas and by Calmette and Salimbeni [35] in plague, in that the excess of immune serum which these authors used is removed from the treated bacteria. It was found, as Besredka notes, that this excess serum tends to produce simply a passive immunity instead of the active immunity which is produced by the cultures treated with immune serum, and washed. Apart from his original experimental work, Besredka did not deal with the practical aspects of sensitized vaccines until the experimental work on typhoid fever in apes was taken up by him in collaboration with Metchnikoff in 1911.[8] In the meantime, however, sensitized vaccines had been tried out apparently with considerable success in at least three instances. Marie [36] had been able to utilize the principle in treating rabies virus, Dopter [37] in vaccination against dysentery, and Theobald Smith [38] in a similar way found that he could produce active immunity by a balanced mixture of diphtheria toxin and antitoxin. This method of active immunization against diphtheria by a balanced (sensitized) mixture of toxin and antitoxin has recently been applied to human beings by von Behring.[39] The principal advantages of this

method, as originally pointed out by Besredka and as apparently proved, are, first, it produces little or no violent reaction on inoculation in instances in which the untreated bacteria themselves are distinctly irritating, as, for example, in plague; second, it gives rise to an immediate though transitory passive immunity; third, it produces, eventually, an active immunity which is as enduring and as rapidly formed as when untreated bacteria are used.

Not a little experimental work was done with sensitized typhoid vaccine before the work of Metchnikoff and Besredka, which will be taken up later. Paladino Blandini [10] made a very careful study of seventeen different typhoid vaccines, comparing their relative immunizing properties. His experiments were carried out on guinea pigs, which were treated by the various preparations and subsequently given an intraperitoneal dose of living typhoid bacilli. The vaccines tested include small doses of living cultures; killed cultures prepared after the method of Pfeiffer and Kolle, and of Wright; several "soluble toxin preparations" of the typhoid bacillus; nucleo-albumins; and extracts of the typhoid bacillus prepared in different manners. Compared with these methods was the sensitized vaccine employed by Besredka, and Blandini was able to demonstrate that the latter method was by all means the most protective. Not only were guinea pigs protected for at least four months, but it was found that their serum, which contains sensitizers, also protects normal animals against infection. Ardin-Del-teil, Negre, and Reynaud [40] find that the use of sensitized typhoid vaccine in rabbits and human beings gives rise to relatively small amounts of agglutinins, but the bactericidal properties of the serum of those treated in this manner are much higher than of those treated by the ordinary cultures. These authors have also obtained very favorable results in treating cases of typhoid fever with this vaccine.

This failure of sensitized or agglutinated cultures to produce potent antibodies had already been noted by Neisser and Lubowski [41] and by Pfeiffer and Bessau.[42] Garbat and Meyer [43] immunized rabbits either with sensitized or with whole cultures

and compared the properties of the serums of the two sets. The serum obtained by immunizing the rabbits with sensitized cultures agglutinated and gave the fixation reaction much less strongly than the corresponding animals treated with unsensitized cultures. The serums of the sensitized animals, however, were more bacteriotropic and protected animals experimentally much better than the serum of the animals treated with the plain vaccine. This fact is incidentally evidence of the unreliability of the agglutinin test as a measure of the grade of protection against typhoid infection, a matter which we shall later consider.

In 1911 Metchnikoff and Besredka [8] first reported their very important work on experimental typhoid fever in anthropoid apes. They found that the chimpanzee and the gibbon when given the dejecta from cases of typhoid fever, or lavishly contaminated food, or, in their latter experiments, when the mouth of the animal is painted with cultures of the typhoid bacillus, after eight days' incubation develop characteristic typhoid fever. Fifteen or sixteen monkeys that were tested gave positive blood cultures on the tenth day, their serum contained agglutinins, and, in three instances, death due to the typhoid infection followed. The temperature of these animals ran as high as 40.8° C. (105.4° F.). Peyer's patches were found swollen but not ulcerated. The only detail in which this experimental disease would seem to differ from typhoid fever in human beings is the fact that the spleen in the chimpanzees is not distinctly enlarged. Having determined in this way that the typhoid bacillus is in reality the cause of typhoid fever and not some adherent filterable virus, as in the case of hog cholera, they turned their attention to methods of immunization against the disease. Their first series of experiments are far from convincing, although they draw from them rather sweeping conclusions. In the first place, the number of animals employed was necessarily small, owing to their expense and the great susceptibility of the animals to extraneous infection, so that in this first series, in which there were five experiments, there was only one vaccinated animal in each case tested with an untreated control. They come to the conclusion from their preliminary attempts that neither Vin-

cent's typhoid vaccine, an autolysate of the typhoid bacillus, nor a *killed* culture sensitized according to the method of Besredka will immunize anthropoid apes against a subsequent infection by the mouth.

Apart from the necessarily small number of animals in each experiment, certain other objections may be made to their experiments and conclusions, part of which Vincent [44] has pointed out. In the first place, the animals were tested very shortly after finishing the immunizing treatment, usually in from four to six days, which may reasonably be regarded as too brief a period for the establishment of the highest grade of immunity; and, second, the dose given the animals by the mouth, which is not stated very definitely, seems in all events to be extremely large, much larger, indeed, than in the natural infection in man. They conclude from their experiments that heated vaccines do not protect anthropoid apes against typhoid infection, and, owing to the analogy of the disease in these animals with the human syndrome, they regard such vaccines as unfitted to protect human beings. Inasmuch as the protective vaccination of man against typhoid fever by means of heated vaccines is generally recognized as reducing the morbidity to one-half or one-sixth of the normal, the logic of their position does not seem sound. It would seem better to have concluded that the immunization or the disease in anthropoid apes, as they have produced it, are not similar to their analogs in human beings.

In their second communication, Metchnikoff and Besredka [45] present experiments which, with the exception of one experiment in which Vincent's vaccine is used, are more convincing. Their second series deals with the protective effect of vaccination by a living paratyphoid B culture, and by sensitized *living* cultures of the typhoid bacillus against subsequent infection of the latter organism in the manner described. These experiments comprise more animals than the previous series, and in addition the infection is not given until from ten to fifteen days after the completion of the treatment. Vincent's culture again failed to protect, whereas the living sensitized typhoid culture and the living paratyphoid B cultures uniformly saved the animals. It would

be far from safe, however, to conclude from these experiments that no preparation of heated typhoid bacilli has the power to protect even anthropoid apes against subsequent infection by the typhoid bacillus.

We have examined the experimental details of the work of Metchnikoff and Besredka, not in a spirit of unfavorable criticism, because, as will later be shown, we personally believe that their conclusions were in part correct, though the data on which they have based them have not convinced us.

We may here examine the results that have already been obtained in immunizing human beings by the living sensitized cultures recommended by Metchnikoff and Besredka. Broughton Alcock,[46] working under their direction, was the first to report on the harmlessness of the method, its freedom from untoward effects, and its apparent protective value. He advocates a dose corresponding to 1/100 of an agar culture, which is equivalent to 500 million typhoid bacilli. Their organisms are sensitized by a few drops of a strong antityphoid serum, the exact potency and amount of which is not stated either by Metchnikoff and Besredka or by Broughton Alcock. After standing for twenty-four hours the clumped bacteria are washed in salt solution and resuspended in an aliquot portion of normal saline. A living sensitized vaccine prepared in this manner keeps for at least four months on ice. Objections have been raised by Vincent and others as to the danger of injecting living micro-organisms into the human body, but Metchnikoff and Besredka [47] have reported that careful tests fail to show that human beings treated in this manner become carriers of the typhoid bacillus or eliminate the micro-organism through the fæces or urine. In view of the uniformly good results that have been obtained, there seems little reason further to suspect the harmfulness of the method. In a recent report Metchnikoff and Besredka [48] have further endeavored to determine the optimal vaccinating dose and interval of this sensitized vaccine, and Besredka [49] reports some of the favorable results that have been obtained. Cadeau [50] vaccinated twenty-five patients, and only two showed slight general symptoms. In an asylum at Briqueville where many cases of typhoid

fever existed in 1912, 516 persons were vaccinated, and in the twelve months subsequent no cases occurred among them, whereas four occurred in the 343 persons who were not immunized. Marie reports similar absence of general reaction. In all, Besredka has dispensed 10,000 doses of the vaccine and no unpleasant results have been reported except when the injections were given intramuscularly. He recommends a dosage of from 500 million to 1,000 million, although larger amounts could be given without harm. It is, of course, too early to draw any conclusive figures as to the actual protective value of this method, although its harmlessness and freedom from untoward symptoms seem to have been proved.

PERSONAL INVESTIGATIONS

In view of the general acceptance of the relative protective value of immunization with killed cultures of the typhoid bacillus, and the claim that Besredka's method of vaccination with sensitized living typhoid bacilli insures all the advantages of the more unpleasant method of treating with dead cultures, and protects as well or better, it would seem desirable to devise some method of animal experimentation which would enable us to settle conclusively this and many other debatable questions that arise in connection with this extremely beneficial process. There are still many questions in connection with typhoid vaccination which remain unsettled, such as the dosage to be employed, the interval at which the injection should be given, and, most important of all, the duration of the protection that is afforded by any particular method and to any particular individual. Animal experimentation has, to be sure, been used, for the most part on guinea pigs that after treatment with various typhoid vaccines are infected in the peritoneal cavity. It seems justifiable, however, to question the transference of results obtained in preventing a banal peritonitis in this animal to human beings. The method of testing typhoid vaccines on anthropoid apes that Metchnikoff and Besredka have described is undoubtedly the ideal one, but practically impossible to employ for the settling

of details of method owing to the expense of the animals and the difficulty in obtaining them in any numbers.

We have already described in some detail our method for testing the immunizing power of a given typhoid vaccine by means of the "rabbit-carrier" condition.[51] This carrier condition, as originally pointed out by Blackstein, may be produced in rabbits by the intravenous injection of certain strains of the typhoid bacillus. It presents a very close analogy to the disease known as typhoid fever in man. It is a bacteriæmia, with a particular localization of the micro-organism in the gall-bladder, which viscus shows the characteristic lesions described in human beings, including the formation of gall-stones under favorable conditions. By employing one-half of a standard 10 per cent. blood agar culture of a certain strain of *B. typhosus*, we have been able to produce this condition in normal rabbits with relative certainty. Thus of thirty control rabbits, twenty-eight, or 93 per cent., either became permanent carriers with positive cultures from blood or gall-bladder, or, in a few instances, died acutely within twenty-four hours with symptoms of intoxication. For further discussion of the experimental advantages of this method over others proposed for this purpose, excepting experiments on anthropoid apes, we refer readers to our previous communication.

As we have just suggested, the presence of antibodies, particularly of agglutinins, is not a measure of the degree of protection against typhoid fever, either in animals or in human beings. Antibodies indicate reaction, which usually parallels protection but is by no means synonymous with it. In typhoid fever in particular the eventual absence of agglutinins in those recovered from and thereby protected from the disease indicates that serum tests are by no means to be confused with protection, which is more likely to be of a cellular than of a serum type. This indicates emphatically the necessity of testing the prophylactic immunizing value of any given vaccine by actual infection of the vaccinated animal rather than by any test of its antibodies. In our previous paper we have put forward our reasons for regarding the artificially produced bacteriæmia in rabbits

as analogous to typhoid fever in human beings, which with increasing agreement is now regarded primarily as a bacteriæmia rather than a purely intestinal disease. The fact that we are able to prevent this carrier condition by previous immunization with one typhoid vaccine, whereas another preparation of the typhoid bacillus in the same amount does not prevent it, is further evidence that the method we have chosen is a delicate one and further suggests the variations in result that have been obtained in using different typhoid vaccines in human beings.

Preparation of Typhoid Vaccines for Comparative Tests.— The first essential in comparing various vaccine preparations seemed to us to lie in a standardization of the amount of substance employed. The methods usually employed in standardizing vaccines for clinical use may be accurate enough in view of the fact that the optimal dose is largely empirical and a little less or more may make no difference in the results. The fact that the usual methods of enumeration of bacteria by counting by the Wright method are being constantly changed is evidence enough that it is a far from satisfactory condition. We had already adopted the far more accurate method of dried, weighed bacterial preparations when the work of Wilson and Dickson [52] came to our notice. These authors have carefully determined the actual weights of the doses of various bacteria commonly employed, checking their weighing results by counting the bacteria in a given volume of suspension. They find, for example, that there are on an average 8,000 million of typhoid bacilli in 1 mg. of a typhoid vaccine dried on platinum foil. We were surprised to find how closely our estimations, although obtained in different manners, agree not only among themselves, but also with the results of these authors. Our method of preparing dead typhoid vaccine has been to add normal saline (0.85 per cent.) in proportionate amounts to suspend forty-eight-hour cultures grown on Blake bottles containing 125 c.c. of 2 per cent. agar (titrated +1) or, in certain experiments, of the same medium containing 10 per cent. fresh defibrinated rabbit blood. To such a suspension is added an equal volume of absolute alcohol, which kills the culture in the proportions used in less

than fifteen minutes, flocculates out the organisms so that they can be easily collected by centrifugalization, and accelerates their drying in an unglazed porcelain plate in partial vacuum over sulphuric acid.

Our experiments on the relation of the number of bacteria to weight agree very closely with those of Wilson and Dickson (8,000 million of typhoid bacilli to 1 mg. of dried pulverized typhoid powder). In all our experiments with vaccine preparations, then, we have used weighed amounts of dried and ground bacilli as a basis for comparison, except in those in which living cultures of the organisms have been employed, in which case comparisons were made in another way.

Methods of Immunization.—Having determined on the use of weighed amounts of dried, ground bacteria for comparative testing of typhoid vaccines, the next essential was to decide on the best method of immunization of the rabbits that are to be tested for protection against the carrier state.

Fornet and Müller [53] and Bonhoff and Tsuzuki [54] have already shown that for the production of certain antibodies an intensive method of immunization may be employed by reinjection at short intervals. In collaboration with Fitzgerald [55] I have still further perfected this method. We were able, for example, to produce high-grade hæmolysins to sheep corpuscles by injecting rabbits intravenously on three successive days with 1 c.c. of washed sheep blood. These lysins we find to be present in high degree four or five days after the last injection. Similar, although not quite so perfect, results were obtained in the production of precipitins and also in the production of bacterial agglutinins by Miss Edna Locke.[56] On the basis of these experiments, we had already determined that, provided a vaccine did not produce too severe symptoms, the period over which immunization extended might be markedly decreased without any corresponding deduction in the intensity of the immunity effected. It is of interest to note that Fornet [11] has also adopted a rapid method of immunization in vaccinating against typhoid. We have ourselves recommended, and Force [57] has employed, immunization at intervals of two or three days in human beings,

as will be mentioned later. The rapid method of immunization at short intervals not only may be shown in the case of rabbits to effect an equally high grade of immunity as determined either by antibody content or by actual resistance to infection, but also has the further advantage of actually producing less harmful effects when large amounts of the untreated vaccine are used. We have repeatedly noted that if a large total amount of vaccine is given to rabbits during a period extending over three weeks there is a larger percentage of mortality due to cachexia than when the same amount of vaccine is given within three days. The immunizing doses have invariably been three in number and have been administered either intravenously or subcutaneously; in all our later experiments it has seemed better to adopt the intravenous method as giving greater uniformity of results.

The advantage of immunization at short intervals as against longer intervals was first tested by comparing the resistance to the carrier state of two sets of rabbits, the one immunized by three intravenous injections on three successive days, the other by three equal doses administered intravenously at three-day intervals. There was found to be no marked difference in relative resistance of these two sets of animals when given the test dose two months later. The agglutinins averaged the same titer in the two sets. In a more systematic experiment, a comparison of three daily with three five-day interval doses was made. The results of this experiment are expressed in the following table:

TABLE 1.—Results of Long- and Short-Interval Methods of Typhoid Immunization in Rabbits

	Died During Vaccination	Carriers	Recovered
Short intervals	0	3	4
Long intervals	4	3	0

This experiment as well as other similar ones would seem to indicate the superior value of the short-interval intensive method of immunization.

Dosage of Vaccine Preparations.—We have no contribution to make on the optimal dose of typhoid vaccine for prophylactic use in human beings. As will be seen later, our choice for such purposes has been largely empirical and has followed accepted usage. In our comparative experimental work, however, it has been necessary not only to use preparations derived from weighed, dried bacteria, but also to determine with increasing accuracy the duration of the protection afforded by a given amount of a standard preparation. In our first experiments we immunized separate series of rabbits each with a given preparation and then tested representatives of each series at several successive periods. Inasmuch as we started with rather large doses of vaccine, the time before the protection wore off was rather long and the method time-consuming. By decreasing the total vaccinating dose we were finally able to estimate with certainty that a given amount of one type of vaccine would protect for five or six weeks; with this point determined, it was easy to compare the immunizing value of other preparations with this one. In nearly all experiments, two or more control normal animals have been injected, although their use is not absolutely imperative since the less well-protected animals become carriers and the percentage of carriers in each series gives the basis of comparison.

Determination of the Typhoid Vaccine of Election for Immunization.—The carrier condition was at first determined in the immunized and control animals by cultures taken from the blood ten and twenty days after inoculation; the results were checked by the post-mortem condition and cultures from the bile when the animals died, or in the case of survivors by chloroforming them from fifty to seventy days later. A later method which gives quicker results is to chloroform all the survivors at the end of ten or fourteen days and make cultures from the blood and bile. A perfectly immunized animal is one that gives negative blood cultures and at death presents no lesions, particularly of the gall-bladder, and gives negative cultures from the bile.

Throughout our experiments it has been our effort to kill our cultures of *B. typhosus* in a manner designed to obviate, so far as possible, the deteriorating effect of heat that has been evidenced by the work of numerous observers mentioned before. The introduction of heat, at all events for the purpose of obtaining sterile cultures, seems to us quite unnecessary, and, as I pointed out a number of years ago,[58] cultures of the dysentery bacillus may be sterilized and preserved by the simple addition of 0.5 per cent. trikresol or phenol, without destroying their toxic and antigenic properties to the same degree as in corresponding cultures that are first killed by heat at 60° C. (140° F.) and then preserved by trikresol. Very little attention appears to have been paid to this observation, although we believe that several have utilized the method with advantage, killing the cultures either with liquor formaldehydi or with trikresol. In the present series of experiments we have desired not only to kill the bacteria with little harm but also to collect and dry them in order that the dosage employed might be reckoned from weighed amounts of the dried bacilli, which had been previously ground in an agate mortar. For this purpose, as already stated, we precipitate our various bacterial suspensions with equal parts of absolute alcohol, which accelerates the centrifugalization and the drying of the preparation. If the culture is to be sensitized, a definite amount of a potent, immune serum from the rabbit is previously added, the dosage being estimated in accordance with the agglutination titer of the serum in question and the number of bottles from which the bacterial suspension was obtained, the amount being 1 c.c. of a serum that agglutinates the organism in question in a dilution of 1:20,000 to the suspended growth from each of the seven Blake bottles. After standing for two or three hours in an incubator at 37° C. 98.6° F.), the sensitized culture is left in the ice-chest over night, centrifugalized, the supernatant fluid removed, the sediment washed in the original amount of normal saline, recentrifugalized, and the volume restored to normal with sterile saline. This washed sensitized culture is then flocculated by

adding an equal volume of absolute alcohol, dried, ground, and weighed as in the unsensitized culture.

We first compared the protective value of sensitized and unsensitized cultures. The results of our experiments are given in full in our fifth article on antityphoid immunization, in the *Archives of Internal Medicine*.[59] When parallel series of rabbits treated with sensitized and unsensitized vaccines are tested some weeks later, it is found that the serum of the unsensitized vaccine animal will agglutinate the typhoid bacillus rather better than the serum of a sensitized vaccine animal. The animals treated with sensitized vaccine are found, however, to be much better protected from becoming carriers on the intravenous administration of living typhoid bacilli.

The method which we have described for preparing and grinding the vaccine is essentially similar to the one described by Besredka for the preparation of endotoxins, and it may, indeed, be shown in the case of the untreated vaccine that the supernatant fluid contains the greater part of the toxic substances of the whole vaccine (Besredka,[60] Stenitzer[61]). It seemed to us important to determine at this point whether or not the supernatant fluid of such a ground and suspended preparation of typhoid bacilli, or the sediment of bacterial bodies left on centrifugalizing this mixture, contains the immunizing principle. In experiments in which the immunizing value of the two preparations is shown, it is very evident that it is the sediment of bacterial bodies deprived to a great extent of toxicity of the whole vaccine which is the essential immunizing principle. This immediately suggests the advisability, from the point of view of symptoms produced and of protecting value assured, of utilizing simply the remnants of the bacterial bodies obtained in such a vaccine rather than the whole vaccine itself. Experiments designed to test out the comparative value of the whole vaccine, on the one hand, and the sediment, on the other, show very distinctly that the sediment possesses not merely as much but apparently actually more immunizing value than the whole vaccine. In comparing the whole suspended vaccine with the sediment derived from such a vaccine, it should be

noted that every technical error necessary to produce the sediment from the whole vaccine would tend to lessen the amount of material in the sediment, and the superior immunizing value of the latter is therefore so much the more striking. In one experiment the percentage of failure to become carriers shown by rabbits immunized with the four different vaccines was as follows:

Rabbits given whole unsensitized vaccine recovered in 33 per cent.

Rabbits given unsensitized sediment recovered in 60 per cent.

Rabbits given whole sensitized vaccine recovered in 66 per cent.

Rabbits given sensitized sediment recovered in 75 per cent.

We seem so far to have determined, then, that killed, sensitized cultures are more protective than plain, unsensitized, killed cultures of the typhoid bacillus and that the protection is due to the bacillary sediment and not to the supernatant endotoxic fluid. These sediments, moreover, seemed distinctly more protective than the whole vaccines from which they were derived.

In view of the assertions of Metchnikoff and Besredka, it remained to determine by our method whether or not living, sensitized cultures have any superior immunizing value over sensitized cultures killed by alcohol. For this purpose we have compared the living sensitized culture first with whole dried sensitized culture and then with the sensitized sediment. In these experiments the original bacterial suspension is sensitized, washed, and divided into two parts, one of which is left intact and the other precipitated by alcohol, dried, ground, weighed, and suspended in the original amount of fluid. Here again technical errors would militate against the immunizing strength of the dried culture, particularly when the latter is carried further to the sediment preparation. Such experiments show, first, that living sensitized cultures have a slight superiority over whole dead (alcohol) cultures, but, second, that dead sensitized sediment protects better than the living sensitized vaccine.

Protective Vaccination in Human Beings by Means of the Modified Sensitized Culture.—Our experimental results had reached a stage nearly a year ago when we felt justified in recommending the whole sensitized culture that we have described for use in protecting human beings against typhoid fever. It seemed demonstrated from the work on rabbits that the sensitized vaccine, washed, precipitated, killed with alcohol, and ground, would protect at least as well and probably better than the ordinary vaccine that had not been sensitized, and we anticipated much fewer untoward symptoms following vaccination by the use of this sensitized vaccine. This absence of untoward effects has been fully justified in experience. Up to the present time over 1,000 students in the University of California have been immunized by Professor Force of the Department of Hygiene. The absence of reaction or the mildness of reaction has been very striking. Force [57] has already reported on the first 261 cases by this method, and less complete analyses of the subsequent cases agree with the results obtained in the smaller number. In these 261 cases—apart from the local symptoms of induration, redness, and slight tenderness for one or two days—general symptoms, even of a mild grade, were very seldom observed. The vaccine was administered on alternate days, the treatment being completed within a week. The very fact that it was possible to give the second and third vaccinations on the second and fourth days after the first indicates strongly that even the local condition was so mild as to allow the subsequent inoculations. The dosage at first employed was $^1/_{16}$ mg. of dried culture suspended in 0.5 per cent. carbolated salt solution, corresponding, as we have determined, to 500 million bacteria. Since this produced little or no reaction, we have since increased it by one-half ($^3/_{32}$ mg.). In only six cases in the total number of inoculations practised (671) did the temperature rise above 38° C. (about 100° F.). The symptoms were somewhat more pronounced in women than in men. In two cases of arrested tuberculosis the reaction was more marked and in a few cases that gave a history of previous typhoid fever the reaction was also slightly more pronounced. More recently we

have employed the sensitized sediment vaccine in accordance with the experimental results that have just been obtained, which show conclusively that this substance is more immunizing than the whole sensitized preparation. This sensitized sediment gives even fewer reactions than the whole sensitized vaccine. Thus in a recent series that Force has vaccinated with sensitized sediment, using a dosage corresponding to 750 million bacteria, including 672 separate injections, malaise was noted in 1.8 per cent.; fever (above 99° F.) in 4.5 per cent.; pain in back in 2.4 per cent.; pain in head in 9 per cent., and pain in the arm in 3 per cent. In cards returned to us from 231 outside patients to whom the vaccine was distributed, 215 report total absence of local and general reactions (93 per cent.). Ten cases were marked as having shown generalized symptoms and three cases of typhoid recoveries and three patients who had been previously vaccinated also showed slight symptoms. These reactions may be compared with the following: Hartsock [62] obtained a mild reaction, by which he specifies "temperature up to 100° F. with slight general reaction, malaise, and considerable local tenderness," in 83 per cent. of men treated with U. S. Army vaccine. Hatchel and Stoner [63] in 1,326 cases vaccinated with a polyvalent vaccine found malaise in over 58 per cent. and fever in 43 per cent. Albert and Mendenhall [64] used the U. S. Army strain in regular doses and intervals and found that the local reaction lasted for from three to five days. Fever as high as 103° F. was usual, and malaise, headache, and insomnia were common.

It must not be understood that we recommend this preparation of vaccine because it produces less unpleasant symptoms on administration but primarily because, so far as can be judged from our animal experiments, it protects better.

Both Wassermann [24] and Vincent [15] have suggested the use of polyvalent vaccines; although the typhoid bacillus has been regarded as an unusually fixed species, the occurrence of minor biologic peculiarities, particularly in the fermentation of sugars, has been noted in the collection of organisms which we have studied. In accordance with these facts we have recommended a polyvalent sensitized sediment for prophylactic immunization

against typhoid fever in human beings. The organisms, five in number, which have been used to produce the polyvalent mixture were isolated from recent cases of typhoid fever in the vicinity. An additional advantage in the use of sensitized over unsensitized cultures is that any number of strains of the typhoid bacillus may be used as in our polyvalent vaccine without producing any more symptoms than with a monovalent vaccine. It is particularly unnecessary to seek a "mild strain" as in the case of the U. S. Army vaccine. Our various strains are grown separately on Blake flasks, and the suspended cultures mixed and treated with a polyvalent immune serum obtained by immunizing rabbits with each of the strains in turn. This polyvalent sensitized sediment vaccine is now being manufactured and distributed free of cost by the California State Board of Health to any physicians in the State who may apply.

THE TYPHOIDIN REACTION

One of the greatest difficulties that has been present in determining the protective value of typhoid immunization as a whole has been the impossibility of determining the protection of a given group of persons by other means than the careful study of morbidity statistics among vaccinated people over a long period of years (Firth [65]). This difficulty delayed the final acceptance of typhoid immunization for at least eight years (1896–1904). Still less have we any means of determining whether or not a given person who has been vaccinated is actually protected against typhoid fever. We have already described in full the skin test which Gay and Force [66] have employed as of presumptive value in testing resistance to typhoid infection.

A new summary of our results with the typhoidin may be of interest: In 44 cases giving a definite history of typhoid fever from four and one-half months to forty-one years previously, 40 have given a clear-cut skin test to the concentrated growth of the bacillus. Of the four negative tests, two gave a strong skin reaction to a similar preparation made from *B. paratyphosus A.* which we have found not to occur in anything like the same

intensity in those cases that react to typhoidin. Only one of the remaining negative cases was further tested with the paratyphoidin solution and therefore only one of two cases was unexplainably negative and 95 per cent. (40 of 42) positive. We have a growing set of observations on comparative tests with two paratyphoidin solutions (A and B) in recovered typhoid and paratyphoid cases, and in those vaccinated against typhoid, which we may wish to present at a later time; they apparently show certain interesting group reactions.

Of the controls giving no history of typhoid fever, 38 out of 44, or 86 per cent., have been clearly negative. Only five of these control cases (11 per cent.) reacted distinctly positively, and we believe may be explained as cases of aborted or mild, undiagnosed typhoid fever. This explanation is rendered probable by the observation, brought to our attention by Dr. Edward von Adelung, of a perfectly controlled clinical experiment on this subject. The observer, Dr. von Adelung, was a member of a family party of four which visited Germany nineteen years ago. About two weeks after drinking at a suspected water source, two of the members came down with a fever which ran the typical course of typhoid and was so diagnosed. The other two members of the party at the same time had mild symptoms, lasting one and three days, respectively, and consisting of headache, fever, malaise, and a sensation of flushing, which they regarded as abortive typhoid. Neither of the latter two persons gave any other history of typhoid fever. When the skin reaction was applied to the two cases that had run the typical typhoid course and to the two that had passed through the "abortive" attacks, all gave positive results. This well-controlled, natural experiment would seem to prove that the supposedly normal cases that react to typhoidin may well be those that have had abortive or undiagnosed typhoid fever in the past.

Our experience with the skin reaction in those who have been vaccinated with various typhoid vaccines, including our own, has shown us that the majority of those who have been treated within a year and a half or two years may be expected to give a positive reaction, provided always that the last injection has

been given at least a month previously. After two years the reaction is less likely to be positive. This experience with the skin test would correspond very closely with what has been found clinically to be the usual duration of artificial typhoid immunity. We have felt justified, then, in recommending to students who have taken the typhoid vaccine previously that they should have a skin test applied subsequently at intervals and that if it turned out to be negative they should repeat the treatment.

Since our last communication on the typhoidin test, we have been led to modify the preparations of the solutions employed. We found that some of the original preparation of typhoidin after four or five months failed to produce a positive reaction in cases in which we had been led to expect it. That this failure was due to a deterioration in the product was evidenced by subsequent positive results obtained in six such cases by means of a new preparation. Although such deterioration does not occur for a considerable time with tuberculin, it is known to take place in mallein and abortin. In the latter two cases and in tuberculin as well, better reactions have been obtained with preparations purified by alcohol precipitation and dried (compare Haring [67] and Meyer and Hardenbergh [68]). A similar preparation has been made by precipitating the original typhoidin with twenty volumes of absolute alcohol, filtering, washing with absolute alcohol and ether, and then drying on porcelain plates over a vacuum with sulphuric acid. The control solution, 5 per cent. glycerin bouillon evaporated to one-tenth volume, was treated in a similar manner. Ten c.c. of the original typhoidin yielded 0.78 gm. of dried powder when the culture had been grown for five days before evaporating. The dried powder from the same amount of a control solution gave only 0.5 gm. There is every reason to believe that this dried typhoidin will keep its potency undiminished for at least a considerable time. Our observations, however, extend only to its trial over a period of two months. We dissolve a small amount in carbolated saline equivalent to the original volume of concentrated typhoidin or even to a double concentrated solution, and find that when kept in a cool

place it gives very good reaction in typhoid immunes for at least a month.

Our suggestion and growing belief that this skin reaction with typhoidin is a real measure of the protection that the person enjoys against typhoid fever is strengthened by observations on our immunized rabbits. We have already shown that the agglutinin titer is no indication of the resistance of a given animal to infection, and observations on the Widal reaction in man tend to the same belief (Ruediger and Hulbert [69]). The typhoidin reaction, on the other hand, is positive in that category of persons who are known to be protected against typhoid fever, namely, typhoid recoveries; it does not occur in persons who give no history of the disease, except in a small percentage that may reasonably be suspected of having had an abortive attack. The reaction further occurs in the majority of those persons who have been vaccinated against typhoid within the last two years and then gradually disappears. We had scarcely hoped to show differences in typhoidin reaction between incompletely and perfectly immunized rabbits as tested by our method of infection, which is, after all, a violent one, but our results in this respect have exceeded our anticipations. It is, however, easy to demonstrate fundamental differences between normal and immunized rabbits by the intradermal reactions. Cutaneous reactions were first tried, but abandoned as unsatisfactory.

With the intradermal reaction, performed by producing a tiny bleb under the skin by a short needle carrying typhoidin, a distinctive reaction is produced in rabbits that have been artificially immunized, but not in controls. In each case a patch on the abdominal surface is shaved the day before the test and two blebs produced under the epidermis by injecting in the one concentrated glycerin bouillon and in the other typhoidin. In the normal animal there is little or no difference between control and typhoidin spot. Both are usually surrounded by a red zone of a few millimetres, and there may be slight induration. In the animal that has previously been treated by typhoid vaccines there is a sharp difference between the two spots. The typhoidin spot, as early as five hours, but with increasing regu-

larity and intensity to twenty-four hours, becomes surrounded by a red areola, is indurated, and quickly forms a firm, hard nodule with a yellowish, slightly softened centre, from 2 to 5 mm. in diameter. This nodule persists at least for several days and may last two or three weeks. It is characterized histologically by an infiltration of lymphoid cells with which are mixed a few polymorphonuclear leucocytes.

Five normal rabbits tested in this manner gave negative reactions. Of 35 more or less perfectly immunized rabbits, 26 (74 per cent.) reacted positively. The differences in reaction between animals effectively immunized against our method of producing carriers and those which are not so fully protected is a relative matter when viewed in the aggregate, but in individual experiments is more striking. Thus, dividing our treated animals in respect to a negative or positive intradermal test and then comparing the results with their carrier condition or recovery on infecting the following day, we find that of nine with a negative intradermal test, seven, or 78 per cent., became carriers; of 24 with a positive intradermal test, 13, or 54 per cent., became carriers, a distinct though only relative indication of agreement between the test and the absolute protection.

In Table 4 is given the comparative immunizing value of four different vaccine preparations. Before the test inoculation, the intradermal test was applied to these animals with the following results as compared with the eventual carrier conditions:

Group 1, vaccinated with whole unsensitized vaccine (three animals): intradermal test, 3 negative; carriers produced, 2; recovered, 1.

Group 2, vaccinated with unsensitized sediment (five animals): intradermal test, 4 positive; carriers produced, 3; recovered, 2.

Group 3, vaccinated with whole sensitized vaccine (three animals): intradermal test, 3 positive; acute death (anaphylaxis), 1; recovered, 2.

Group 4, vaccinated with sensitized sediment (four animals): intradermal test, 4 positive; carriers produced, 1; recovered, 3.

These results show a distinct relation between a positive intradermal test and recovery and between a negative intradermal test and establishment of the carrier state.

Mechanism of the Typhoidin Reaction.—We do not intend, at

this time, to discuss fully the experimental evidence and the hypotheses that have been brought forth to explain the generalized and localized reactions that follow the injection or application of bacterial extractives like typhoidin in infected or immunized individuals. Of these reactions, the one to tuberculin has naturally been most studied. Some of the most interesting and debatable questions that seem to have arisen in connection with the tuberculin reaction are, first, whether or not it is in reality a reaction exactly similar to the anaphylactic reaction with serum, and, second, whether it is due to an interaction of antigen and antibody accompanied, it may be, by alexin fixation (Wassermann and Bruck). As regards the local, cutaneous test we have the further possibility that the antibodies, hypothetically furnished to unite with the antigen, may be furnished from the general circulation or due to local cellular response (von Pirquet). There is certainly evidence in the case of typhoid recoveries that the protection is cellular rather than humoral, and the relative inefficiency and short duration of artificial vaccination against typhoid fever may well be due to the fact that the immunity is not sufficiently cellular in type.

It seemed possible to attack certain of these problems in relation to the localized typhoidin test. The first question that arose was whether or not the reaction was due to the local combination of antigen and antibody. As has already been shown, the sensitized vaccine which we have employed gives less reaction, both general and local, than the untreated vaccine when used in the prophylactic treatment of human beings. We should not, therefore, expect that the local application of a sensitized vaccine in normal individuals would call forth any, or, at any rate, not so marked, a response as the untreated vaccine. And yet, it was argued, if the local typhoidin reaction in an immunized individual is really due to a combination of antigen and antibody, sensitized vaccine, which is just such a combination, might produce a local reaction in a normal individual.

When comparative intradermal tests with sensitized and unsensitized vaccine were made on two normal rabbits, one of the two showed a much more marked reaction to the sensitized

vaccine than to the unsensitized, the sensitized vaccine reaction resembling in all respects that produced in an immunized animal by the injection of typhoidin.

Of four normal human beings that were similarly tested by intradermal blebs, three showed a very characteristically increased reaction to the sensitized vaccine over the unsensitized vaccine. In only one case were the reactions produced by the two suspensions similar, and both were so marked as to suggest the possibility of an overlooked typhoid history. We regard this experiment, then, as distinctly indicating that the local reaction to typhoidin is due to an interaction of antigen and antibody.

One further point on the mechanism of the typhoidin skin test has been brought out by experiments on rabbits. Having reached a logical assumption that the skin test is due to the interaction of antigen and antibody in the immune individual, it remained to show whether the antibody furnished for this combination comes from the circulating blood or is due to local tissue reaction. If the first hypothesis is true, immune serum should transfer the reactionability to a normal animal, and an immune animal that reacts should at least partially lose this power if exsanguinated and transfused with normal rabbit blood. Both these conditions were found possible of realization.

Experiments show clearly that the abstraction of blood from an immune animal that reacts strongly to an intradermal test of typhoidin renders it almost completely insusceptible, whereas a control immune reacts a second time on corresponding days. The blood of the immune animal transfers the susceptibility to the typhoidin reaction to a normal animal, which has previously reacted negatively. We may conclude, therefore, that in the case of immunized rabbits, and perhaps also in the case of human beings artificially immunized against typhoid fever, the skin reaction may be accounted for by the interaction of the antigen with antibodies in the circulating blood. We do not wish at all to suggest that the skin reaction in typhoid recoveries is due to the same mechanism. The absence of demonstrable antibodies in the majority of these cases, as well as their superior

type of immunity, would lead one to think that the skin reaction may be cellular rather than humoral in character.

Before summarizing our results in respect to the actual method advocated for vaccination against typhoid fever which has been the chief object of these experimental studies, I should like to refer briefly to two other aspects of typhoid infection that have been incidental to this work and to some extent investigated.

AGGLUTINABILITY OF BLOOD STRAINS OF THE TYPHOID BACILLUS [70]

The diagnosis of *B. typhosus* isolated from the blood or bile of the suspected carrier rabbits, in the experiments to which reference has been made, depends not only on cultural tests but on the clumping of such an organism by a known antityphoid serum. In our earlier experiments we were surprised to find bacilli isolated from these carrier rabbits that resembled typhoid bacilli in all respects culturally but failed to be agglutinated by a potent antityphoid serum which agglutinated the stock cultures in so high a dilution as 1 to 20,000. Such a serum had been produced by immunizing rabbits with cultures of *B. typhosus* grown on agar. It was soon found that the failure of the recently isolated cultures to be clumped by this serum is owing to the fact that they have become accustomed to growth in the blood, and similar results may be produced by growing the agglutinable stock cultures in media containing either blood or bile for two or three generations.

Of greater interest is the fact that these blood and bile cultures, which are inagglutinable by means of the ordinary antiserum, are readily clumped by means of a serum produced by immunizing rabbits with cultures grown on a blood-agar medium. This antiblood-serum also clumps the plain agar growths of bacteria. Subcultures of the blood-agar growth render it agglutinable again by the plain antiagar serum.

These facts are of considerable theoretical interest as showing the essential lability of an antegenic property, but are also of practical diagnostic importance. Recently isolated strains of *B. typhosus* from human cases are at times temporarily undiagnosible because they fail to agglutinate with a diagnostic serum

until repeatedly subcultured. Such strains, as we have shown, are readily clumped by an antiblood-typhoid serum which should therefore be used for such purposes.

THE PHENOMENON OF SPECIFIC HYPERLEUCOCYTOSIS [71]

The importance of polymorphonuclear leucocytes in combating bacterial infection has been particularly emphasized by Metchnikoff and is now generally admitted in at least some of its aspects by all observers. In studying the fate of typhoid bacilli injected into the blood-stream of normal and of immunized animals we have found certain striking quantitative differences in the response of these important cells to the invading bacteria. In the case of the normal rabbit the injection of typhoid bacilli is followed by a marked fall in the leucocyte count in three or four hours, followed by a rise to two or three times the original number (12,000 per m.m.), which reaches its height between twelve and eighteen hours after injection. A similar hyperleucocytosis occurs also after the injection of any foreign protein substance such as serum or red blood-cells.

In the typhoid immune animals the injection of the specific micro-organism is followed by a hyperleucocytosis of far greater degree. This hyperleucocytosis is in the form of a crisis or rather of two crises which occur usually at 12 and 18 hours with a preceding leucopænia. The leucocytic count on the second and higher of these crises frequently reaches 100,000 to 150,000 per m.m. and in one instance reached over 270,000, a figure which has never been experimentally obtained in any other way so far as we are aware. This exaggerated leucocytic response is specific; that is to say, it occurs in a typhoid immune rabbit only on the injection of typhoid bacilli and not of some other micro-organism.

We have found, moreover, that this specific hyperleucocytic phenomenon occurs in other forms of protein immunity; it may be produced in animals that have been immunized against foreign red blood-cells or serum on injection of the specific substance. In all these instances the increase of leucocytes seems to affect only those of the polymorphonuclear type.

The well-known tropic action of immune serum as an adjuvant to phagocytosis suggested early in our studies that we might here be dealing with a similar phenomenon, the tropins in the circulation of the immune animal rendering the injected bacteria more attractive to leucocytes and, therefore, increasing their response. If this hypothesis were true, we should expect to be able to evoke a similar response in normal animals on the injection of sensitized or tropinized bacteria or red blood-cells. Such has proved to be the case as may be readily shown by experiment.

The work we have done with the specific leucocytic crisis, particularly the last fact, that it may be induced in normal animals by the use of sensitized (tropinized) cultures, immediately suggests therapeutic possibilities. Although our attention has hitherto been directed largely toward the prophylactic use of various typhoid vaccines, our preliminary experiments on animals in a curative way may be of suggestive value.

Metchnikoff has assumed that the principal locus of origin of the immune bodies is in the leucocytes. Our own experiments with determinations of the hæmolytic and precipitin titer in blood- or serum-immunized animals, paralleling the leucocyte response on reinjecting the antigen, indicate that the crisis is followed by an increase in antibodies which rapidly restores the titer to as much or more than the original strength, although at first it always suffers a temporary fall. In other words, the leucocytic crisis (and destruction?) would seem to liberate antibodies. We have already indicated that coincidently with the crisis in well-immunized typhoid animals the body sterilizes itself of the injected bacteria.

Similar processes are recognized as taking place in pneumonia, which is characterized by a crisis followed by rapid recovery. Dochez [72] has found that the protective value for mice of serums from human cases of pneumonia is increased after the crisis. Hektoen [73] also suggests the function of the leucocytic crisis in pneumonia as of curative value.

We have in a few instances been able to abort the usual carrier condition in rabbits that follows the injection of our standard dose of blood agar culture of *B. typhosus* by injections

of a sensitized typhoid vaccine, following the initial infection. Further study in this direction is at present engaging our attention.

SUMMARY AND CONCLUSIONS

The history of the development of artificial immunization against the typhoid bacillus in animals and human beings is reviewed, and some of the many preparations of the typhoid bacillus used for this purpose are discussed. Particular attention is drawn to the reputed advantages of living sensitized typhoid vaccine (Besredka) as opposed to other types of vaccine. The many vaccines that are still being advocated indicate that the best vaccine has not yet been found and that the best method of proving which is the best vaccine has not been determined.

In the present article we explain again the method of testing typhoid immunity by means of an artificial typhoid carrier state in the rabbit, and again advocate it as the best test for this purpose short of experimentation on anthropoid apes. We find this method admirably suited to indicate slight differences in typhoid preparations as regards their ability to produce an immunity against a subsequent typhoid carrier condition produced by intravenous injection of living typhoid bacilli. In successive series of experiments we have been able to show by this method, first, that untreated bacteria, killed and precipitated by alcohol, dried, ground, and weighed, do not protect so well as typhoid bacilli that have been sensitized by an immune serum and subsequently treated in the same manner. Second, it has been possible to show in the case of the unsensitized, dried bacteria that the sediment of bacterial bodies freed from the supernatant, endotoxic fluid as prepared from these dried cultures contains the immunizing principle almost in its entirety. The sediment of either sensitized or unsensitized cultures protects not only better than the supernatant fluid from these sediments, but actually better than the whole, unseparated mixture. Third, we find that alcohol-killed, sensitized cultures protect almost as well as living, sensitized cultures and that the sediment

of alcohol-killed, sensitized cultures protects better than living, sensitized cultures.

Sensitized cultures of the typhoid bacillus, whether whole or as sediment, produce little or no reaction in human beings, with the possible exception of those who have previously suffered from typhoid fever or have been immunized. By comparing our results with those of certain other observers, we conclude that a considerable degree of reaction, both local and general, is avoided by the use of these sensitized cultures, which possess the further advantage, so far as our experimental work can determine, of producing a more durable type of immunity.

We recommend vaccination at short intervals (two days) in human beings, which is rendered quite possible with the mild vaccine we employ, and is evidenced from animal experimentation as giving rise to less toxic effect and to fully as durable an immunity as vaccination at larger intervals. This type of vaccination has the further advantage of completing the prophylactic treatment of three injections within a week.

We further believe that a polyvalent vaccine derived from strains of the typhoid bacillus isolated in the vicinity of cases that are to be treated is advantageous, judging largely from the work of other observers. A further advantage in the use of sensitized cultures is that a polyvalent vaccine, no matter how recently the strains may have been isolated, is also almost entirely free from untoward effect. We have no direct experimental evidence bearing on the superior immunizing value of such a polyvalent vaccine. The dosage of vaccines in human beings which we recommend is empirical and based on the usage of other experimenters.

We recommend, then, for prophylactic immunization against typhoid fever, three injections of the sediment of a dried, ground, sensitized culture of several local strains of the typhoid bacillus mixed together, given at two-day intervals and in a dosage of $^{3}/_{32}$ mg., of the original dried culture, which corresponds, as has been determined, to a dosage of approximately 750 million living typhoid bacilli.

Our own experimental and clinical results, as well as the

work of many other investigators, leads us to regard the agglutination reaction with the serum of immunized animals and human beings as by no means indicative of the degree of protection afforded against infection with the typhoid bacillus. Of far more prognostic significance is the skin test with the typhoidin solution, which Gay and Force have recently described. Further summaries of the results with this test show that this reaction is positive in the majority of recovered cases of typhoid (95 per cent.), even as far back as forty-one years, and that it occurs in only about 11 per cent. of persons who give no history of typhoid or of typhoid immunization. Distinct evidence is brought forth that indicates that these supposed normals reacting to typhoidin have suffered from an abortive or undiagnosed typhoid fever. Persons immunized with various types of typhoid vaccine react in the majority of cases for about two years and then become more frequently negative. We regard a negative skin test after vaccination as indication for revaccination.

That a positive typhoidin test bears a distinct relation to protection against typhoid fever is further indicated by similar reactions produced intradermally in our immunized rabbits. In spite of our more violent method of artificial infection as against natural infection in typhoid fever, we find that animals that resist becoming carriers show a higher percentage of positive intradermal tests to typhoidin administered the day before; whereas those that are not immunized sufficiently to resist the carrier state more frequently show a negative typhoidin reaction.

We suggest the use of dried, alcohol precipitate from typhoidin solution as a stock from which the test solution may be prepared, since it has been found that the original typhoidin solution deteriorates within a few months.

Experiments dealing with the mechanism of the local typhoidin reaction indicate that it is due to an interaction of antigen and antibody, as is shown by the fact that the *intradermal* test of a sensitized vaccine will produce a characteristic reaction in human beings, whereas the corresponding amount of an unsensitized vaccine produced no such reaction. The paradox

of producing a more severe local reaction with the sensitized vaccine, which has been found to produce less violent reactions when used for immunization, is only apparent. Further experiments with rabbits indicate that in the condition of artificial immunization against the typhoid bacillus, the antibodies which unite with the antigen to produce the local test are in the circulating blood, as is indicated by the passive transfer to a normal rabbit by means of serum from an immune rabbit of susceptibility to this reaction. This is further evidenced by the extraction of blood from the immunized rabbit and a corresponding loss of reaction. We do not regard this experiment, however, as indicating the circulatory nature of the antibodies in the recovered typhoid persons who almost invariably react to typhoidin.

We refer briefly to two phenomena that have been met with in connection with our studies which deal more directly with prophylactic immunization. Strains of the typhoid bacillus grown on blood media or isolated directly from blood and bile are inagglutinable by the ordinary antityphoid serum. They are readily clumped by a serum produced by immunizing animals with blood cultures. These facts are of value in the diagnosis of recently isolated inagglutinable strains of *B. typhosus* from human cases.

The phenomenon of a specific and extreme hyperleucocytic crisis produced on re-injecting an animal that has been immunized against a foreign protein with the same antigen is referred to. In the case of typhoid immunity this crisis bears a definite relation to the recovery of the immunized animal from typhoid infection and its possible significance in the specific therapy of typhoid fever is suggested.

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THE EXCITATION WAVE IN THE HEART*

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MAY I preface what I have to say to-day by telling you how much I appreciate your invitation to deliver this Harvey lecture. How clearly the great physician, with whose name this Society associates itself, to whose name it delights to do honor, saw that the natural and safe advance of medicine should follow its advance guard, physiology. Has he not fitly been named the Father of that Science? Is it not a matter of profound satisfaction and pride to us that this pioneer of experimental physiology should have been of our profession and that his greatest discovery should have been prompted by observations upon the human being? As Harvey by physiological study laid the foundation of medicine as an exact science, so, to-day, if we have learned the lesson which his writings should teach us, we shall maintain this tradition, preserving the closest intimacy between our conceptions of the physiology and pathology of mankind.

If we as students of the heart are exponents of such new methods as clinical electrocardiography, should we not, in aspiring to become the humble disciples of this great teacher, first probe the normal phenomena of the heart's electric forces to the utmost? Can we who are met to emphasize the name and works of this man, for all time the first exponent of the heart's mechanism and function, more fittingly employ ourselves than by earnestly considering the natural heart-beat? In these questions you will find my reason for attempting to explain to you the origin and course of the natural excitation wave. Harvey, our master, chose as his illustration the heart of a king's deer; we, his disciples, may select a less regal beast, the dog.

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* Delivered October 24, 1914.

GENERAL PRINCIPLES

That the heart-beat is accompanied by an electric discharge was first clearly shown by Kölliker and Müller in 1856. These workers laid upon the beating ventricle the nerve of a nerve-muscle preparation, and noted that with each contraction of the ventricle the nerve became excited. In that simple yet ingenious experiment our knowledge of the excitatory process commences. Since that time, a great number of workers have examined the mammalian ventricle from the point of view of the electric currents found in it. It will not be possible in this address to do justice to them, for the last chapters of the story are in themselves of undue length. I do not propose, therefore, to treat these questions historically, but to describe to you in language as simple as possible the results of recent observation. For some five years my laboratory has been engaged in the study of this question, and I have been joined in the work by a number of collaborators. These collaborators have been, for the most part, your countrymen, and it is a lively pleasure to me to know that several of them are here to-night. The Drs. Oppenheimer of this city published with me one of our first papers. Dr. Meakins, of Montreal, and Dr. White, of Boston, joined in these researches at a later date; and most recently I have had the help of Dr. Rothschild of Mt. Sinai Hospital.

Let us in the first place examine the electric events which are associated with the contraction of a simple strip of muscle, and formulate the general laws which are to guide us in our examination of so complex a structure as the heart. If we connect a simple strip of muscle (*P–D*, Fig. 1) by means of non-polarizable electrodes to a sensitive galvanometer, and stimulate one end of this muscle (*P*), the galvanometer exhibits two deflections. The meaning of these deflections, together forming when recorded a diphasic curve, is known. The first deflection accompanies the commencement of muscle activity in the neighborhood of *P* (Fig. 1, *a*), and this deflection has the same direction as has the deflection obtained when the zinc terminal of a copper-zinc couple replaces the active muscle *P*. *When muscle*

is active, therefore, it is in a state of relative negativity, in precisely the same sense that the zinc of a battery is relatively negative. This negativity and the passage of a current from the inactive point through the galvanometer to the active point produces the first swing of the string recorder, a swing which is upright in our curves. It is a large swing because it is unbalanced; the region of the other contact being for the time being inactive. The second deflection of which I have spoken is similarly conditioned; it is produced after the excitatory process, associated with the contraction, has travelled in a wave

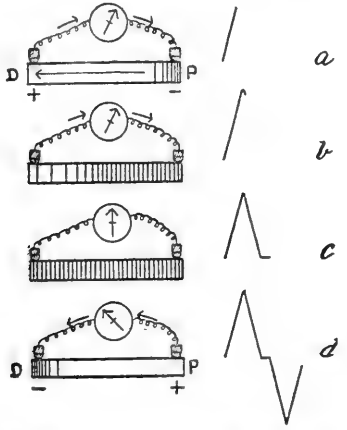


FIG. 1.—A diagram, illustrating the development and subsidence of activity (and negativity) in a single muscle strip, responding to a stimulus applied at *P*. The corresponding and successive phases of the galvanometric curve are shown in the four lines *a*, *b*, *c* and *d*.

from the proximal (*P*) to the distal end (*D*) of the muscle strip. *D* is active while at *P* activity has subsided (Fig. 1, *d*). The portion of the muscle which has the electric charge of similar kind to our zinc terminal becomes transferred as the wave of activity passes from *P* to *D*. Consequently after the active process reaches *D* the swing of the galvanometer is reversed; a current passes from *P* through the galvanometer to *D*, and develops the second deflection which is of opposite direction to the first. Thus the two phases of our recorded curve are due to a change in the relation of the active point to the two leading

off contacts, and this change produces a reversal of direction, the two deflections together comprising a diphasic effect. The culmination of the first phase can be shown theoretically and experimentally to coincide with the arrival of the excitatory process at the distal end, in short strips of muscle (Fig. 1, *b*). For when this occurs a balance begins to be established between the electric state under the two contacts. Evidently when the first phase is complete and the curve is about to cross the base line, activity is exactly equal under the contacts (Fig. 1, *c*). Now this is a simple experiment and easily understood, once it

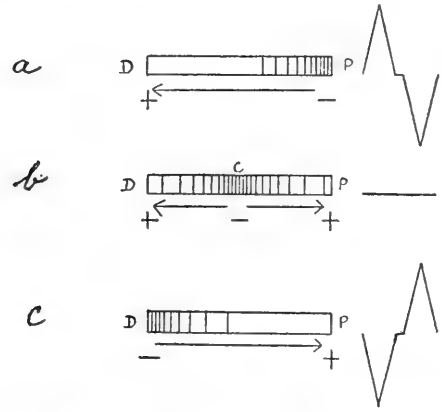


FIG. 2.—A similar diagram, illustrating the inversion of the curve when the order of contraction is reversed, and its isopotentiality when the ends of the strip are activated simultaneously. The directions in which contraction is driven are indicated by the arrows.

is recognized that electrically active muscle is relatively negative to inactive muscle. Simple as it is, it is fundamental to electrocardiography; the whole of our interpretations are ultimately based upon it.

It leads us immediately to our second law, which tells us that the *direction which the excitation wave takes, governs the form of the resulting curve.* And when I speak of excitation wave, you should recollect that this wave is intimately bound up with the contraction wave; it precedes it by an extremely short interval, and is presumably the result of those physico-chemical processes which at any point immediately precede actual con-

traction. That the shape of our curve is governed by the direction of the wave is readily shown by our simple strip of muscle, for let us reverse the direction of contraction by stimulating the originally distal end (D), and forcing the wave to travel from D to P. We still obtain a diphasic curve; but compared with our first curve, each phase is now reversed in direction (Fig. 2, a and c); it is easy to see why this is so, for having reversed the contraction, we have reversed the order in which the ends of the strip become relatively negative. But supposing that the same strip is stimulated at its centre point (Fig. 2, b), and that the contraction wave travels with equal rapidity to the two ends, where our contacts are arranged. Each end then becomes nega-

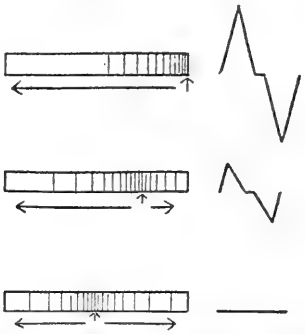


FIG. 3.—A similar diagram; to show that maximal excursion of the galvanometric recorder is obtained when the interval of delay between the arrival of the excitation wave at the contacts is greatest.

tive at the same instant and the two effects neutralize each other. In these circumstances there will be no swing of the recording instrument. Further, supposing that the excitation wave is started now at P and then at a series of regularly placed points up to a centre point (Fig. 3), then a graduated series of curves will be obtained, from a simple and large diphasic curve at one end of the scale, through curves of gradually diminishing amplitude, to a horizontal line at the other. From observations of this kind it is clear that the amplitude of the first phase is greatest when the time interval between the receipt of the excitation at the two contacts is greatest. If the time interval is nothing, a state of isopotentiality is established, and as the time interval

is longer and longer, so the effects are more and more unbalanced, and the culmination occurs later and later, and has more and more opportunity to develop. When we deal with a sheet of muscle, for example the auricle, as opposed to a strip, then the same statement applies; if a point is stimulated on the surface and a pair of contacts is arranged at a little distance away, then the amplitude is greatest when the contacts are radial [1] to the point of stimulation (Fig. 4, *a*), for with these conditions the interval between the arrival of the wave at the two contacts is maximal. In these observations we have the basis of our first investigations of the mammalian heart.

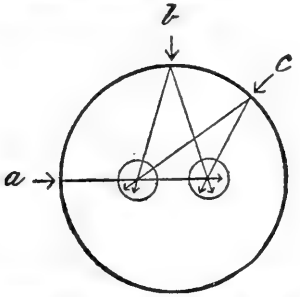


FIG. 4.—The circle represents a sheet of muscle, excited at *a*, *b* or *c*, and examined at two central contact points. The excursion of the recorder is greatest when the contacts are in the line of the excitation wave; *i.e.*, when the muscle is stimulated at *a*; it is least when stimulation is at *b*, for in this circumstance activity is simultaneously developed at the contacts.

I. THE ORIGIN OF THE EXCITATION WAVE IN THE AURICLE

The Point Which is Relatively Negative to All Outlying Ones.—If we place a pair of contacts upon given areas of the mammalian auricle and rotate them through an angle of 90 degrees, our curve varies in amplitude. We are able quickly to isolate a line which yields the greatest amplitude when the contacts are placed along it. Such lines are the favorable lines from which to lead, and we may conclude that such lines approximately represent the lines along which the natural excitation wave travels. Such lines in the mammalian auricle converge to a point in the neighborhood of the angle of superior vena cava and right appendix, where, as you know, the chief part of the sino-auricular node is situated. A specially favorable line is that

[1] As Engelmann has shown, the excitation wave radiates from a point of stimulation.

of the tænia terminalis. We have, therefore, a preliminary evidence that the excitation wave radiates from a central position, the region of the angle named.

We take our second step. If the natural excitation wave spreads along radiating lines from the upper regions of the sulcus terminalis and we place one contact in this region and the other upon a circle of points surrounding it, we should, according to a rule which has been formulated, obtain a series of curves of good excursion, and if activity is always developed under the central contact first, this contact should always be primarily negative to all other points. That is to say, if we arrange such radiating leads, maintaining our central contact upon the point

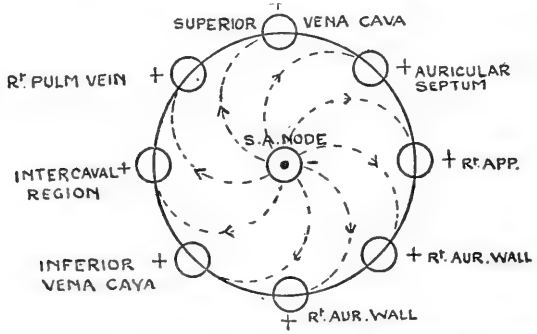


FIG. 5.—A diagram, illustrating a method of examining a sheet of muscle. A central contact lies over the region giving rise to the excitation wave, the second contact is placed at outlying points successively. In these circumstances the central contact always exhibits primary negativity.

at which the excitation wave arises, a series of curves should be obtained, of which the first phases are always of a given sign; the direction of the deflections should always indicate primary negativity of the central point (Fig. 5), *i.e.*, the first deflections should all be upright.

There is but one portion of the superficies of the mammalian auricle which exhibits these electric relations during normal contractions. If one contact is placed on the upper reaches of the sulcus terminalis and the other contact is moved along a circumference surrounding this centre, it matters not where this second contact lies, the first deflection obtained with auricular systole is upward in direction, indicating relative negativity of the centre point. Such are the events when the centre contact lies in appo-

sition to the head and superficial part of the sino-auricular node (Fig. 6). Now this experiment is a striking one, for the auricular muscle in this neighborhood is thin, and the *S–A* node lies in what we may regard as the centre of a muscle sheet, in the sense that a complete circle of points may be arranged around it. There is little or no possibility that this region of the heart receives the excitation wave from some deeper structure, for all possible paths to the node may be investigated. The conclusion that this centre is the centre in which the excitation wave originates is most strongly suggested. The observations upon which this proposition rests were made by Wybauw and by observation

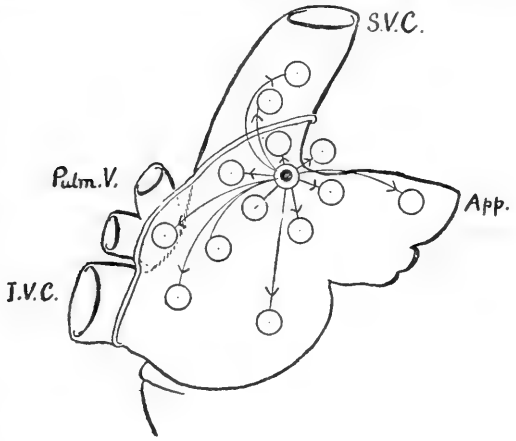


Fig. 6.—The contacts as applied to an auricle. The central contact, which is invariably relatively negative to outlying points when activity in the auricle starts, overlies the *S-A* node.

in my laboratory in conjunction with the Drs. Oppenheimer. They are observations which I have repeatedly confirmed since our original publication, and which have been recently confirmed by Eyster and Meek, independent workers in this land.

In the pig's heart, the sino-auricular node lies further up the sulcus than in the dog, and in one animal of this species I have found the point of relative negativity to be in a corresponding position. It lay, as Ivy Mackenzie subsequently showed histologically, immediately over the sino-auricular node in this particular animal, as it had lain in all our dogs.

Forcing a Natural Excitation Wave.—There is a second and

distinct method of approaching the same subject. Suppose that we start contractions from various regions of the auricle and observe the type of curve which each yields, using a given and fixed lead, and compare such curves with those of the normal heart-beat. I have said that the shape of the curves will be controlled by the direction which the excitation wave takes in the muscle. If, then, as we stimulate the auricle to contract, we discover some region of it which yields curves which are identical with the normal, we may be sure that the natural excitation waves and those propagated from stimulation of the area in question follow similar paths. They can only follow similar paths if the region which we stimulate is the region from which the normal excitation waves are propagated. In the case of the mammalian auricle, as I have shown, there is but a single area which answers to these conditions (Fig. 7). It is the area immediately surrounding the upper reaches of the sulcus terminalis, the region which contains the *S–A* node. Our second evidence, therefore, accords with our first; both indicate the *S–A* nodal region as that in which the excitation wave has its birth.

Extrinsic and Intrinsic Deflections.—I pass to a consideration of what are termed "outlying leads," that is to say, leads in which neither contact lies over the *S–A* node. In leading directly from the heart muscle, the chief deflections are produced by the arrival of the excitatory process immediately beneath the contacts. The contacts are exposed to the full force of this electric discharge. Such leads are very different from those utilized in human electrocardiography, for in them the contacts are upon the limbs and not upon the heart. Curves of the excitation wave may be obtained under each condition, and, for purposes both of description and of investigation, the direct and indirect effects should not be confused. Especially is this the case in leading from the heart itself. In such leads, the contacts lie on the muscle and the deflections are of two kinds.

1. There are deflections which result from the arrival of the excitation process immediately beneath the contacts; these we term *intrinsic deflections*. They are deflections, as you may suppose, which represent considerable electrical potentials and which have considerable amplitude.

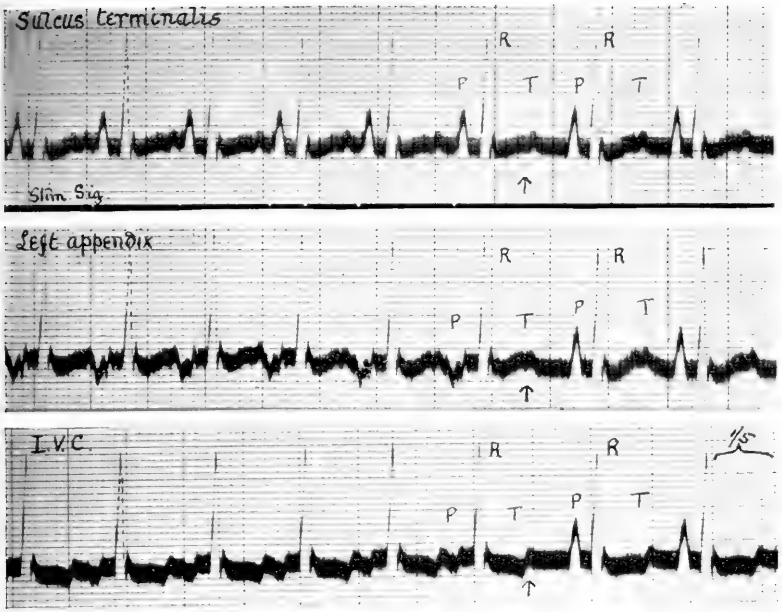
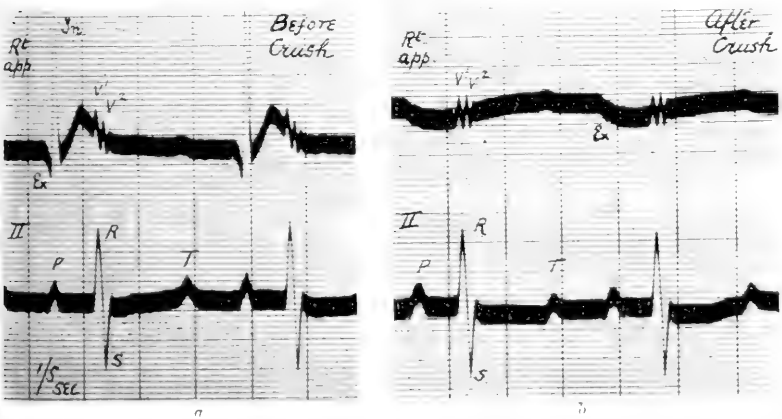
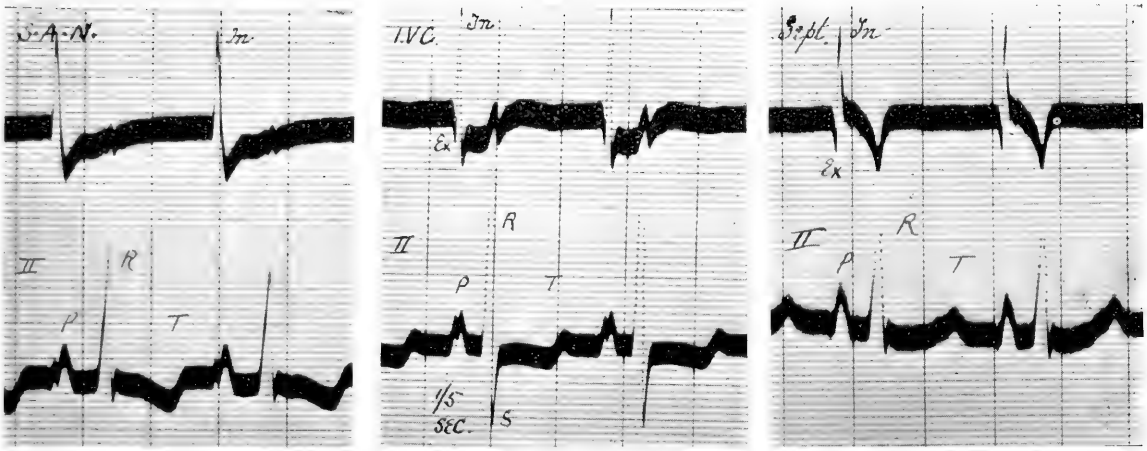


FIG. 7.—Three electrocardiograms from a dog (Lead II). The last two cycles in each curve are natural heart-beats. The remaining cycles are in response to stimulation over (1) the upper part of the sulcus terminalis, (2) left appendix, and (3) inferior cava. The natural beats are simulated when stimulation is in the region of the S-A node.



a b

FIG. 8.—Simultaneous electrocardiograms. The upper curves from the appendix, the lower curves from Lead II. Showing the effect of crushing the base of the appendix and rendering the tissue under the contacts inactive. The chief or intrinsic deflection (*In*) is abolished; the extrinsic deflection (*Ex*) remains, as do the ventricular deflections (*v*).



a b c

FIG. 9.—Examples of simultaneous electrocardiograms. The upper curves were taken from the region of the node, the inferior cava and the septum respectively; the lower curves are from the right forelimb and left hind limb (Lead II) in each instance.

2. There are also deflections which are yielded by the excitation wave, travelling in distant areas of the muscle. To these we apply the term *extrinsic deflections.*

A simple example of intrinsic and extrinsic deflections is the following: If we place two contacts upon the sulcus terminalis, at each beat of the auricle a large intrinsic deflection is produced by the arrival of the excitation wave beneath the proximal contact. When the ventricle contracts, the same contacts pick up smaller electric discharges from the last-named chamber. These extrinsic effects are records of muscle activity at a distance. But the same double effect is noticed in the auricle itself. For example, if we lead by two contacts from the right auricular appendix, we obtain a curve of the form shown in Fig. 8, *a*. You see the usual tall spike, but it is preceded by a small downward deflection. That this initial deflection is not produced by activity in the appendix, and that the chief deflection is, can readily be demonstrated by crushing the base of the appendix. In this manner the appendix is rendered inactive, and when this is accomplished the type of curve changes. The extrinsic effect, the small initial deflection, remains, while the intrinsic effects disappear (Fig. 8, *b*). Now this is a fundamental demonstration, for it permits us to analyze those curves which are obtained from outlying leads. All such leads give curves of this composite form, consisting of a main deflection, which corresponds to the arrival of the excitation process beneath the contacts, and diminutive initial deflections, which are due to the passage of other portions of the auricular muscle into the excitatory state. In considering the course of the excitation wave, as opposed to its origin, we shall focus our attention upon these chief or intrinsic deflections, for they will alone concern us.

The Point of Primary Negativity.—It has been said that a single point of the auricular muscle shows negativity relative to surrounding areas when the auricle first becomes active, and it has been concluded that this is so because this area first develops negativity or activity. Recently we have been able to demonstrate (Lewis, Meakins and White) that such is indeed the case by a direct and most conclusive method. We take simultaneous electrocardiograms (Fig. 9), either from two direct

leads or, preferably, from a direct lead and a standard lead (Lead *II*). By using exact methods of mensuration we have been able to reduce our customary error below a thousandth of a second and to measure the time of onset of the excitation wave in various regions of the auricle with great accuracy. Having searched the whole of the superficies of both auricles and the septum internally in a large number of animals, we can affirm that the first appearance of the excitation wave is over the head of the sino-auricular node and that it appears at later times in all other regions.

The same observations provide this and another important evidence that the *S–A* nodal region originates the excitation wave. It is the only region of the auricle from which curves are obtained in which there are no initial deflections (Fig. 9, *a*), the reason being that when the intrinsic deflection is obtained from this lead, the whole of the rest of the auricular tissue is in a state of inactivity, while in all outlying leads the intrinsic deflection, which represents activity, is preceded by initial movements of the string (*Ex.* Fig. 9 *b* and *c*), which represents currents from the preceding activity of the *S–A* nodal region and surrounding areas.

We may sum up this, the first part of my address to you, in the statement that we have abundant and conclusive evidence that the excitation wave commences in the immediate neighborhood of the head of the sino-auricular node.

II. THE COURSE OF THE EXCITATION WAVE IN THE AURICLE

When we examine the intrinsic deflections in curves from outlying leads, if the contacts are arranged radially to the *S–A* node, the direction of the intrinsic deflection is always the same, indicating that of the two contacts the proximal always receives the excitation wave first (Fig. 10); we have taken many hundreds of such curves without noting a single exception to this rule. From the direction of the intrinsic deflection alone we may conclude that the excitation wave spreads from the *S–A* node in all directions radially, that it runs down the tænia terminalis, into the tip of the right appendix, along the intra-auricular band to the tip of the left appendix, and down the

septum; that it runs into all the veins, caval, coronary and pulmonary, against the blood-stream.

And these conclusions are completely substantiated by our readings of the times at which the excitation wave reaches particular points. If a series of contacts is placed upon the auricle in a direction radial to the *S–A* node, and the times at which the excitatory process arrives in each is estimated (Figs. 11 and 13),

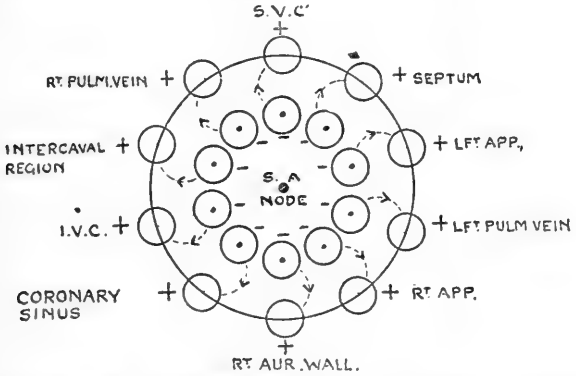


FIG. 10.—A diagram illustrating a system of "outlying" leads. A pair of contacts is arranged radially to the node in various regions. The proximal contact always receives the excitation wave first, as shown by the direction of the intrinsic deflection.

a contact proximal to the *S–A* node is always found to receive the excitation wave before a point more distal; and if the contacts are equidistant from each other, the times at which the excitation wave appears at the individual contacts of the series

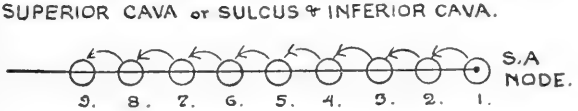


FIG. 11.—A diagram illustrating leads from serial contacts. If the *S-A* node lies at one end of the series, the time at which the excitation wave appears recedes uniformly throughout the series, starting at the nodal end.

increase in a regular order. The excitation wave passes up the superior vena cava and flows along it to a point well outside the pericardium (Fig. 15); it ends where the heart muscle ends and the venous muscle begins. It passes down the inferior cava to the edge of the cuff of muscle which is in places intrapericardial, in places extrapericardial (Figs. 13 and 14).

Knowing the distances between our contacts and the *S–A*

node, we are able to estimate the rates of conduction of the wave to all parts of the auricle. The average transmission times, distances, and rates are given in the accompanying table. The transmission rates are wonderfully uniform from node to all

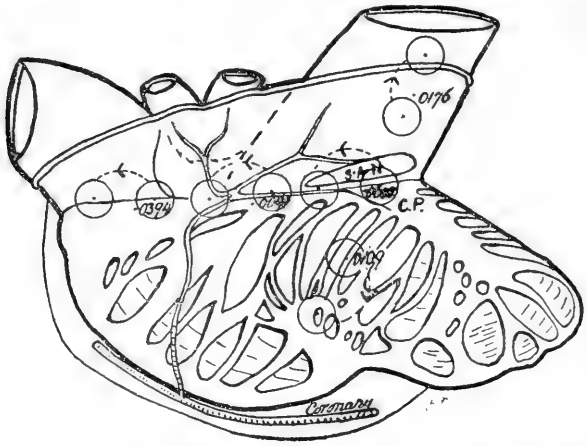


FIG. 12.—Outline of an auricle in an actual experiment; showing the arrangement of the muscle bands, the concentration point (*C. P.*), and the outline of the *S-A* node. The diagram is accurately to scale, and illustrates the method of leading off by paired contacts and the subsequent orientation.

parts of the auricle; such differences as occur are readily accounted for by small errors of measurement, or by the arrangement of the muscle bands, for it seems as if the rate of trans-

Region	Distance in mm.	Transmission time	Transmission rate	Number of observations
Intercaval	15.2	.0139	1232	18
Intra-auricular band	12.9	.0126	1252	6
Superior vena cava	8.2	.0136	588	11
Septum (mid and low)	31.5	.0305	1059	11
Right appendix	28.0	.0314	955	11
Right auricle	16.0	.0206	859	7
Right pulmonary vein	24.0	.0254	1121	4
Inferior vena cava	31.5	.0325	998	18
Coronary sinus	43.9	.0412	1096	5
Left pulmonary vein	45.2	.0412	1118	5
Left appendix	44.6	.0446	996	7

Average heart rate 158.4

mission is greatest where the muscle bands are straight. The solitary exception to the statement that the rate of transmission is uniform and approaches 1000 mm. per second is found in the superior cava; here it is lower and we are inclined to attribute this difference to the direction of the muscle-fibres in this vein; they are arranged for the most part obliquely across the vein, while our transmission rates have been estimated up and down it. We are unable to obtain evidence of hindrance to the passage of the wave from node to auricle at any point; the rate of travel appears to be uniform, the direction of travel radial in all directions. Neither can we find any evidence of an increased rate

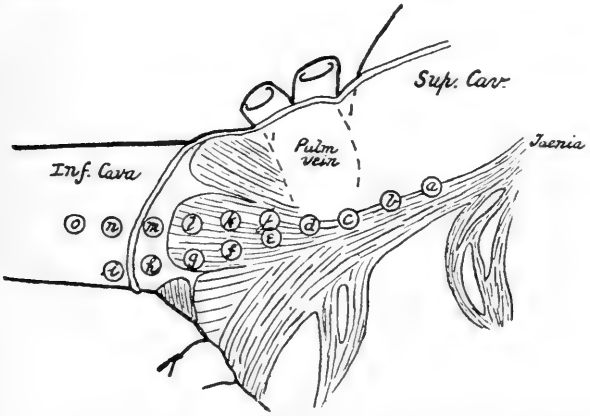


FIG. 13.—A scale drawing from an actual experiment; showing a number of contacts used for leads from sulcus and inferior cava. Examples of the curves are shown in Fig. 14.

of conduction to the *A–V* node; our calculated rates are the same for all parts of the auricular septum as for the rest of the auricular tissue. We have also used special methods of estimating the transmission time from node to node by a method which I do not propose to consider in detail, and find the interval to be long.

The excitation wave in the auricle may be likened to the spread of a fluid poured upon a flat surface—its edge advances as an ever-widening circle, until the whole surface is covered (Fig. 16); such variation as exists in the rate of travel along various lines in the auricle is fully accounted for by the simple anatomical arrangement of the tissue. If we examine the

arrangement of the muscle bands of any mammalian auricle, we shall agree, I think, that they are ordered upon a definite plan. Immediately below the *S–A* node the fibres collect from all parts

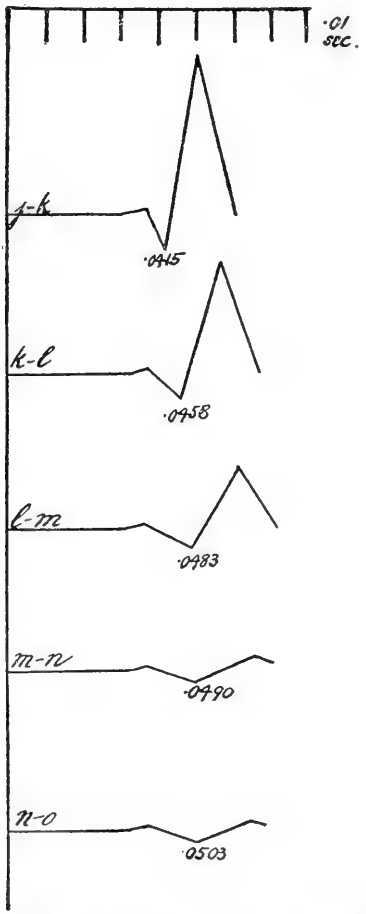


FIG. 14.—A diagram showing outlines of the curves obtained from five of the leads of Fig. 13. Charted in relation to the first appearance of the excitation wave in the auricle (*S.A.N.* line). The intrinsic deflection gradually recedes in time as the lead is taken lower on the vein, until the edge of the muscle is reached; the intrinsic deflection is then lost.

of the superficies of the right auricle in a curious fan-shaped manner, to join in a knot of tissue which we term the *concentration point* (Fig. 12, *C.P.*). The *S–A* node is placed in the most

advantageous position possible for a quick distribution of the contraction wave to all parts of the auricle, the fibres stream into this region of the heart from all the chief outlying regions. The chief fibres of the right appendix run direct to the head of the sulcus; the tænia runs from bottom to top of sulcus; the intra-auricular band runs across from the angle to the left appendix; other fibres run down the intra-auricular septum.

To sum up: the excitation wave, which has its origin in the

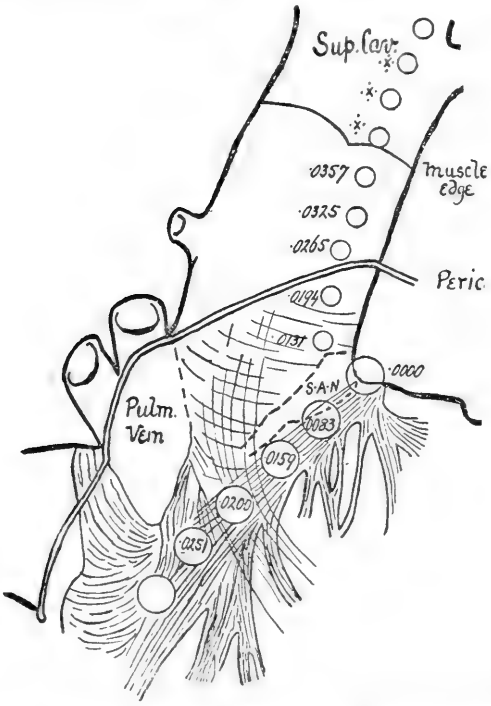


FIG. 15.—Serial leads from sulcus and superior cava in another auricle. The times at which the excitation wave appeared at the several contact points are given. From the highest *S.V.C.* (contacts marked *) no intrinsic deflections were obtained.

S–A node, spreads immediately and at rates ranging around 1000 mm. per second along the chief muscle tracts which radiate from the neighborhood of this node; it courses throughout the whole of the auricular tissue, up to its ending upon the chief veins, and courses down the septum at a similar speed to reach the *A–V* node, whence it is transmitted to the ventricle. Is it

not true to say that one auricle contracts before the other; the excitation wave appears in some portions of the right auricle before some portions of the left, and *vice versa?* The spread may be likened to the spread of fluid poured upon an almost flat surface.

III. THE EXCITATION WAVE IN THE VENTRICLE

We have followed the course of the excitation wave from the sino-auricular node, throughout the auricle and to the auriculoventricular node. I do not propose to deal with the evidence for the transmission of the impulse from auricle to

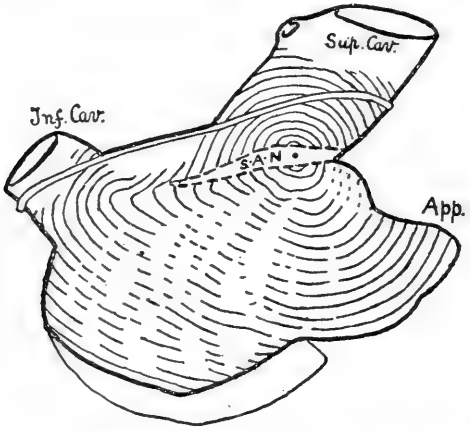


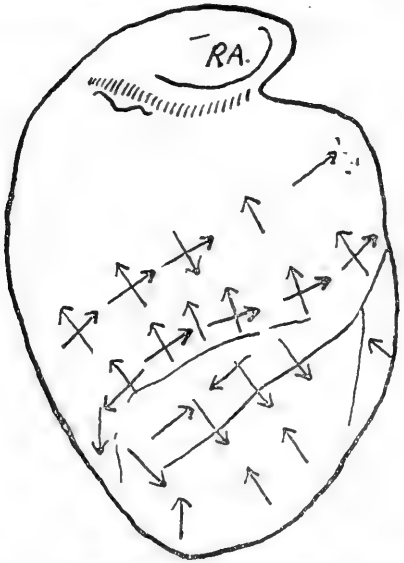
FIG. 16.—A diagram illustrating the spread of the excitation wave over the surface of the right auricle. The spread is almost uniform and follows the chief muscle bands.

ventricle. We know as a result of recent investigations that it passes through the auriculoventricular bundle; and there is strong evidence that it is distributed in the ventricle through that intricate and almost universal subendocardial network, the Purkinje system.

We pass to a study of the excitation process in the ventricle itself. In discussing this subject I propose to take a somewhat unusual course. At a somewhat later date it is proposed to publish a full paper on this subject; the work of Dr. Rothschild and myself is still in progress but is sufficiently advanced to bring before you in the form of a preliminary communication.

Our observations have been conducted along lines similar

to those for the investigation of the auricle. We estimate the time of the appearance of the excitation wave, relative to *R* in a standard electrocardiogram, in the various areas of the musculature. The problems are more difficult than those connected with the auricle. In the last-named chamber, as we have seen, the wave spreads in uniform and diverging lines. At an early stage of our observations we found that the spread in the ventricle happens in an entirely different fashion. If we



FIGS. 17 and 18.—Outlines of the front of the heart in an actual experiment. Upon Fig. 17, arrows have been drawn; they depict the direction of spread on the front of this heart, investigated by means of closely paired contacts. Here the direction of the deflection was the index.

examine a series of points upon a given superficial band of ventricular muscle, for example, the conspicuous fibres which sweep from the conus across the upper part of the interventricular groove and around the left border of the heart to the apex, we immediately ascertain that the cause of the wave does not follow these bands. Fig. 18 serves as an illustration: the excitation wave appears at a series of points along the right border of this diagram at time intervals, .0241, .0231, .0198, .0150, .0146, .0187 and .0196 seconds after *R*. It appears almost simultaneously

at all these points, although they overlie the same muscle band. We have conclusive evidence that the excitatory process takes little or no consideration of the anatomical arrangement of the musculature of the ventricle. If we cover the front of the heart with contacts and estimate the order of the excitation process, we constantly discover an area in the region of the anterior attachment of the wall of the right ventricle (shaded area in Fig. 18) in which the excitatory process first commences so far

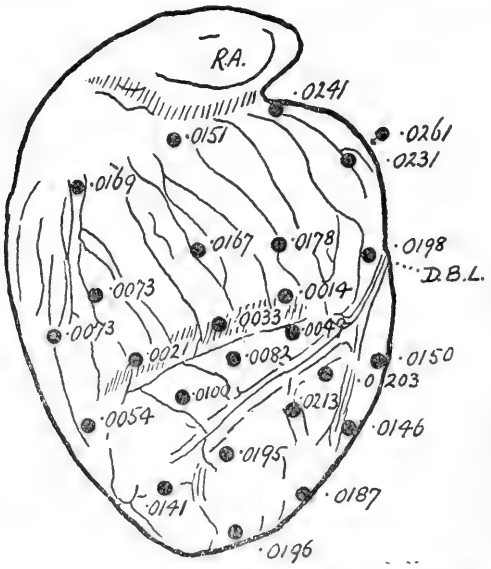


FIG. 18.—The times at which the excitation wave appeared on the front of the same heart, related to the upstroke of *R.* in Lead II. *R.A.*, right appendix; *D.B.L.*, descending branch of left coronary artery.

as the superficies of the heart is concerned. But there is this remarkable fact, that there exists in this region a considerable area, though it is of variable extent, in which the excitatory process commences almost simultaneously. If we examine the underlying structures we shall find that this exposed region is the most directly supplied by the right branch of the *A–V* bundle, and we have little doubt that the distribution of the right branch to this region is responsible, in part at least, for its early and simultaneous excitation. Examining the whole super-

ficial surface of the heart in a number of animals, we find a close resemblance in the distribution from beast to beast. The superficial area which passes earliest into a state of excitation is almost always that which I have already indicated, namely, the portions of the conus where these join the interventricular groove. This is the portion of the wall overlying the large anterior papillary muscle of the right ventricle. The rest of the right ventricle becomes active later; the latest region is the upper wall of the conus directly below the pulmonary valves; the base of the right ventricle at its fusion with the fat in the *A–V* groove, and that portion which lies along the posterior interventricular groove, are almost but not quite so late.

Yet although there is this almost constant order, the time differences between the onsets of activity in the several parts of the right ventricle are remarkably small. In the case of the auricle, the wave takes from start to finish 4 to 5 hundredths of a second to complete its course. In the ventricles, although these chambers are so much longer, the whole course is usually completed in dogs of the same size in less than 3 hundredths of a second. The order in the left ventricle is equally definite, though at present I shall not enter into detail. The earliest point is the vortex of the left ventricle or the extreme apex, and this region sometimes successfully rivals the right ventricle in the race; a hundredth of a second later the neighboring points are activated. The appearance of activity over the remainder of this chamber is practically simultaneous, the time differences are usually to be measured in a few thousandths of a second. The basal attachment is, generally speaking, latest of all and practically coincident with the activity in the conus region.

No system of spread from point to point of the muscle-fibres in a definite order can be imagined which will explain this distribution. We are forced to assume that the ventricular wall is reached by an impulse travelling along a large number of paths of distribution. These paths, as we are able to show, are the Purkinje paths. If we examine a series of points, such as those shown in Fig. 19 before and after section of the right branch of the *A–V* bundle, we find clear evidence for this statement. After

section of this branch, as you are aware, a conspicuous change occurs in the form of the electrocardiogram; this change interferes to some extent with our absolute standard of measurement, but we are able to ascertain the relative order before and after the interference with precision. It is found that prior to section the right ventricle becomes active before the left in such a series of contacts as is figured, but after section the order changes. The relation of points to each other over the left ventricle remains unaltered, while activity in the right ventricle is materially delayed and progresses from left towards right. The

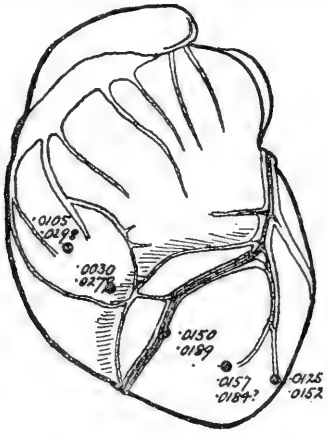


FIG. 19.—Outline of the front of a dog's heart upon which five contact points were investigated, before (top figures) and after (bottom figures) section of the right branch of the *A-V* bundle. It will be noted that over the left ventricle the order remains unchanged; but over the right, whereas before section the excitation wave appeared earliest in this region, after section it appeared latest.

Purkinje system is thus proved to be concerned in the distribution.

But allowing this to be the case, we have still to explain a great deal. Even where we take into account this branching system, we cannot fully explain the time relations over certain regions unless we assume that ventricular conduction is much more rapid than conduction in the auricle. To take an example: there are no free branching strands to the region of the conus; the subendocardial network in this region is spread as a continuous sheet; yet conduction to the muscle beneath the pul-

monary valves is extremely rapid. To meet these difficulties of explanation we have devised special experiments. It has been necessary to measure the rate of conduction through various tissue areas when an artificial excitation wave is propagated across contacts in line with each other. The natural rate of conduction in the auricle is fairly uniform and at about 1000 mm. for a second. The rate of conduction in the ventricle varies with the region examined. *It is highest and approaches or surpasses 2000 mm. per second where the muscle is thinnest.* It is lowest and approaches 400 mm. per second where the muscle is thickest. The reason for this variation is clear to us. The rate of conduction through ventricular muscle is slow, the rate of conduction through Purkinje substance is at least five times as fast. When we excite the pericardial surface, the difference in the

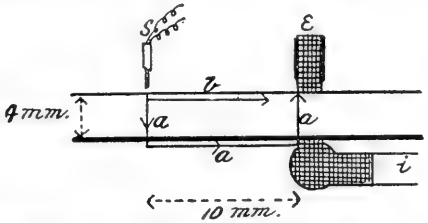


FIG. 20.—Two contacts (*e*, external, *i*, internal) are placed on the epicardium and endocardium respectively, and the heart wall is stimulated at *S*, 10 mm. away. The excitation appears at the internal contact first and at a natural interval, before it appears at the external contact; it travels therefore by way of the Purkinje substance.

times at which the excitation wave reaches the contacts in line with the stimulation point depends upon whether the excitation wave has time to travel through and into the Purkinje substance, and along it and out again through the muscle to our contacts, before it passes directly to our contacts through the muscle alone. Evidently the thicker the tested muscle, the less likelihood is there of quick penetration. That this explanation is valid is clearly shown by two further experiments.

If two contacts are placed opposite to each other, one on the epicardial the other upon the endocardial surface, the natural excitation wave always reaches the internal contact first (Fig. 20). It also reaches the internal contact first, *and by precisely the natural time interval, when an excitation wave is provoked*

from the outside, provided that the point of stimulation is sufficiently far removed. Thus in Fig. 20 *e* represents an external and *i* an internal contact, and the epicardium is stimulated 10 mm. away. The excitation wave appears at the internal contact first and at the external contact after the natural interval. It has travelled, therefore, along the path *a a a*, through 8 mm. of muscle and 10 mm. of the Purkinje system before it has travelled through 10 mm. of muscle (path *b*). Thus it prefers to pass through 10 mm. of Purkinje substance rather than through 2 mm. or less of muscle.

The second experiment is similar in kind. Two contacts are

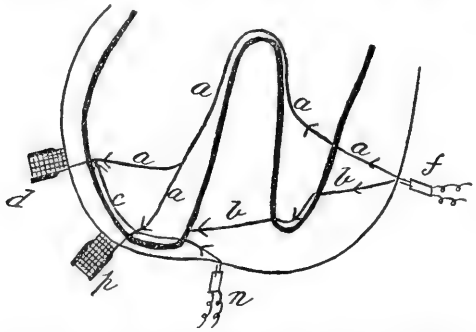


FIG. 21.—A diagram of the ventricles, seen in vertical section. Two contacts, *p* and *d*, are placed on the right ventricle. The ventricle is stimulated at *n* and the excitation wave passes along the path *c*. The ventricle is stimulated at *f* and the excitation wave now appears at *p* and *d* at such times as to suggest that it travels through Purkinje tissue and bundle branches *a*, *a*, in preference to the shorter branch *b*, *b*.

placed on the right ventricle (Fig. 21) and the heart is stimulated at a point in the neighborhood of the septum (*n*) some 10 or 15 mm. away. The interval between the arrival of the excitation process at *p* and *d* is relatively long. (The path taken is along *c*.) But if the point of stimulation is moved 30 to 40 mm. away (*f*) and the experiment is repeated, the interval is materially reduced; in a number of such experiments it is reduced *until it reaches the interval displayed by the natural heart-beat.* In such instances the quicker though far longer path is over the septum through the main divisions of the bundle (*aa* as opposed to *bb*).

Let us sum up our findings. The spread of the excitation

wave in the ventricle is controlled by the Purkinje system; it is hastened by the early branching of this system, especially in the left ventricle. The Purkinje system has a high rate of conduction as compared to ventricular muscle, and this quality also favors quick distribution.

Evidently, before we may end our search, we have to investigate the endocardial surface of the heart; this presents great difficulties, but is in the course of completion. The excitation wave appears early inside the ventricle, and the time intervals appear to be very small between different endocardial regions. We believe that the earliest region of all is the upper part of the septum on the left side, and that other regions become active according to their distance from the main distributing tracts; but this has not been convincingly proved. Why then does the front of the right ventricle become active so long before superficial regions of the ventricle overlying the left papillary

FIG. 22.—A diagram illustrating the spread of the excitation wave in the auricle from a central node. The spread is along the muscle bands.

muscles? For a simple reason, namely, because the muscle is thinner. We have data of a very suggestive kind which appear to show that, as Purkinje conduction is extremely rapid, the excitation process starts almost simultaneously over the whole interior of both ventricles, and that the appearance of activity upon the superficies, while partially controlled by the distance from the main Purkinje strand, is chiefly controlled by the thickness of the muscle overlying the Purkinje substance. It is chiefly to this cause that we attribute the early appearance of the excitation wave over the front of the right ventricle, near its attachment, and at the vortex of the left ventricle; for these are the thinnest points of the ventricular walls. According to our view the excitation spreads in the ventricle along the Purkinje system, and appears on the surface by directly piercing the whole thickness of the wall (see Fig. 23); this piercing of the wall is aided by the penetration of the wall by isolated strands of the end arborization of the Purkinje system.

The observations which I have briefly surveyed will, I trust, take us far toward a final explanation of the normal electrocardiogram, for we are now in a position to state the regions which are excited when given deflections of the normal curve are inscribed; but this subject I shall defer. Our view of the distribution in the ventricle will also help us, so we believe, to understand the curious alterations of electrocardiograms met with in the hypertrophies; for a thickened left ventricle should delay the activity of the special musculature by delaying penetration and deepen and prolong *S* in axial leads. However, this view is at present in the stage of tentative hypothesis.

Finally, a word on conduction rates in various regions of the

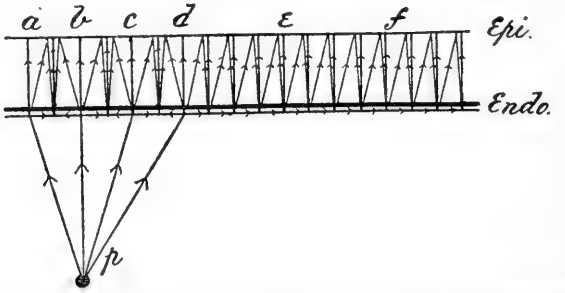


FIG. 23.—The spread in the ventricle as it is conceived. The spread is from *p*, through branches of the Purkinje system, subsequently the spread is along the endocardial network and from this at right angles through the ventricular wall.

heart. We find the conduction rate to increase as we pass from: (1) ventricular muscle to (2) auricular muscle to (3) Purkinje substance.

It is to be remarked that the glycogen content of these tissues increases in the same order; but more interesting at present is the physiological significance of these variations in activity.

Distribution of the excitation wave in the auricle is expedited by the central position of the sino-auricular node and by a relatively high conduction rate, a relatively simple plan. In the muscle of the ventricle conduction is slowest because its function of distribution is a minor one; on the other hand, this, the driving chamber, is provided with a special system of distribution, clearly ordered to provoke almost simultaneous contraction; this special system is endowed with conduction powers of the highest order.

CERTAIN ASPECTS OF BIOLOGICAL OXIDATION*

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THE subject of oxidation presents one of the most fascinating themes in the entire domain of chemistry. The various views which have been held regarding the nature of combustion and oxidation have always exercised a profound influence on chemical thought and on biological science and medicine. Modern views regarding the nature of oxidation date from the work of Lavoisier, who observed that in the process of oxidation oxygen adds itself to the substance oxidized, and that the resulting one or more products weigh more than the original material by exactly the weight of oxygen required to effect the oxidation.

It is not within the scope of this lecture to recount the views which have been held regarding the mechanism of oxidation in general. It would also be quite futile to review for you the whole subject of vital oxidation in the time at our disposal. I shall therefore confine my remarks to a small part of the field in which I have been particularly interested for several years.

The subject of vital oxidation may be divided into the following phases:

1. The mechanism by which oxidative processes are brought about in living material.
2. The nature of the substances oxidized by living organisms.
3. The relation between oxidation within the body and the energy requirements of the organism.
4. The relation of oxidative processes to other chemical processes in the body.

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* Delivered November 8, 1914.

5. The effect of various substances and of various conditions on vital oxidation.

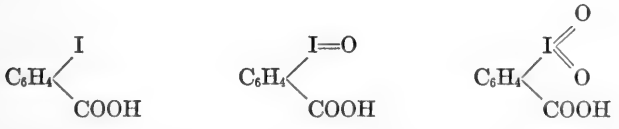
6. The relation of vital oxidation to functional activity.

Notwithstanding the great interest connected with the first four phases of oxidation here enumerated, I shall have very little to say regarding them except as they bear directly upon the last two phases.

It was recognized by Lavoisier that oxidation is constantly occurring in the body, and it was surmised that the body derives its heat from oxidation. Ever since the establishment of the principle of the conservation of energy the body has been regarded as an engine which converts the latent chemical energy of food-stuffs by oxidation into heat, mechanical work, and all the manifestations of life. Oxidation within the body was looked upon for many years as quite similar to combustion outside the body. It is perfectly evident, however, that great differences exist between oxidation within and without the organism. Thus proteins, carbohydrates and fats are oxidized within the body at a temperature of 37° C., whereas it requires a far higher temperature to oxidize these substances in the air. In the attempts to explain this fact the group of oxidizing enzymes or oxidases was discovered. Much work has been done to indicate the great differences between oxidation within and without the body. It has been shown that many substances which are readily oxidized outside the body are either very incompletely oxidized or not oxidized at all within the organism. Under this heading may be classed carbon monoxide, oxalic acid, and many others. The selective oxidation of many organic substances in the body is another very remarkable characteristic of vital oxidation. Thus very slight changes in the structure of a substance may completely prevent its oxidation in the body. A great deal of work has been done on the relation of chemical constitution to the property of undergoing oxidation in the organism. I shall take occasion later to refer to another remarkable difference between oxidation within and without the body.

In 1901 Kastle and I [1] showed that certain of the organic peroxides give practically all of the reactions of certain oxidases

widely distributed in living matter. We found, for instance, that benzoyl peroxide gives the well-known guaiacum reaction, and that the bluing of guaiacum by benzoyl peroxide can be inhibited by hydrocyanic acid in the same manner that this substance inhibits the bluing of guaiacum by the oxidases. On the basis of this work and on the presumption that the oxidases play a rôle in pathological as well as physiological processes, I was led to study the pharmacological action of benzoyl peroxide and also any therapeutic uses to which it might be put.[2] The substance proved to be markedly antiseptic and extremely useful in the treatment of local infection; in fact, its effect in local infection was so remarkable as to suggest that its beneficial effect could not be attributed entirely to its antiseptic action. This naturally suggested that the active oxygen contained in benzoyl peroxide had some peculiar effect on the tissues, increasing their resistance to infection. The nature of this increased resistance was not determined at that time. I shall refer to this again later. Benzoyl peroxide is but slightly soluble in water, so that its effect on general infections could not be studied. In casting about for a substance soluble in water which might contain oxygen in a physiologically available form, and which could be injected intravenously, we decided to make a careful pharmacological study of the sodium salts of iodbenzoic, iodosobenzoic, and iodoxybenzoic acids. These acids which were first prepared by Victor Meyer and his co-workers[3] have the following composition:



Iodbenzoic acid contains no active oxygen. Iodosobenzoic acid contains 6.06 per cent. of active oxygen. Iodoxybenzoic acid contains 11.43 per cent. of active oxygen. By active oxygen I mean oxygen which in the chemical sense is very active outside the body and which in the compounds under consideration is the

oxygen bound to the iodine. Grove and I [4] sought to determine whether the oxygen contained in these substances is physiologically available, that is to say, whether the oxygen bound to iodine in these substances can be utilized by the organism for purposes of physiological oxidation. We found that the sodium salts of iodosobenzoic and iodoxybenzoic acids instantly oxidize hæmoglobin to oxyhæmoglobin. We found that sodium iodosobenzoate can furnish the oxygen for at least one peroxidase reaction. It was found that while neither blood nor sodium iodosobenzoate is capable of oxidizing phenolphthalin to phenolphthalein, together they are capable of effecting this oxidation. Dilute solutions of sodium iodosobenzoate and sodium iodoxybenzoate taste very much like solutions of hydrogen peroxide. All of these facts indicate that the oxygen which they contain is physiologically available.

Arkin showed that sodium iodosobenzoate and sodium iodoxybenzoate are from one hundred to two hundred times as highly bactericidal as sodium iodbenzoate on *Bacillus typhosus*.[5] This difference can only be attributed to the oxidizing action of the former substances. On intravenous injection it was found that sodium iodosobenzoate and iodoxybenzoate markedly depress the respiratory and vasomotor centres, whereas the iodbenzoate has no such effect. Therefore the effects of the two former substances on these centres must be attributed to the physiologically active oxygen which they contain. It would thus appear that a substance which apparently increases oxidation within the respiratory and vasomotor centres depresses them. It has been known for a long time that hydrocyanic acid is one of the most powerful stimulants of the respiratory centre. It is also well known that hydrocyanic acid depresses vital oxidation. Therefore it seemed interesting to determine whether an antagonism exists between the action of hydrocyanic acid and iodosobenzoate. In order to determine this, cannulas were placed in both femoral veins of a cat. In the left femoral vein a solution of sodium iodosobenzoate was injected and its effect determined. After a short time sodium cyanide was injected into the right femoral vein, in order to determine the effect of a given dose

in the animal under experimentation. Then both substances were injected simultaneously into the opposite veins. The complete antagonism of the two substances in their action on the respiratory centre was readily seen.[6] This further indicated that iodosobenzoate increases oxidation in the centre, since it is capable of antagonizing a substance known to inhibit vital oxidation.

Eyster and I [7] showed that sodium iodosobenzoate, when perfused through the isolated mammalian heart has a marked effect on the size of the beat even in very dilute solutions, whereas iodbenzoate has very much less action. We found that most of the active oxygen of the iodosobenzoate is removed from the solution on perfusing through the heart, and that the more vigorously the heart is beating the greater is the amount of oxygen taken up by the heart from this compound. Although iodoxybenzoate is apparently as active on the respiratory centre as iodosobenzoate, it is much less active in altering the activity of the isolated heart. The heart also takes up far less oxygen from a solution of iodoxybenzoate than from iodosobenzoate, which again clearly indicates that it is the active oxygen in these compounds which accounts for their pharmacological action. It is also very interesting as showing how futile it is to predict from knowledge previously gained with one tissue what effect a given oxidizing substance will have on another tissue. In fact, the longer one works at the problems of vital oxidation, the less willing he becomes to make predictions. The effect of any new factor or condition cannot be foretold. It must be ascertained by experiment.

The action of iodoso- and iodoxybenzoate on the circulation and respiration seemed so interesting that we determined to step aside from the original object of the investigation, namely, the quest of a substance for the treatment of general infection, long enough to determine the relation between physiological activity and changes in the rate of oxidative processes. Strange to say, this question had never been carefully attacked as such, and the knowledge on the subject which was more or less desultory did not elucidate the problem satisfactorily. The reaction of the

animal to asphyxia has been investigated for the last sixty years. Asphyxia is, however, a complicated condition. There exists both a lack of oxygen and an excess of carbon dioxide, and the relative parts played by these two factors in producing the symptoms of asphyxia have been the source of endless controversy. Furthermore, products of incomplete oxidation having an acid character arise during asphyxia, and to these the symptoms of asphyxia have also been ascribed in part. The striking symptoms of asphyxia however produced are the following: increase in the rate and depth of the respiration, rise of blood-pressure, slowing of the pulse, cessation of respiration, general convulsions followed by paralysis, marked and progressive fall of blood-pressure, death.

I cannot review the previous work on the subject of asphyxia, and must confine myself to recounting certain experiments which seem to us to demonstrate conclusively that the symptoms of asphyxia can be brought about by decreased oxidation. Gasser and I [8] produced decreased oxidation by methods which did not permit of any accumulation of carbon dioxide or of acid products in two ways: (1) by injecting sodium cyanide intravenously; (2) by injecting pure carbon monoxide into the trachea without interfering with the respiration in any way.

Artificial respiration was given when the natural respiration was depressed, thus preventing the accumulation of carbon dioxide. In the rabbit the injection of sodium cyanide into the jugular vein stimulates the respiratory centre, on the average, within three to four seconds. Stewart found that it requires 2.8 seconds for the blood to pass from the left jugular vein to the right carotid in the rabbit. Thus the latent period of stimulation of the respiratory centre by sodium cyanide is practically the time required for the substance to reach the centre. This extremely rapid action precludes all possibility of explaining the stimulation as due to excess of carbon dioxide or the accumulation of acid products. It proves beyond peradventure that the cells respond with stimulation to a decrease in their own oxidative processes directly. Similarly the vasomotor and

cardio-inhibitory centres are stimulated, but the latent period is longer than in the case of the respiratory centre. The order of stimulation is, first, respiratory centre, second, the vasomotor centre, and last, the cardio-inhibitory centre. With carbon monoxide quite similar results were obtained, but a little longer time was required. Here the stimulation of the respiratory centre occurs within six and one-half seconds. The extra time required to produce stimulation by carbon monoxide can readily be accounted for by the time required for the gas to be drawn into the lungs and pass through the endothelium into the blood.

The difference between the action of carbon monoxide and hydrocyanic acid in these reactions is this: The carbon monoxide by combining with hæmoglobin and forming carbon monoxide hæmoglobin reduces the amount of oxygen which the cells receive, whereas hydrocyanic acid does not interfere with the supply of oxygen but interferes with the consumption of oxygen by the tissues through its inhibiting action on the oxidases. This fact illustrates that *decreased oxidation may occur when the supply of oxygen is not diminished and that the term decreased oxidation is not synonymous with oxygen want. Oxygen want is but one means of producing decreased oxidation.* Every phase of the pharmacologic action of hydrocyanic acid and carbon monoxide can be explained on this basis.

These results clearly prove that decreased oxidation leads first to increased functional activity or stimulation and later to depression. The effect of reducing oxidation in these centres depends on three factors: first, on the extent to which the oxidative processes are reduced, second, the suddenness with which they are reduced, third, the condition of the centres.[9] If oxidation is reduced below a certain level, stimulation will not be observed. The more suddenly the rate of oxidation is reduced, the greater will be the stimulation. If the reduction of the rate of oxidation is very slow, no stage of stimulation will be noted, as the irritability of the cells will then decrease faster than the stimulus increases. Finally, the better the condition of the centre the more readily is the stage of stimulation demonstrable,

and, conversely, the poorer the condition of the centre the more difficult it is to elicit a stage of stimulation.

To summarize, then, we have found that a depression in the rate of oxidative processes in the medullary centres leads primarily to stimulation, whereas our work on iodosobenzoate and iodoxybenzoate showed that an increase in the rate of oxidative processes causes decreased activity or depression. In other words, the functional activity varies inversely with changes in the rate of oxidative processes.

We [10] have attempted to form some conception which would account for this inverse relationship between functional activity and changes in the rate of oxygen utilization by the cell. Verzár,[11] working on the gaseous metabolism of muscle, and Barcroft and Piper [12] on the gaseous metabolism of the submaxillary gland, have shown that the period of increased utilization of oxygen outlasts by several minutes the period of increased functional activity following stimulation. Barcroft and Piper found that following certain forms of stimulation maximum utilization of oxygen occurs when the saliva almost ceased to flow and conclude, "Probably, therefore, gland, like muscle, is a mechanism in which oxidation serves to replenish a store of potential energy which is liberated in the act of secretion." It would seem that we have two sets of processes going on within cells, one of which requires the acquisition of oxygen from without the cell. Since the cell continues to fix oxygen at a greatly increased rate for a time after functional activity has ceased, and since heat continues to be liberated after the contraction of a muscle as shown by A. V. Hill,[13] it would seem that the fixation of oxygen by the cell must be the beginning of a series of oxidations. Let us designate this phase of oxidation within the cell as the " R " processes, since " R " will suggest rest and recovery. The essential feature of the " R " processes is that they require oxygen from without the cell. From our viewpoint functional activity is the external manifestation of a set of chemical reactions occurring within the cell. Since energy is liberated during functional activity, we must assume that these processes are also oxidative at least in part.

Let us designate the processes of which function is the external expression as "A" processes, since "A" will suggest activity. We conceive that neither the "A" nor the "R" processes are ever in complete abeyance during life, but that their relative intensity determines the relative state of activity of the cell.

The work with iodosobenzoate and iodoxybenzoate indicates that a stimulation of the "R" processes (oxygen fixation) retards the "A" processes and depression results. On the other hand, if the "R" processes are depressed, as by the use of carbon monoxide or sodium cyanide, it would seem that the cell must derive its energy from the "A" processes and increased functional activity must result. This is the only conception we have been able to form to account for the reciprocal relationship of oxygen fixation and functional activity. We know that an increase in the "A" processes entails an increase in the "R" processes, but since the latter outlast the former it would appear that the "R" processes lag behind. Since recuperation apparently does not occur under anæsthesia, it would follow that both the "A" and the "R" processes are depressed in this state. Our work indicates also that when the "R" processes fall below a certain level, all functional activity ceases. According to our point of view, the stimulating action of carbon dioxide is due to its power to depress the "R" processes just as hydrocyanic acid does. We believe that under physiological conditions the oxidative processes, and therefore the functional activity of the respiratory centre, are conditioned by the carbon dioxide tension and not by the oxygen tension. This follows from the work of Haldane and Priestley.[14]

We have recently turned our attention to another phase of the proposition that decreased oxidation primarily stimulates cells. The previous work showed that the cells of the respiratory centre are apparently more sensitive to alteration in the rate of their oxidative processes than any cells in the body, and respond most readily with stimulation to a decrease in their oxidative processes. This is what we should expect since the respiratory centre, by controlling the entrance of oxygen into

the body and the exit of carbon dioxide, really controls two of the fundamental conditions for tissue oxidation. From the lungs to the tissues the oxygen must be carried by the hæmoglobin. Since the red blood-corpuscles and hæmoglobin are produced by the red bone-marrow it is obvious that the red bone-marrow supplements the action of the respiratory centre in supplying the tissues with oxygen. Paul Bert [15] thirty-six years ago showed that the oxygen-carrying power of the blood in the case of animals living at high altitudes is much greater than in the case of animals at lower altitudes. He explained this as due to a reaction of the organism to accommodate itself to the decreased pressure of oxygen in the atmosphere. The facts brought out by Bert as well as his explanation of the blood changes have been the subject of much controversy. The majority of investigators, however, have found that there is an increase in erythrocytes and hæmoglobin at high altitude. I cannot review the views which have been held regarding these phenomena but will merely enumerate some of them: (1) the increase is insignificant; (2) the erythrocytes and hæmoglobin always increase proportionately, and the increase is due to concentration of the blood either by loss of water from the body through evaporation or by the passage of plasma from the blood to the tissues. Again, the blood changes have been attributed to various physical factors such as (1) a displacement of the diaphragm upward and an alteration in the pulmonary circulation, (2) lessened vital capacity of the lungs at reduced pressure, etc.

There are obviously many factors which may play a rôle in the effect of reduced atmospheric pressure on the blood. From our point of view, the increase in the hæmoglobin and the red blood-corpuscles is probably due to a stimulation of the bone-marrow by decreased oxidation within the bone-marrow itself. I should like to describe to you briefly the apparatus which Mr. A. C. Kolls and I have devised in order to attack this problem.* This apparatus was devised for the purpose of keeping animals in an atmosphere of reduced oxygen tension and at the

* A description of this apparatus will soon be published.

same time to maintain them at the normal atmospheric pressure and thereby exclude all of the physical factors of high altitude. It consists of a respiratory chamber, the air of which is kept at a constant composition. The arrangement for absorbing carbon dioxide and water which the animal produces is that devised by Professor Benedict,[16] and the oxygen supply is automatically controlled by a mechanism quite similar to that used in Professor Lusk's laboratory and described by Williams.[17] We have introduced certain modifications which greatly facilitate the work that we have in hand. The results which Mr. H. C. Dallwig, Mr. A. C. Kolls and I have obtained with this apparatus may be indicated by citing two or three experiments out of a large number.**

REDUCED OXYGEN EXPERIMENT (RABBITS NOS. 4 AND 5)

Length of experiment, 132 hours.
Average oxygen tension, 11.98 per cent.
Average carbon dioxide, 0.08 per cent.
Average per cent. humidity { Inside, 35 per cent. / Outside, 33 per cent.

	Hæmoglobin (g. per 100 c.c. blood)	Erythrocytes
No. 4:		
Before experiment..	13.98	6,678,000
After 132 hours....	16.97 (+21.4 per cent.)	8,417,000 (+26 per cent.)
No. 5:		
Before experiment..	14.39	8,384,000
After 132 hours....	17.43 (+21 per cent.)	8,490,000 (+1.3 per cent.)

CONTROL EXPERIMENT (RABBITS NOS. 9, 10 AND 11)

Length of experiment, 167.5 hours.
Average oxygen tension, 20.9 per cent.
Average carbon dioxide tension, 0.21 per cent.
Weight:

No. 9 (Albino) before control....	852 g.	After.... 970 g. (+118 g.)
No. 10 (Albino) before control....	861 g.	After.... 935 g. (+ 74 g.)
No. 11 (Albino) before control....	790 g.	After.... 898 g. (+108 g.)

** A complete account of these experiments will soon be published.

	Hæmoglobin (g. per 100 c.c. blood)	Erythrocytes
No. 9:		
Before control....	10.9	5,293,000
After control	11.58 (+6.2 per cent.)	5,772,000 (+9 per cent.)
No. 10:		
Before control ...	13.1	6,389,000
After control	13.74 (+4 9 per cent.)	6,512,000 (+1.9 per cent.)
No. 11:		
Before control ...	12.66	6,152,000
After control	11.54 (−8.8 per cent.)	5,827,000 (−5.3 per cent.)

REDUCED OXYGEN EXPERIMENT (RABBITS NOS. 9, 10 AND 11)

Length of experiment, 147 hours.
Average oxygen tension, 10.98 per cent.
Average carbon dioxide tension, 0.19 per cent.
Weight:

No. 9.	Before experiment.....	970 g.	After.....	1060 g. (+ 90 g.)
No. 10.	Before experiment.....	935 g.	After.....	1070 g. (+135 g.)
No. 11.	Before experiment.....	898 g.	After.....	973 g. (+ 75 g.)

	Hæmoglobin (g. per 100 c.c. blood)	Erythrocytes
No. 9:		
Before exp.	11.58	5,772,000
After exp.	15.68 (+35.4 per cent.)	7,544,000 (+30.7 per cent.)
2 days after exp..	16.70 (+44.2 per cent.)	7,707,000 (+33.5 per cent.)
10 days after exp..	15.26 (+31.8 per cent.)	7,442,000 (+28.9 per cent.)
23 days after exp..	11.8 (+ 1.9 per cent.)	6,000,000 (+ 3.9 per cent.)
No. 10:		
Before exp.	13.74	6,512,000
After exp.	16.38 (+19.2 per cent.)	7,301,000 (+12.1 per cent.)
2 days after exp..	15.82 (+15.1 per cent.)	7,186,000 (+10.3 per cent.)
10 days after exp..	16.76 (+22 per cent.)	7,635,000 (+17.2 per cent.)
23 days after exp..	15.77 (+14.8 per cent.)	7,533,000 (+15.7 per cent.)
No. 11:		
Before exp.	11.54	5,827,000
After exp.	17.02 (+47.5 per cent.)	7,266,000 (+24.7 per cent.)
2 days after exp..	14.70 (+27.4 per cent.)	7,200,000 (+23.6 per cent.)
10 days after exp..	14.83 (+28.5 per cent.)	6,918,000 (+18.7 per cent.)
23 days after exp..	14.47 (+25.4 per cent.)	6,872,000 (+17.9 per cent.)

REDUCED OXYGEN EXPERIMENT (RABBITS NOS. 15 AND 16)

Length of experiment, 260.5 hours.
Average oxygen tension, 10.6 per cent.
Average carbon dioxide tension, 0.2 per cent.
Weight:
No. 15 (gray and white), before exp. 1050 g. After. 1130 g. (+ 80 g.)
No. 16 (Albino), before exp..... 1010 g. After. 1150 g. (+140 g.)

	Hæmoglobin (g. per 100 c.c. blood)	Erythrocytes
No. 15:		
Before exp.	10.6	5,376,000
3 days run	13.32 (+25.7 per cent.)	5,920,000 (+10.1 per cent.)
6 days run	16.76 (+58.1 per cent.)	6,620,000 (+23.1 per cent.)
11 days run	15.92 (+50.2 per cent.)	5,715,000 (+ 6.3 per cent.)
No. 16:		
Before exp.	11.16	5,952,000
3 days run	12.28 (+10 per cent.)	6,075,000 (+ 2.1 per cent.)
6 days run	14.96 (+34 per cent.)	7,018,000 (+17.9 per cent.)
11 days run	15.26 (+36.7 per cent.)	6,850,000 (+15.1 per cent.)

It is noteworthy that in rabbit No. 5 the large increase in hæmoglobin was not accompanied by a decided increase in the red blood-corpuscles, showing clearly that the result could not be due to increased concentration of the blood, in which case the red blood-corpuscles and hæmoglobin would have increased proportionately. Blood smears stained with Jenner stain showed a large number of basophilic macrocytes. The blood smears also left no doubt that we were here dealing with a stimulation of the bone-marrow. In some of our experiments we have allowed animals to remain in the box at the normal oxygen concentration and, under these conditions, no increase in the red blood-corpuscles and hæmoglobin was noted. (See control experiments with rabbits Nos. 9, 10 and 11.) In some cases we obtained marked increase in the red blood-corpuscles with relatively slight increase in the hæmoglobin. In other cases we obtained, as in rabbit No. 5, a marked increase in the hæmoglobin with very slight or no changes in the number of red blood-corpuscles. Some animals are very resistant to decreased oxidation and show but small increases in the red blood-corpuscles and hæmoglobin. An increase in the carbon dioxide in the atmosphere of the cham-

ber with the normal oxygen concentration produces relatively little effect on the blood count in comparison with atmospheres poor in oxygen. We have some indication, however, that increased carbon dioxide here, as in the case of the respiratory centre, may stimulate the bone-marrow. In the case of the bone-marrow, however, the stimulation is slight, whereas it acts powerfully on the respiratory centre.

It is interesting then that the respiratory centre and the bone-marrow conduct themselves alike with regard to alteration in their own oxidative processes, since these two tissues provide the primary conditions for tissue oxidation. The work is interesting further in connection with polycythæmia as observed clinically. It would seem that we might expect to find polycythæmia in any chronic respiratory or circulatory condition which would result in the bone-marrows receiving an insufficient supply of oxygen.

We have made certain observations in the box on the oxygen concentration required for a continuance of life, and compared with this the oxygen concentration required to support the flame of various combustible materials. It was shown by Clowes [18] that various combustible gases and liquids would burn only at definite minimum oxygen concentrations. We have confirmed certain phases of Clowes's work. Alcohol ceases to burn when the oxygen concentration falls to 15 per cent. The Madison illuminating gas ceases to burn in an oxygen concentration of about 13 per cent. At about this same point a flaming pledget of cotton saturated with ether is extinguished. The hydrogen flame is extinguished at about 6.6 per cent. oxygen. Below this point no combustible substances which we have studied will produce a flame. Animals continue to live, however, in an oxygen concentration far below this point. Thus we have found it necessary in order to produce alarming symptoms and death in rabbits to reduce the oxygen to between 3 and 3.5 per cent. This brings out one of the most striking differences between vital oxidation and ordinary combustion. If the atmosphere of the earth should become altered so as to contain only one-half the amount of oxygen, animal life would not be interfered with in any way,

but it would be impossible to run a locomotive or to burn illuminating gas and many of the substances which we consider dangerously inflammable would be as non-inflammable as water.

To return for a moment before closing to the original subject of the effect of physiologically active oxygen on inflammation and on immunity processes, I should like to refer briefly to two pieces of work. Prof. L. Hektoen [19] has investigated the effect of iodosobenzoate and iodoxybenzoate on certain immunity reactions. He injected these substances intravenously in dogs and found that they greatly increase the production of specific hæmolysin following a single injection of 10 per cent. suspension of goat's blood. The results were striking. The increase in hæmolysin in the animals receiving the treatment was from 12 to 60 times as great as in the control animals. Iodbenzoate without active oxygen is without effect. Whether physiologically active oxygen will similarly stimulate the production of other immune bodies remains to be determined by further experiment. It is sufficiently obvious that if we could similarly increase the production of diphtheria antitoxin it would be of great value. Dr. S. Amberg and his co-workers [20] in a series of investigations have shown that iodosobenzoate and iodoxybenzoate injected intravenously or intraperitoneally have the power of markedly inhibiting the local inflammatory reaction as produced by mustard oil or bacterial toxins injected intracutaneously or instilled into the conjunctival sac, whereas iodbenzoate possessing no physiologically active oxygen is entirely without effect. The results are very striking indeed.

Many attempts of an unscientific character have been made to use oxygen in some form, such as ozone, compressed air, etc., in the treatment of disease. I need only refer to the extensive and disastrous use of potassium chlorate internally which was in vogue for many years. It was supposed that since it is a very strong oxidizing agent outside the body that it would facilitate oxidation within the organism. This substance, however, does not lose its oxygen in the body but is excreted unchanged in the urine. Dr. Abraham Jacobi [21] pointed out the great danger attendant upon its use internally. The investigations which I

have reviewed for you indicate that at some future time we may find important therapeutic applications of the results of studies in vital oxidation, but we hope that the next attempt will be founded on a basis of fact and will be subjected to careful scientific scrutiny before it is given to the profession.

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NUTRITION AND GROWTH*

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THE NATURE OF GROWTH

GROWTH receives its impetus, in a general way, from two distinct sources of influence. There is an *internal* factor, representing in good part the hereditary features, among which the inherent growth impulse or capacity to grow is conspicuous. The other influence—the *external* factor—involves the environment of growth, and includes, among other agencies, the food supply. No single factor is entirely independent of the other. Of the growth impulse Rubner has subtly said: "Ernährungsphysiologisch drückt er sich in dem Verhältniss der Ansatzgrösse zum Stoffwechsel aus." Nutrition can only give the growth impulse free play; neither can succeed without the other. The nutrition factor is controllable; the growth impulse is inborn and, in part at least, not subject to regulation at will.

If growth were merely the resultant of the assimilation of food, the problems attending it would be somewhat simplified. The pathology of growth may, however, be manifested in the midst of perfect nutrition and teaches plainly that something more than the food supply is here concerned. Abnormal growth and perverted nutrition are by no means always coincident. I have discussed elsewhere some of the other aspects of growth (Mendel 1914a, also 1914b); the present review will be confined to the food factor.

THE ENERGY FACTOR

No modern discussion of nutrition during growth can overlook the energy aspects of the subject. The energy metabolism of youthful, *i.e.*, growing, individuals is said to be somewhat greater than that of adults, whether it be calculated on the basis of units of body weight or of skin area. To justify such state-

* Delivered November 28, 1914.

ments it is, of course, necessary to have some dependable standard of comparison. Growing individuals are of very variable size. For many years it has been customary, following Rubner's lead, to emphasize the significance of the relationship supposed to exist between the metabolism and the body surface rather than between the metabolism and the body weight (*cf.* also McCrudden and Lusk, 1913). Uncontrollable muscular activity, diet, and psychic disturbances introduce difficulties that make comparisons almost impossible, particularly in the case of infants who have served as subjects in many experiments on the energy metabolism during growth. Not long ago Murlin and Hoobler (1914) announced that metabolism in different children was much more nearly proportional to the weight than to the surface area, and when the weight was first multiplied by the specific gravity the agreement was even better. More recently Benedict and Talbot (1914) have concluded, from an elaborate study of infants, that the basal metabolism cannot in any wise be considered a direct function of the body weight and the body surface, and particularly has no relationship with body surface on the basis of the law of cooling bodies. They are inclined to believe that the "active mass of protoplasmic tissue"—a quantity which cannot yet be measured directly—determines the fundamental metabolism. A precise formulation of the energy requirement of the growing organism, expressed in general terms, must abide further investigation.[1]

Aside from its energy aspects, the food requirement during growth is peculiar in that the intake of certain groups of nutrients, such as the proteins and inorganic salts, must exceed the demand for wear-and-tear, so that some excess will be available for the formation or completion of new cells and the elaboration of the tissues that especially develop in the period of growth. For the most part the materials included in the above requirement are merely those which are demanded in the usual maintenance of the grown adult, though perhaps in smaller proportions. It is not improbable, however, that the food needs of

[1] *Cf.* Murlin and Hoobler: Am. Jour. Dis. Child., 1915, ix, 81; Benedict: Proc. Nat. Acad. Sc., 1915, i, 105.

the growing organism may in part be specific and peculiar, calling not only for a little more lime or iron or protein than the non-growing individual requires, but for special substances in addition.

HISTORICAL ASPECTS OF NUTRITION IN GROWTH

To gain some conception of the changes which progress in the science of physiology has wrought in the theory of nutrition during growth, we may turn back to the classic monograph published in 1881 by Carl Voit. This marks the beginning of what may be called the modern generalizations on this subject. Voit gave expression to the then current belief that the period of early growth is one characterized by a comparatively large food requirement and intensity of metabolism. "It is generally believed," he writes, "that the youthful organism is the seat of a particularly active metabolism" (1881, p. 532). This was stated at a time when the energy features were not emphasized as they were later, and at a period when the consideration of the metabolism in growth centered primarily in the transformation of the nitrogenous compounds. The predominant interest in the metabolism of protein had a certain justification in that growth (which means increase of protoplasm), in some degree involves a deposition of nitrogenous derivatives in the organism.

Voit realized that although a deposition of flesh ("Ansatz") can occur in the fully developed adult, the comparable phenomenon of tissue increment in the growing individual is much more conspicuous. To account for this unlike physiological behavior in the two stages of the organism—the adolescent and the adult—he came to the conclusion that there are differences in the destructive metabolism of these two periods of life. Quoting Voit: "It is demonstrated, therefore, that in an organism still in process of growth the conditions for the disintegration of protein are incomparably less favorable than they are in adults. With this fact is associated the rapid growth of the organs" (1881, p. 536).

How is the alleged lessened protein catabolism in growth to be accounted for? Voit replies: "We must have recourse to the assumption that the growing organs rapidly withdraw circulat-

ing protein and by organization into tissue protein protect it from degradation. The youthful organs behave like a secreting mammary gland or a rapidly developing neoplasm, whereby likewise protein is fixed and spared from destruction. . . . In consequence of the increasing size of the growing organs little by little more protein is used up; but the extent of growth of the cells also gradually declines, so that less and less protein is withdrawn from the circulating media and more and more is destroyed. Accordingly larger amounts of protein are subsequently required to accomplish tissue production'' (1881, pp. 537–538).

These contentions of Voit have been reviewed critically by Rubner (1908) in the light of several decades of subsequent investigation. After pointing to the now recognized importance of making comparisons in the total metabolism on the basis of suitable units—according to Rubner, in terms of the surface area of the body—he rejects the idea of a greatly heightened total metabolism in the period of adolescence.[2] Rubner likewise denies that tissue deposition (''Ansatz'') is essentially unique in the growing organism.[3]

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[2] "Mit dem Begriff Wachstum hatte man unwillkürlich, indem man sich die wichtigen morphologischen Veränderungen der Zelle und die Aktion des Zellkerns vor Augen hielt, immer den Gedanken an einen enorm gesteigerten Stoffwechsel verbunden und der jugenlichen Zelle wies man auch sonst in dieser Richtung eine besondere Stellung zu. Durch meine Untersuchungen ist hier Klarheit geschafft worden. Die jugendliche Zelle hat einen Kraftwechsel, der sich schon aus der 'Kleinheit' jugendlicher Organismen ableiten lässt und selbst wachsend, das sieht man aus den berichteten Beobachtungen, beansprucht sie ein sehr bescheidenes Mass von Nahrung, das über die direkt zum Ansatz verwendeten Stoffe nur unwesentlich hinausgeht." (Rubner, 1908, p. 90.)

[3] Ich habe gesehen dass aber unter ähnlichen Nährstoffverhältnissen wie es beim jungen Tier die Regel ist, auch beim ausgewachsenen länger dauernder Ansatz erzielt wird, aber eines versteht sich von selbst, die Variante des Erfolges der Aufspeicherung von Eiweiss ist verschieden. Dass der Ansatz beim Ausgewachsenen eher zum Stillstand kommt als das Wachstum ist etwas ganz Selbstverständliches. Beim Wachstum wird eben von der Zelle immer wieder Platz für die Eiweissablagerung geschaffen, weil neue Zellen gebildet werden und bei der Rekonstruktion füllen sich

In distinction from his predecessors, Rubner insists that there is neither decreased essential protein catabolism nor proportionately much greater protein consumption requisite in growing individuals. Thus he writes "I therefore believe the conclusion that growing animals as a rule consume a large amount of protein and destroy extremely little—a conclusion based on experiments dating from the developmental period of the science of nutrition—is untenable" (1908, p. 94).

These quotations may suffice to contrast the views of two masters of the physiology of nutrition in successive generations. Rubner has further made it evident by his researches, particularly with O. Heubner, that the diet of the growing infant is in general comparatively low in protein, rather than the reverse which is commonly assumed. How low the protein intake may remain is exemplified by the following data (Rubner and Heubner, 1905):

for growth ..... 7 per cent. of the total energy intake,
for maintenance ..... 5 per cent. of the total energy intake.

With an abundance of calories the child can deposit nitrogen and grow as soon as the intake contains the slightest excess of protein above the small need set by the wear-and-tear of the organism.

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nur solche Zellen, in denen ein Mangel vorhanden ist. Das wachsende Tier vermehrt allmählich sein Gewicht auf das 20 bis 30fache des Neugeborenen, die sich rekonstruierende Zelle kommt selten über die Verdoppelung der Masse hinaus. Damit wird aber kein neuer Gesichtspunkt gewonnen, denn dass nur junge Tiere wachsen und alte nicht, bedarf keiner weiteren Erläuterung. Ueber den Kernpunkt der Frage, ob nämlich die Anziehung für das Eiweiss der Nahrung in der Jugend eine andere ist als später, ist aus dem Umstand der grossen Länge der Dauer des Wachstums gegenüber dem kürzer währenden Ansatz gar nichts zu schliessen. Das Wachstum könnte durch dieselben, auch sonst beim Ansatz wirkenden Kräfte vermittelt werden, und der grosse Zuwachs nur das Produkt der länger dauernden Ansatzmöglichkeit sein. Für entscheidende Experimente auf diesem Gebiete müssten ganz besondere Voraussetzungen gemacht werden, man kann grossen Ansatz nur sehen, wenn die Zellen durch Hunger stark heruntergekommen sind und dann wieder genährt werden. Hiermit müsste man unter genauer Einhaltung der physiologischen Versuchbedingungen dann normale Fütterungsversuche am wachsenden Tiere anstellen." (Rubner, 1908, pp. 91–92.)

It seems sometimes to be forgotten, particularly in connection with animal nutrition, that large addenda of protein in the diet do not guarantee growth. As Rubner has remarked: "Growth is not proportional to the quantity of protein in the diet. Growth is a *function of the cell;* it can be rendered *latent* by an insufficient supply of protein, but protein cannot raise the rapidity of growth above the limits set by nature. As the amount of protein in the diet increases, a smaller percentage is utilized (for growth) and the excess of the intake is merely consumed in place of an equivalent of non-nitrogenous food fuel. The strong attraction between protein and growth decreases in the course of the period of development and is greatest in early life" (1908, p. 110).

From Rubner's standpoint, and with particular reference to the human suckling that is not overfed, the destruction of protein is confined in the first period of growth to the wear-and-tear quota. "This behavior of protein during growth," Rubner writes, "is a biological necessity; the relative importance of the physiological functions involved determines the order in which they are filled. First, losses are replaced; next, growth ensues; third, the usual metabolism of protein for the production of heat occurs" (1908, p. 111).

NEWER FEATURES OF THE PHYSIOLOGY OF GROWTH

In all of the foregoing one will search in vain for any indication of those newer conceptions of metabolism, in the development of which the name of Abderhalden has been so prominent. "We must assume," says Rubner, "that the food material must be in excess of a threshold value before growth can proceed. Whether it is the degree of concentration of protein in the tissue fluids that is the determining factor, or whether the body hoards materials and keeps them in reserve for its purposes cannot be determined at the present day" (1908, p. 113). Rubner did venture the statement, in 1908, that "in growth *all* protein compounds which are essential for cell construction are taken up" (p. 111); and he adds that it is not certain whether the same procedure is essential to tissue repair. Meanwhile has

come a newer conception which no longer pictures the food products entering unchanged, or at most only slightly altered, into the cycle of metabolism. The significance of the nutrient units—the "Bausteine"—has come into the foreground. The tissues are independent of the gross peculiarities of the ingested foods; they select their constructive units for growth and repair and their fuel out of the shattered remains of the alimentary contents. "Der einzelne Baustein verrät nicht, welche Rolle seine Muttersubstanz dereinst im Zellgetriebe gespielt hat. So wird die einzelne Körperzelle in weiten Grenzen unabhängig von äusseren Einflüssen" (Abderhalden, 1912a).

A careful comparison of the various proteins which may serve as food proteins, and of which the biological properties and chemical make-up are to-day known in some detail, at once excludes the probability of a direct relation between them and the body proteins which they must be supposed to replace or augment. The differences are too conspicuous.

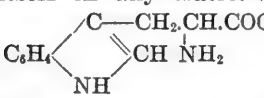
QUANTITATIVE COMPARISON OF AMINO-ACIDS OBTAINED BY HYDROLYSIS FROM PROTEINS[4]

(Compiled by T. B. Osborne, 1914)

	Casein	Ovalbumin	Gliadin	Zein	Edestin	Legumin (Pea)
Glycocoll	0.00	0.00	0.00	0.00	3.80	0.38
Alanine	1.50	2.22	2.00	13.39	3.60	2.08
Valine	7.20	2.50	3.34	1.88	6.20	?
Leucine	9.35	10.71	6.62	19.55	14.50	8.00
Proline	6.70	3.56	13.22	9.04	4.10	3.22
Oxyproline	0.23	?	?	?	?	?
Phenylalanine	3.20	5.07	2.35	6.55	3.09	3.75
Glutaminic acid	15.55	9.10	43.66	26.17	18.74	13.80
Aspartic acid	1.39	2.20	0.58	1.71	4.50	5.30
Serine	0.50	?	0.13	1.02	0.33	0.53
Tyrosine	4.50	1.77	1.61	3.55	2.13	1.55
Cystine	?	?	0.45	?	1.00	?
Histidine	2.50	1.71	1.49	0.82	2.19	2.42
Arginine	3.81	4.91	2.91	1.55	14.17	10.12
Lysine	5.95	3.76	0.15	0.00	1.65	4.29
Tryptophane, about	1.50	present	1.00	0.00	present	present
Ammonia	1.61	1.34	5.22	3.64	2.28	1.99
	65.49	48.85	84.73	88.87	82.28	57.43

[4] These analyses are combinations of what appear to be the best determinations of various chemists.

No one would gainsay that if calcium or iron is indispensable for growth it must be furnished in the dietary. But it has required not a little investigation, and much more still is needed, to make an equally convincing statement with reference to the individual protein structural units, the amino-acids. The reasons for this uncertainty are not hard to find. The inequalities of the proteins from the amino-acid standpoint had not been demonstrated clearly until very recent times; and, what is more important, it has not been possible to say what capacity the animal organism may have to synthesize anew the different amino-acids—eighteen or more in number—which find a place in the construction and functions of the body.

It is plain, then, that we must know what nutrient units of any nature are indispensable and, further, whether a complete lack or deficit of them in the intake can be made good by direct synthesis. Thus it has already been demonstrated that glycocoll  can be manufactured anew in the body. Lack of it in the diet would therefore not necessarily betray itself in any nutritive upset. The amino-acid tryptophane  on the other hand, apparently cannot be produced (at least not in sufficient abundance) *de novo* by mammals, if one may judge by the disastrous results which follow a tryptophane-free dietary and the prompt recovery which the restitution of the amino-acid entails. The situation is further complicated by the probability that new tissue construction, such as growth involves, demands structural units which are either conserved in the wear-and-tear of ordinary maintenance without growth, or are not required for, or destroyed in, the maintenance metabolism.

THE PROTEIN FACTOR

Restricting our considerations for the moment to the protein requirement, we shall not err in identifying it to-day with the specific amino-acid needs for the growing organism. A direct

way to study this consists in administering the nitrogenous components of the diet (in so far as they are ordinarily furnished by proteins), in the form of mixtures of the known amino-acid derivatives. The technical difficulties of such a procedure are almost insuperable at present. Abderhalden (Abderhalden, 1912b; also Abderhalden and Hirsch, 1912) alone has attempted, with any degree of success, to feed young dogs with predigested foods. In some of his experiments considerable gains in weight —in one case 1000 grammes, in another 1200 grammes—were made. Prolonged growth is an admirable index of protein synthesis and of the adequacy of a dietary. But a temporary or transient gain of weight, or one which follows the depletion of the body by previous unsuitable nutritive conditions, cannot be taken as evidence of true growth; for repair may be accomplished without necessarily implying actual synthetic processes in the sense intended. Real growth, consistently continued, manifests itself in characteristic increments of weight and size as exhibited in typical curves of growth.

Another way of approaching the problem of what nitrogenous units are essential for growth consists in comparing the nutritive efficiency of individual proteins and particularly such as are known to differ widely from the typical tissue protein of animals in their structural composition. Obviously this cannot be done by additions to the usual mixed diet which commonly contains a diversity of proteins. Even milk, which is looked upon as a comparatively simple food, furnishes at least two proteins—casein and lactalbumin—decidedly unlike in structure and amino-acid yield, and present in widely different proportions in the mammary secretion of different species. It is necessary, therefore, to devise a ration in which all of the essential food ingredients except proteins or amino-acids are present in abundance, and to which these nitrogenous food substances can be added one by one and tested. In this way the protein factor becomes the sole variable in the diet.

Despite numerous earlier failures, the possibility of maintaining animals on mixtures of isolated food substances and of

inducing growth thereon has at length been demonstrated.[5] Röhmann has been a pioneer in this field (Röhmann, 1902).

T. B. Osborne and I (Osborne and Mendel, 1911b, p. 80) were able to facilitate the study of the rôle of the individual proteins and amino-acids in growth by introducing the use of what we have termed "protein-free milk" in the artificial ration. This consists of the dried residue of milk after removal of fats and proteins. It contains, aside from traces of unremoved proteins, all of the milk sugar and inorganic elements along with small amounts of incidental components most of which remain unknown, yet evidently furnish an essential non-protein factor to the diet. On mixtures of "protein-free milk," sugar, starch, and purified fats along with selected isolated proteins, young white rats—and in some cases mice (Wheeler, 1913)—have grown to maturity and in turn produced young even in the third generation. The catalogue of the individual proteins with which, in suitable concentration, normal growth has been secured, at least for considerable periods of observation if not until completed maturity, includes:

Proteins of Animal Origin	Proteins of Vegetable Origin
Casein (milk)	Edestin (hemp-seed)
Lactalbumin (milk)	Globulin (squash-seed)
Ovalbumin (hen's egg)	Excelsin (Brazil-nut)
Ovovitellin (hen's egg)	Glutelin (maize)
	Globulin (cotton-seed)
	Glutenin (wheat)
	Glycinin (soy bean)
	Cannabin (hemp-seed)

Failure to induce growth has attended our trials with

Legumelin (soy bean)	Hordein (barley)
Vignin (vetch)	Conglutin (blue or yellow lupine)
Gliadin (wheat or rye)	Gelatin (horn)
Legumin (pea)	Zein (maize)
Legumin (vetch)	Phaseolin (white kidney bean)

[5] The earlier literature is reviewed in Osborne and Mendel, 1911b.

In the case of some of these proteins, notably gelatin, zein, gliadin and hordein, an explanation for the failure of growth was at once suggested by the known deficiencies of each of these

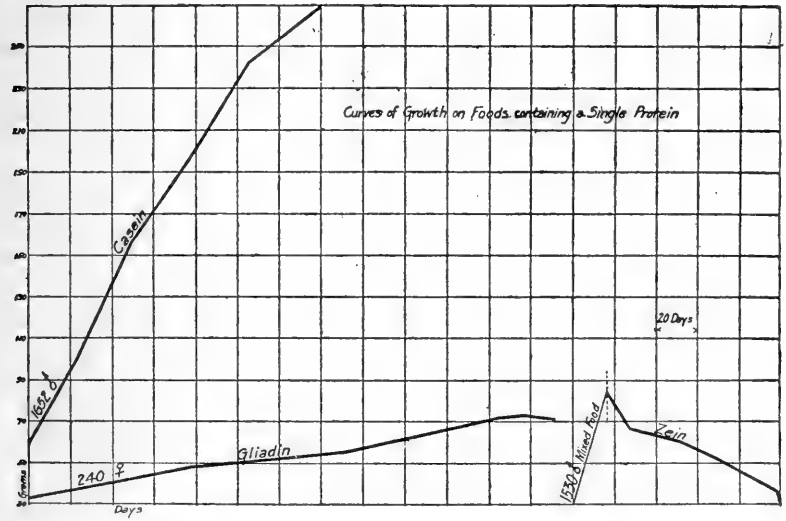


CHART I.—Showing typical curves of growth of rats maintained on diets containing a single protein. On the casein food (devoid of glycocoll) satisfactory growth is obtained; on the gliadin food (deficient in lysine) little more than maintenance of body weight is possible; on the zein food (devoid of glycocoll, lysine, and tryptophane) even maintenance of body weight is impossible.

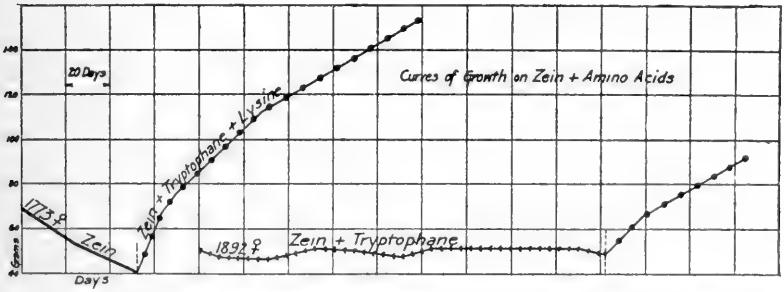


CHART II.—Showing the effect of the addition of the amino-acids, tryptophane and lysine, to zein which fails to yield them. With zein alone (rat 1773) there is nutritive decline. The addition of tryptophane (rat 1892) permits maintenance without growth on foods containing zein as the sole protein. The addition of tryptophane and lysine to zein enables the animals to make considerable growth.

It is interesting to note, in relation to rat 1892, that the growth of this animal was inhibited for six months without material change in its body weight. That the capacity to grow is not lost by prolonged dwarfing on imperfect food is shown by the subsequent growth of the animal when lysine was added to the food containing zein and tryptophane.

substances in respect to one or more amino-acids already ascertained to be yielded by the adequate proteins. The tryptophane group (with its indole nucleus) is missing in gelatin and zein;

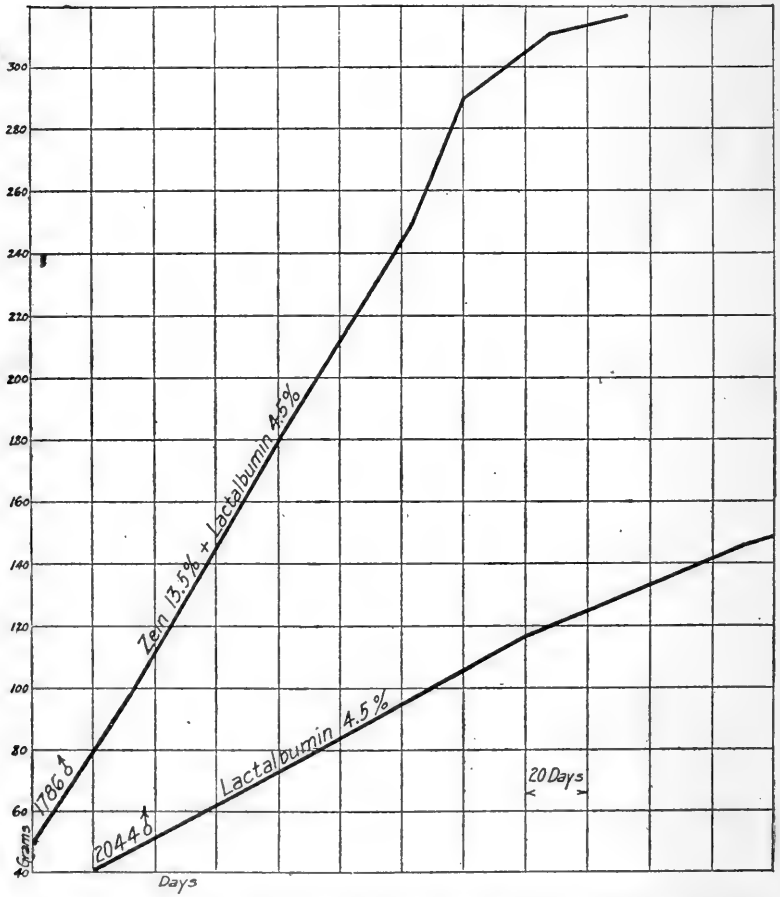


CHART III.—Showing the favorable effect upon growth by supplementing a protein (zein), incapable of maintaining animals when it is the sole protein furnished in the diet, with a more "perfect" protein (lactalbumin). The proportion of the lactalbumin used —4.5 per cent.—was of itself insufficient to promote growth well. It evidently furnished the amino-acid groups lacking in the zein.

the diamino-acid group of lysine is lacking, or nearly so, in zein, gliadin, and hordein. Other shortcomings are the lack of the tryosine group in gelatin and the glycocoll group in zein.

That glycocoll is either not indispensable or else that it can be furnished by direct synthesis in the organism is shown by the excellent growth attending the use of the glycocoll-free casein.

If we analyze the situation as revealed in the charts of some actual experiments, it becomes apparent that both lysine and tryptophane are unquestionably necessary as constructive units in growth. The decline brought about by the zein food can be stopped by the addition of tryptophane, as such, to the diet. This results in maintenance; but no growth ensues until lysine also is added.

The significance of other amino-acids derived from proteins needs to be studied in comparable ways. Experimentally such studies are complicated by the fact that most available proteins are not entirely devoid of the important amino-acid nuclei. The supply of the missing amino-acids need not be in the form of the isolated compound. Suitable proteins which yield them answer in the same way.

In these illustrations the supply of the various proteins was a liberal one. When, however, the protein is offered in more restricted amounts, the indispensability of certain amino-acids may make itself apparent in most surprising ways. Casein, for example, is comparatively deficient in its yield of the sulphur-containing amino-acid cystine. Where the amount of casein in the diet is plentiful, all the amino-acids are evidently afforded in adequate amount to permit the maximum synthesis of (cystine-yielding) tissue allowed by the capacity to grow—the hereditary factor. But when the supply of casein is limited, the curve of growth is altered. This does not mean that the growth is limited by the lack of sufficient protein *per se;* for the addition of cystine at once raises the nutritive efficiency of the diet. Growth has here been limited by the supply of the (relatively) least abundant essential amino-acid—in this case, cystine.

The same story is told in the case of edestin, a protein adequate for growth when fed in abundance, but revealing its comparative poverty in lysine groups as soon as the intake is restricted. Here the addition of lysine, instead of cystine, makes it possible for the organism to use the remaining more abundant

amino-acids effectually for growth even when the total protein supply is not large.

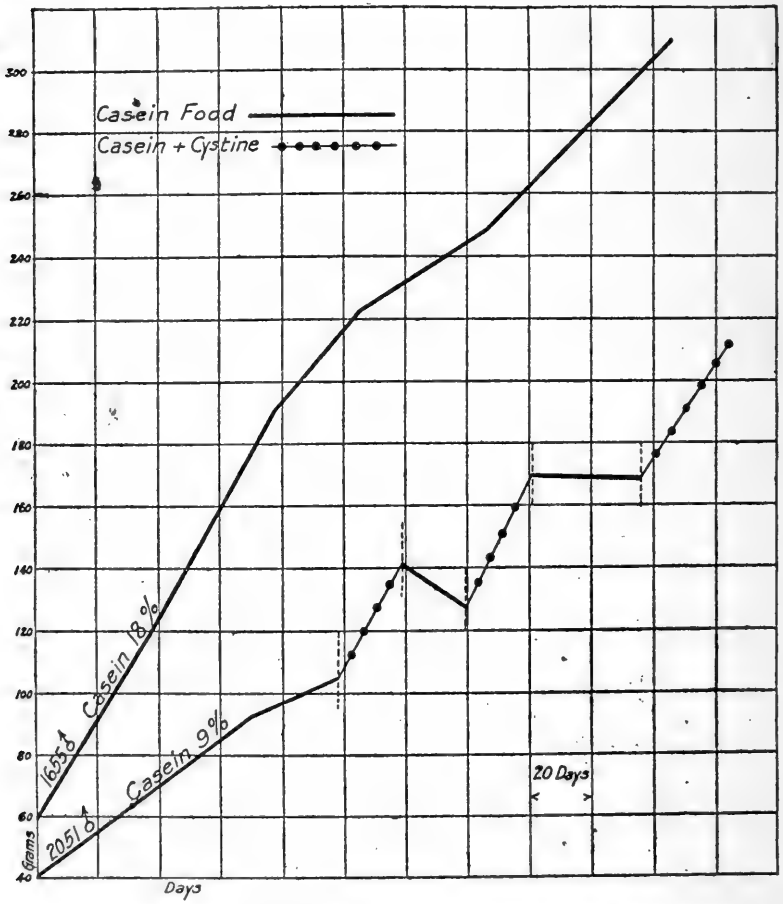


CHART IV.—The curve for rat 1655 shows the satisfactory growth obtained when 18 per cent. of casein was present in the diet as the sole protein. With a smaller amount of casein (rat 2051)—9 per cent.—much less rapid growth ensued. That the insufficiency of the smaller amount of casein is essentially due to its relative deficiency in cystine-yielding groups is shown by the marked accelerating influence upon growth brought about by the addition of the amino-acid, cystine, to the food containing 9 per cent. of casein and the prompt contrary effect when the cystine was withdrawn from the diet.

The possibility of growth and the extent to which it is accomplished are limited by the supply of each essential amino-

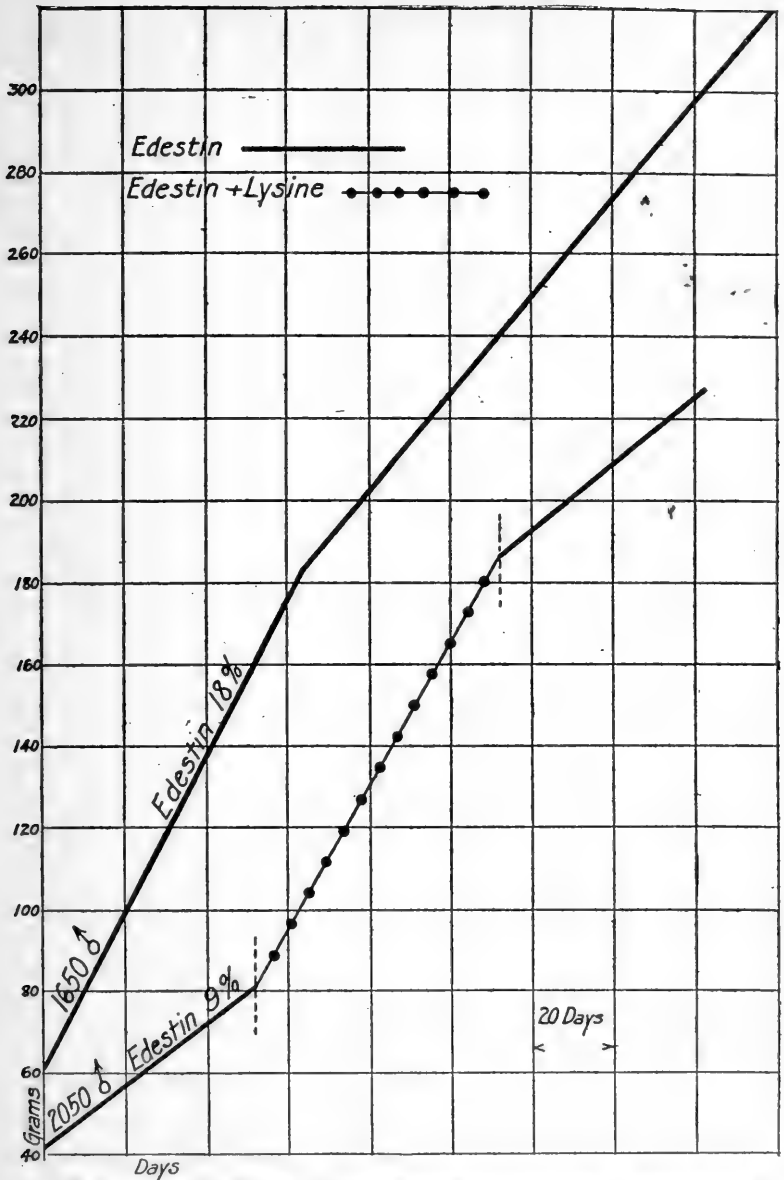


CHART V.—The curve for rat 1650 shows the satisfactory growth obtained when 18 per cent. of edestin was present in the diet as the sole protein. With a smaller amount of edestin (rat 2050)—9 per cent.—much less rapid growth ensued. That the insufficiency of the smaller amount of edestin is essentially due to its relative deficiency in lysine-yielding groups is shown by the marked accelerating influence upon growth brought about by the addition of the amino-acid, lysine, to the food containing 9 per cent. of edestin and the less rapid growth when the additional lysine was withdrawn from the diet

acid. It matters not whether this is exhibited as such or in the guise of protein; in either event the "law of the minimum" is exemplified. The amino-acid shortcomings of one protein can be made good by supplementing it with another protein in which they do not exist to the same degree.

Let us consider what these new observations mean for the problem of protein minimum. It may now be preferable to speak of amino-acid minima. The differences between proteins appear in a new light. The presence of lactalbumin along with casein in milk furnishes a mixture of proteins which is preferable, gramme for gramme, to casein alone. The relative amino-acid shortcomings of the casein, as exhibited in the low content of cystine, are averted by the lactalbumin. From the standpoint of economy, therefore, it is advantageous to learn the amino-acid make-up of all available food proteins, so that they can be exhibited in proportions furnishing a balanced total amino-acid make-up as nearly ideal as possible. This means far more in a practical way to animal production in agriculture than to human nutrition, where we are more lavish with our resources. The proteins of the comparatively cheap maize kernel, for example, consist largely of zein, which of itself fails to maintain nutritive equilibrium (Osborne and Mendel, 1913a, 1914b, and Osborne, 1913). Corn and the by-products of the maize kernel are notably insufficient for good feeding results unless they are supplemented by other protein-containing foods. Osborne and I have found different proteins, like casein, lactalbumin, and edestin, or even the maize glutelin,—the companion protein of zein in the maize kernel,—not equally efficient as a supplement to zein in promoting growth. As we have written elsewhere: "The foregoing experiences bring into new light certain problems related to the economy of foods and commercial fodders. Corn forms the cheapest basis for the feeding of farm animals in food production. Inasmuch as the rate of growth is limited by hereditary, rather than nutritive, conditions, it is futile to furnish more energy, and particularly more protein, than is essential for normal development. An inadequate but cheap protein

can be supplemented advantageously by one which supplies the needed factors, *i.e.*, amino-acids. The relative economy of these additions of supplementary proteins to an inefficient but inexpensive ration depends not only on their quantity but likewise on their amino-acid make-up. A very small addition of a protein, like lactalbumin, may be far more advantageous, when the cost per unit of gain is considered, than larger amounts of cheaper proteins which supplement less perfectly the amino-acid deficiency of the standard diet. It is perhaps not too utopian to expect that the day may come when amino-acid concentrates may serve to render perfect the mixtures of proteins in a fodder like maize or its commercial by-products'' (Osborne and Mendel, 1914b, p. 10).

The maize kernel is not only quite low in protein, the major part of which fails to yield certain important amino-acids, but it is markedly deficient in calcium, which is obviously demanded in abundance in the ration of growth. Evvard, Dox, and Guernsey (1914) have lately found that the addition of calcium carbonate and blood protein to a basal ration of corn and salt fed to pregnant gilts resulted in more advantageous growth, *i.e.*, in new-born pigs having greater size, more vigor, bigger bone, increased coat quantity, better coat color and higher condition. It requires little scientific imagination to translate such experiments with maize into viewpoints applicable in human feeding.

CARBOHYDRATES AND GROWTH

Without carbohydrate in the diet the nutritive functions of a growing individual are menaced quite as readily as they are during adult life. Metabolism exhibits pathological manifestations in the lack of carbohydrates. The isolated occurrence of lactose, a carbohydrate peculiar to milk, in the food which nature has provided for growing mammals has quite naturally given rise by teleological reasoning to the belief that milk sugar has some special virtue in the nutrition of growth. On the other hand, sucrose, maltose, glucose, and even starch and dextrins

have found champions, particularly among pediatrists who, above all others, have become interested in ascertaining which, if any, is the best carbohydrate to furnish to growing infants. There are indications, founded on rational experimentation, of inequalities in the value of the different substances referred to. Perhaps these are to be associated with the relative preparedness of the alimentary tract at different ages (or in different species) to digest the various carbohydrates prior to their absorption. Suggestions of inequalities in this respect are not entirely wanting (*cf.* Mendel and Mitchell, 1907). But the strict contrast of the comparative value of the familiar sugars can only be made under experimental conditions in which the number of other variables is reduced to a minimum. Despite the large amount of literature on this subject a critical examination of it leaves the impression that the evidence at hand will not justify a dogmatic conclusion which discriminates unqualifiedly for or against one of the familiar dietary sugars (*cf.* also Calvary, 1913).

FATS, "LIPOIDS," AND GROWTH

There is, at present, a dearth of conclusive information as to whether true fats are an actual requirement for the maintenance of a healthy, normal organism. Fats are, of course, commonly found in greater or lesser abundance in every dietary; but to what extent they represent a permanently indispensable need of the animal remains to be learned. As has been pointed out elsewhere (Osborne and Mendel, 1912), the reason why this apparently fundamental question in nutrition has not been answered before is presumably attributable to the experimental difficulties inherent in its solution. Fats or fat-like substances are present to some extent in the majority of the familiar food materials, from which they can be completely removed only with the expenditure of considerable effort and care; and the attempts to maintain animals on artificially prepared mixtures of isolated food substances have, until lately, met with little success.[6]

[6] A discussion of earlier attempts in this direction will be found in Osborne and Mendel, 1911a, 1911b.

There is an added reason why it has been practically impossible to solve conclusively the question of the dispensability of the true fats. In food materials these glycerides are almost invariably accompanied by substances of similar solubilities and physical properties: phosphatides, cholesterol, pigments, etc., for which the heterogeneous designation "lipoid" has been devised.[7] These are found in some quantity in every active cell; but although this fact suggests that some, at least, of the so-called "lipoids" have a pre-eminent biochemical importance, it by no means follows that they are essential to the diet during growth or at other periods. They are synthesized by plant cells and it is not impossible to conceive of them likewise as being manufactured in the animal organism. McCollum (McCollum, 1909, McCollum and Halpin, 1912, also Fingerling, 1912) has demonstrated that the phosphorus needed by an animal for phosphatide formation can be drawn from inorganic phosphates, and that phosphatides can be synthesized anew in the animal body. Röhmann (1908b, 1914) asserts the possibility of lecithin synthesis in mice which were maintained into the second generation on lecithin-free food.[8]

Renewed interest has been infused into the question of the rôle of fats as indispensable factors in the diet by the observations of Stepp (1909, 1911a, 1911b, 1912a, 1912b). In attempting to ascertain whether animals are dependent upon their food supply for lipoids or can furnish them by synthesis, like plants, he fed materials extracted with ether and alcohol to mice and observed the effect on the nutritive equilibrium of the animals. Obviously this method of preparing the food eliminated true fats from the diet at the same time. Stepp's observations and conclusions deserve to be carefully examined in connection with the problem at hand. He noted that without exception his mice succumbed in a few weeks when offered otherwise adequate food

[7] For a general description of the so-called lipoids, their occurrence and possible biochemical significance, see Bang, 1907 and 1909.

[8] The protocols (Röhmann, 1914, p. 58) merely show the absence of phosphorus-containing protein in the diet. The composition of the "margarine" always used is not given.

mixtures that had been thoroughly extracted. The deduction was made that the nutritive failure is due to the lack of certain "lipoid" substances, because the addition of alcohol-ether extracts of materials known to be rich in this type of compound sufficed to keep the animals alive. The lacking substance is assumed not to be inorganic, since the addition of the ash of the lipoid extracts made from the food material failed to maintain the mice. Furthermore—and this calls for emphasis here—the sustaining component is asserted not to be ordinary fat inasmuch as the addition of so typical a fat mixture as butter failed to replace the missing life-sustaining factor. More recently Stepp (1913) has re-emphasized his belief that the alcohol-ether-soluble food components, in the absence of which mice regularly succumb, are not fats. They are said to be soluble in alcohol but not in ether (*cf.* also Oseki, 1914). Tripalmitin, tristearin and triolein did not resuscitate Stepp's malnourished mice.[9]

Some time ago Osborne and I (Osborne and Mendel, 1912) believed that we had obtained evidence of the dispensableness of true fats for growth of rats. Our foods for these experiments may presumably be designated as fat-free, even if it is perhaps not permissible to speak of them as "lipoid"-free; for, according to the current definition, the so-called "lipoids" include substances soluble in hot alcohol which may not dissolve in ether. None of our isolated food materials were subjected to extraction with hot alcohol. Undoubtedly such treatment would remove other substances as well as lipoids from such a mixture as the "protein-free milk." Although the animals grew for some time on the "artificial" diets thus devised, subsequent experience leads us to believe that the experiments, though extending in some cases over four months, were nevertheless of

[9] Cooper (1914) has lately suggested that the deleterious effect of lipoid-free diets observed by Stepp is due not to the deficiency of lipoid but to the mechanical removal of vitamine (to which reference is made later in the present paper) during the alcohol-ether extractions. The contribution of MacArthur and Luckett (Lipins in Nutrition, Jour. Biol. Chem., 1915, xx, 161) appeared since the preparation of this manuscript for the press.

too brief duration to permit a final answer. Sooner or later all the animals failed to grow further or thrive on such fat-free diets.

This nutritive decline does not, however, necessarily involve the lack of true fats, but presumably concerns a new and hitherto unappreciated aspect of nutrition in growth which deserves consideration in some detail. I refer to the part that may be played by substances which are not identical with the ordinary nutrients and which, despite the minimal amounts thereof present in the diet, may nevertheless be indispensable for growth and the maintenance of life. Hopkins has suggested the term "accessory diet factors"; Hofmeister, "akzessorische Nährstoffe" (*cf.* Oseki, p. 160); Funk, "vitamines" for the factors here involved.

ACCESSORY DIET FACTORS—"VITAMINES"

It would take us too far from our immediate theme to review, at this time, the development of the evidence that, besides the food-stuffs in the ordinary sense, there exist other constituents of our food which are of the very greatest importance for life.[10] The idea has largely been the outcome of the modern study of so-called deficiency diseases, notably beriberi and scurvy. It was scarcely possible to study the problem under ideal conditions until satisfactory methods were devised for feeding mixtures of isolated food substances with some degree of success. Like many new and suggestive hypotheses, the vitamine theory has found a ready acceptance and has been applied in explanation of all sorts of pathological manifestations, in some cases solely on the basis of analogy and quite in advance of any scientific evidence therefor.

The idea of the existence of accessory factors or specific requisites for growth has only of late taken a more concrete form. Friedenthal (1911) has designated as "mitosone" hypothetical products of internal secretion which accelerate the rhythm of cell division. This sort of factor concerns the food supply indirectly at best. The studies of Röhmann (1902, 1903,

[10] For the literature on this theme see Funk, 1913a, 1914.

1908a, 1912, 1914), Hopkins (1912), Hopkins and Neville (1913), McCollum (1909), McCollum and Davis (1913a, 1913b, 1914), Funk (1913a, 1913b, 1914), Funk and Macallum (1914), and Osborne and Mendel (1913b, 1913c, 1914a) in particular, have raised new questions in respect to the possible function of accessory diet factors or "growth vitamines" in growth. Briefly they show that all attempts to grow animals on diets consisting of carefully purified isolated food-stuffs—not the highly complex food mixtures such as constitute the familiar rations of every-day life—sooner or later result in failure. The shortcomings of some of the supposed earlier successful experiments with such "artificial" diets, *e.g.*, mixtures of casein, fat, sugar, starch, and inorganic salts, were masked by the brief duration of the observations.

Both Osborne and I and McCollum and Davis (1914) have experienced numerous instances of growth over periods of many weeks on diets similar to that just outlined; but growth invariably ceased if the trials were not interrupted too early; and the development was resumed as soon as suitable changes in diet were instituted. Röhmann, who has achieved a considerable degree of success in inducing mice to *grow* as well as be maintained on mixtures of isolated food-stuffs, hesitates to accept the vitamine hypothesis in explanation of the failures. He inclines to the possibility of an unsuitable relationship in the proportions of the nutrients used.[11] Hopkins (1912) found that he could remedy the shortcomings of the "artificial" mixtures which he fed to rats by the addition of milk in quantities far too

[11] "Diese Futtergemische sind also für die Aufzucht junger Mäuse den natürlichen noch nicht vollkommen gleichwertig. . . . Worauf diese Unvollkommenheit der künstlichen Nahrungsgemische beruht, will ich nicht entscheiden. Ueberaus bequem ist ja die Erklärung mit Hilfe der Vitamine. Aber sind denn wirklich schon einwandfreie Beweise für deren Vorhandensein gebracht? Ich bin vorläufig mehr geneigt anzunehmen, dass in der von mir verwendeten künstlichen Nahrung zwar alle für die Ernährung und das Wachstum erforderlichen Stoffe vorhanden waren, aber dass das Mischungsverhältnis oder die Form der Nahrungsstoffe nicht derartig war, um die künstliche Nahrung der natürlichen vollkommen gleichwertig erscheinen zu lassen." (Röhmann, 1914.)

small to have significance from the standpoint of their contribution to the energy of the ration. Osborne and I (Osborne and Mendel, 1911b) found, in what we have termed "protein-free milk," a more satisfactory substitute for the less efficient salt mixtures or the ash of milk. This product contains, besides lactose and inorganic salts, very small amounts of unknown compounds. Our attempts to imitate the "protein-free milk" in an artificial way have, like those of Röhmann (1903) and of McCollum and Davis (1913a, 1913b) with salt mixtures, given limited growth at best, though in occasional instances this has been surprising in extent. For such exceptional successes one may offer the hypothesis that the young organism sometimes, if not always, possesses a store of the as yet unknown "chemical determinants" which suffice for some time in the absence of a suitable supply in the food intake. Sooner or later this becomes exhausted and nutritive equilibrium and growth cease. The organism does not synthesize the essential "vitamine," if we prefer to express the situation in terms of this hypothesis.

McCollum and Davis (1914) summarize an extensive experience with rats fed on rations made up of purified casein, dextrin and salt mixtures from reagents by calling attention to a marked difference in the ability of individual animals to grow on such diets. They state that normal growth during a period of somewhat more than one hundred days can be attained only by exceptional individuals. Many fail entirely to grow, others grow at decidedly under the normal rate; and McCollum and Davis believe that they have in such rations a means of measuring the vitality of individuals in a manner more satisfactory than any hitherto employed.

It does not follow directly, however, that the abnormality of the diet in such instances of failure of growth as have been referred to—whether we call it a lack of "vitamine" conceived to be organic in nature (with Funk), or attribute it to absence of some essential inorganic agent, or an inappropriate balance of the nutrients—inevitably involves the lack of a *growth* factor. Adequate growth postulates a satisfactory condition of maintenance before any continued gains in weight can be made. It

might be supposed that milk, "protein-free milk," and other addenda to the ineffective "artificial" diets promote growth solely because they furnish something essential for normal metabolism, the basis upon which tissue construction and expansion are superimposed.

But this is not the whole story. Osborne and I have kept rats through two generations upon a diet consisting solely of whole-milk powder, lard and starch. When we attempted to grow young animals upon a comparable diet of isolated milk protein, "protein-free milk," carbohydrate, and lard there was a suspension of growth, sometimes quite sudden and usually more gradual, before adult size was reached. The essential difference between the adequate and the inadequate ration just described lies in the absence of the milk-fat or cream element of the latter. We found that addition of unsalted butter (Osborne and Mendel, 1913b), or of butter-fat (Osborne and Mendel, 1913c) to the inadequate diets in which lard formed the sole fat component, prevented the suspension of growth in ungrown rats and promptly restored growth where it had failed. Milk fat—which includes all the milk constituents soluble in the fats proper—therefore contains something essential for growth.[12]

Prior to the publication of our results McCollum and Davis (1913b) showed that the failure of rats to make further growth after reaching a "critical" point on mixtures of isolated food substances could be remedied by supplying the ether extract of egg or of butter. They state that rats, in which growth was suspended, may remain in an apparently good nutritive con-

[12] It has been said, in criticism of such conclusions, that the "accessories" merely represent something which induces a larger food intake with a more adequate maintenance or consequent growth, as the case may be. Obviously a failure to eat must result disastrously. We recognize the pertinence of such criticism and its applicability to many instances of nutritive decline: but the very extensive records which we have show numerous cases of decline despite liberal food intake. The ideal plan of feeding measured quantities of food cannot be carried out satisfactorily on many species over long periods; even in birds, which can be "stuffed" with food, it meets with limitations.

dition for many weeks and still be capable of responding to the growth-promoting effect of the ether-soluble substances as well as to the mixed foods of nature. McCollum and Davis in no case obtained a beneficial result by feeding lard or olive oil. To the instances of the favorable influence of butter fat and egg fat Osborne and I have added the equally satisfactory effect of cod-liver oil and, in lesser degree, of beef fat. The results with the "efficient" fat mixtures (in contrast with the inefficient ones) are so striking and prompt as to constitute a unique demonstration of the potency of the hitherto unsuspected factors in diet. By fractioning butter fat we have now been able to demonstrate that the more liquid portions, or what we may term the butter oil, contain the effective ingredient. That it is not universally present in natural oils has also been demonstrated alike by the observations of McCollum and Davis and our own evidence of the inability of fats, like almond oil and olive oil, to replace the butter oil in promoting resumption of growth. The nutrition-promoting properties of cod-liver oil were tested owing to the wide-spread popular and medicinal use of this product. Our experiments afford, we believe, direct evidence that cod-liver oil is something more than a mere nutrient. Experiments now in progress to ascertain how much butter oil is necessary to prevent nutritive disaster, or to induce restoration where decline has resulted, make the quantities appear to be surprisingly small.

Quite recently McCollum and Davis (1914) have found that the property of inducing a resumption of growth in rats which have grown as far as possible on a fat-free ration can be conferred on olive oil by shaking the latter with a solution of the soaps prepared by completely saponifying butter fat in a non-aqueous system with potassium hydroxide, according to the method of Henriques. The results indicate that the substance or substances present in butter fat which exert such a marked stimulating action on growth are sufficiently stable to withstand the conditions of saponification.

The beneficial effects of some of the fat additions manifest themselves not only in the resumption of growth but also in the

alleviation of incidental nutritive disorders and evidences of lowered immunity to disease (Osborne and Mendel, 1913c, p. 431). Experimental animals frequently develop an infection of the eye during the periods of their nutritive decline. This has been noted by various investigators, and is perhaps represented by comparable phenomena in the malnutrition of children. The simple addition of butter fat or of cod-liver oil to the diet, without other change, leads to a prompt disappearance of the symptoms of eye disease.

No amount of butter fat or cod-liver oil will induce growth on dietaries in which the proportions and nature of the inorganic salts are inappropriate, in which suitable carbohydrate is missing, or the quantity and character of the protein is inadequate. The nutrient units—the "Bausteine"—must not be overlooked in our enthusiasm for the newer features. The work of Stepp, McCollum, and Osborne and Mendel agrees in indicating strongly that mere absence of fat from the diet is not the cause of suspension of growth; or rather we should say that the ordinary fats *per se* are not the substances which promote the growth. The naturally occurring fats can dissolve substances other than triglycerides. The chemistry of the problem awaits solution.

THE DETERMINANTS OF GROWTH

It is not unlikely—to speak conservatively—that there are at least two "determinants" in the nutrition of growth. One of these is furnished in our "protein-free milk," which insures proper maintenance even in the absence of growth. When this was fed we have maintained rats without growth for very long periods. Without this "determinant" (as, for example, in diets of isolated food substances containing artificial substitutes for "natural" protein-free milk) the special components of butter fat or cod-liver oil or egg fat induce only limited gains at best. Another "determinant" is furnished by these natural fats (or fractions more recently prepared therefrom by Osborne and myself or the saponification product of McCollum and Davis). Either of the determinants may become "curative";

both are essential for growth when the body's store of them (if such there be) becomes depleted (Funk and Macallum, 1914). It is too early to attempt a tenable conclusion.

PRACTICAL APPLICATIONS

In the domain of practical medicine these recent investigations are likely to awaken fruitful speculation and beneficial applications. The popularity of the milk fats may be revived from a new standpoint. Quite recently, and apparently without knowledge of the American studies in this field, Niemann (1914) has advocated the use of washed butter as an adjuvant to the dietary of malnourished infants and has reported signal successes from its employment. The dietotherapeutic claims of cod-liver oil appear in a new light. The use of egg yolk emulsion in the case of growing individuals finds justification. The claims of milk or fractions of milk, such as whey, etc., to a place in the ration of the young are emphasized, as they always have been whenever proprietary infant foods containing milk products have been compared with those devoid of them (*cf.* Wheeler and Beister, 1914). The contrast of skimmed or cream-free milk with whole milk is brought into new relations.

Growth is more than a mere energy problem. Insufficiency of food and individual food-stuffs may be contrasted with *specific* deficiencies. We have yet to learn where the essential substances are to be found aside from the few products already mentioned. The possible potency of plant products remains to be ascertained. Even the question of unheated *versus* heated milk, and the problem of the influence of preservation by heat upon canned foods, may be concerned with the stability of the "accessory factors" toward physical agents. Perhaps in the refinement of modern food preparation we are dealing with an induced deficiency of actually known substances. One readily thinks of the possible rôle of traces of iodine or silica or manganese hitherto unrecognized.

With activators and hormones, vitamines and food accessories, mitosones and products of internal secretion brought to our attention day by day, I am conscious, in the midst of our

enthusiasm, of a warning given by Rubner in a protest against the creation of a new scientific vocabulary and the danger of transforming the natural sciences into a play of words. "The science of nutrition," he says, "must never tolerate the substitution of unhealthy speculation for what is admittedly a laborious undertaking, namely, experimentation."[13]

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[13] "Es ist sehr bedauerlich, dass in der Literatur des letzten Jahrzehnts überhaupt sich an allen Ecken und Enden die Tendenz geltend macht, bei Experimenten, bei denen weder die wirksamen Substanzen, noch die physikalischen Bedingungen genauer bekannt sind, zu sofortiger Namensgebung zu schreiten. Aus den ersten Hypothesen werden weitere Hilsfshypothesen mit wieder neuer Nomenklatur. Den Lesern kommt gar nicht mehr zu Bewusstsein, dass die Namen, die er hört, nur hypothetische Körper oder nur Namen für einen Vorgang sind, der vielleicht nur bei gewissem Quantitätsverhältnisse des Stoffes in die Erscheinung tritt, bei anderen nicht. Die allerwenigsten der Leser wissen heute noch die Genesis solcher Worte. Der kleinste Teil kennt die Experimente, auf welche die Namensgebung zurückzuführen ist. Die einfachsten Binsenwahrheiten werden dann in der Form hochtrabender Spezialausdrücke zu neuen Errungenschaften, die Literatur ist heute auf manchen medizinischen Gebeiten, man möchte sagen, ohne die Zuhilfenahme besonderer Lexika für Fachausdrücke und Synonyme ungeniessbar. Die Medizin muss hier endlich einmal wieder Halt machen. Hypothesenbau und Theorie haben auch ihr Gutes, sie dürfen aber nicht hypertrophisch werden und das klare durchsichtige Experiment verdrängen. Die Naturwissenschaft darf nicht in ein Spiel mit Worten sich verlieren. Am allerwenigsten ist es aber in der Ernährungslehre angebracht, eine ungesunde Spekulation an Stelle der allerdings mühseligen Experimente zu setzen." (Rubner, 1908.)

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THE EXCRETION OF ACID IN HEALTH AND DISEASE*

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BIOLOGISTS and philosophers alike have found the description of organic regulation a difficult task, for this characteristic of life is one of the genuine riddles of nature. When the man of science reflects upon regulation he must take account of the preservation and development of forms, the restitution of structures, and the maintenance of all bodily equilibria, including the simplest of physicochemical states. Thus he is led to perceive the explanations of functions and of organic activities. He conceives their ends. On the other hand it sometimes appears to the philosopher as if the very nature and essence of the organic, its chief if not its only differentia, were revealed in the seemingly teleological processes of regulation.

The history of this problem of regulation is lost in antiquity. Aristotle saw it clearly. Few great biologists since him have escaped it. Yet I think that little progress toward its final mastery has been made, and only recent scientific researches put the subject on the sound basis of experiment. In general biology the modern definition seems to derive, strange to say, from Herbert Spencer. His famous "Conception of Life" as "the continuous adjustment of internal relations to external relations" is indeed vague. But when one falls back upon the analytical discussions from which Spencer arrived at it, I think one may truly say that the "Conception" comes to little else than a question of equilibrium, involving the totality of organic regulations. As such it seems to have been recognized by Wilhelm Roux. Roux, however, the school of Entwicklungsmechaniker, and other experimental biologists have done for

* Delivered December 12, 1914.

the problem what Spencer could not do. They have made it a central problem of research. Thereby they have made it known, and in a measure they have made it understood. Yet it must be confessed that their results have in turn at least one important deficiency. They lack a precise quantitative foundation upon the abstractions of physical science. Such morphological researches have advanced biology not a little. They have greatly aided in the sound analysis of phenomena, and they have provided a remarkable and substantial basis for the questionable vitalistic theories of Driesch, but they have not satisfied the physical scientist. The physicist, perhaps, will not easily be content with anything which appears to be the sole basis, not only of Driesch's vitalism but of all the vitalisms of the day; not at any rate until it has received its physicochemical formulation.

Driesch and Roux, among biologists, appear to have devoted most pains to the question of what regulation is. I think Driesch's conclusion is the more convenient. This is it: "We shall understand by regulation any occurrence or group of occurrences in a living organism which takes place after any disturbance of its organization or normal functional state, and which leads to a reappearance of this organization or this state, or at least to a certain approach thereto." Regulation, then, is a physiological process. It is hardly different from metabolism, viewed in one light, and it involves, among other things, such physical abstractions as "states," *i.e.*, temperature, osmotic pressure, and alkalinity. Accordingly it is sometimes open to accurate quantitative measurement and experimentation of a kind which the more general experimental biology hardly knows, and in these investigations it need involve no vague concepts.

The problem of regulation has had its independent development in physiology. The study of the control of body temperature long ago taught us how to investigate regulation quantitatively. Nothing is more familiar to the physiologist and the physician. Why then should one take a long way to say so little? Because we greatly need the exact description, not only of regulation, but, I repeat, of *what regulation is,* and I think

we shall find it, if at all, only with the help of physical chemistry and by induction.

A preliminary attack upon this problem, an effort to analyze a part of organic regulation as a physical and chemical process, has been made by von Wendt in his interesting little essay which serves as introduction to the treatment of inorganic metabolism in Oppenheimer's "Handbuch der Biochemie." In metabolism, so Wendt points out, there may be distinguished certain logically independent activities; fundamentally these come to two, utilization and regulation. Both of these phenomena, no doubt, constitute regulation in the larger and necessarily less analytical definition of Driesch. But Wendt's distinctions appear to be well founded and, on the whole, I believe, capable of precise definition. Accordingly, let us consider his ideas of the nature of regulatory metabolism. These, though in fact regulation is inextricably bound up with the other phenomena of metabolism, are not at all difficult to understand if only we pursue the analysis. Then regulatory metabolism is seen to be made up of four important activities: first, restitution, whether exogenous or endogenous; second, hoarding or storing up; third, physicochemical regulation; and, last, excretion.

No such classification of regulatory functions in metabolism is likely to be final, for it is reasonable to suppose that, when the fundamental abstractions of physical science shall be more generally employed in physiology, a truly rigorous treatment of the subject, such as we cannot to-day accomplish, will become possible. Meanwhile it must be again pointed out that this classification is inapplicable to certain phenomena which, for the general biologist, are regulations, and that in other cases it is decidedly ambiguous; but, on the whole, it will serve our purpose for this evening.

One genuine advantage, at any rate, is to be gained from following von Wendt. We shall be concerned with the description of the processes by which regulation is accomplished, rather than with the problem of its determination or control. The latter question, as I have said, seems to be the origin of all forms of neovitalism and other anti-mechanistic views in gen-

eral; it is of surpassing difficulty; and, whatever we may think of its fruits, it is a problem which no one has ever successfully met.

I now ask you to consider with me the regulation of the neutrality of the body, and in particular its final phase, the excretion of acid. The most general and elementary regulation of which we have knowledge is probably the regulation of volume. Next to this, I think, comes the regulation of neutrality. This phenomenon is far more general than the regulation of temperature or of molecular concentration, and it seems to be quite the most exact of all regulations. Moreover, it is known to be closely related to a very large number of physiological processes, such as changes of molecular concentration, changes of volume, osmotic exchanges, the transport and excretion of carbonic acid, the control of respiration, certain processes during fever, the metabolism of active muscle, and many others. Finally the causes of these manifold physiological influences are not wholly unknown. In the first place, the ions of hydrogen and hydroxyl are quite the most active of all ions. Secondly, every acid and basic substance in every phase of the body has, in its own appropriate degree, a share in the reaction of its own phase. And in turn every phase is related to every other phase, directly if it be in contact with it, if not, indirectly, by the heterogeneous equilibria which involve these ions. Such is the chief reason for the increasing importance of the physiology of the hydrogen ion. Upon turning to von Wendt's classification of regulatory processes, it is easy to see that the several subdivisions are of unequal interest. The restitution of basic and acid substances, which are used up in the physicochemical equilibria of neutrality, is such a simple process, and has been so long recognized, at least in its general characteristics, that it must be familiar to everybody. There is an exogenous restitution, which depends upon the presence of large amounts of unchangeable basic and acid substances in the food. This process is ordinarily left to chance and a mixed diet, but in case of need it may be usefully controlled. The recent investigation of

Blatherwick upon the diet illustrates this possibility, while the value of soda when administered in acidosis is a more familiar instance. There is also an endogenous restitution. This involves the necessary, and, in normal metabolism, unvarying production of carbonic acid, phosphoric acid, sulphuric acid, and other substances, and secondly, the very variable manufacture of ammonia, instead of neutral material, during the course of nitrogen metabolism. The latter process is not well understood. In such conditions as diabetic acidosis a large increase in the production of ammonia is perhaps the chief means of prolonging life, but, on the other hand, there are also conditions of acidosis in which the production of ammonia is actually diminished below the normal amount.

Far less familiar than the questions which the processes of restitution raise are those concerned with such storing up of alkaline material as may occur in the organism. In the course of long experience with the problems of acid-base metabolism a conviction has gradually been established in my mind that such reserves of alkali, in substantial amounts, must exist and not infrequently suffer mobilization. But I confess that the evidence is far from conclusive, while the problems raised by this question, involving the most difficult of colloidal phenomena and all sorts of heterogeneous equilibria, are of unusual complexity. One thing at least is certain: the normal equilibrium between acids and bases in the aqueous solutions of the body is so efficient, or, if you will, so elastic, in regulating the reaction that in effect it is, of itself, a great reserve of alkali which always remains instantly and perfectly available.

The present knowledge of the hoarding of alkali is, however, not quite so slight as it might seem. For if we turn to the special case of the blood we shall find, as Spiro and I have rather imperfectly explained, that, with respect to the plasma, the red cells constitute an alkaline reservoir. They take up acid from the surrounding fluid as carbonic acid increases and displaces it during the course through the body, they give it back once more as carbonic acid escapes in the lungs. These phenomena

depend on far-reaching readjustments of equilibria between the two phases, but concern especially the union of protein material with alkali. Our knowledge of this subject depends upon numerous researches which have followed up the truly surprising original observation of Zuntz. Though they concern the blood alone, there can be no doubt that in some measure similar processes are always involved, and are, therefore, of general physiological importance, wherever cells are bathed by fluids. Accordingly it may safely be said that a special manner of storing up alkali, which depends upon the very nature of protoplasm, is characteristic of all organic structures. There is one fact concerning this store of alkali to which I would call your attention: it seems not to become available through the operation of regulatory physiological processes, but to be liberated automatically and merely through the disturbance of physicochemical equilibria. This observation leads to the third division of our subject, physicochemical regulation.

The immediate regulation of the reaction of body fluids is, in truth, nothing but the underlying equilibrium just mentioned, whose disturbance calls into play the stored alkali of the protein compounds. This rests upon a simple, homogeneous chemical reaction, which, like all such reactions, can be influenced only by changing the concentrations of the substances concerned, or by changing the temperature. Thus it is wholly removed from direct physiological control. The organism plays upon the equilibrium, so to speak, or, if you will, utilizes it, by the processes of restitution and excretion. But these processes no more control the equilibrium than the muscle controls the force of gravitation.

The chemical reactions whereby all substances are at once neutralized, the chemical reagents which aid in neutralization, the shares of important substances in the process, and its efficiency, the changes in chemical equilibria, including resulting changes in hydrogen and hydroxyl ion concentrations, all, *so far as they concern true solution,* are known with a fair approach to certainty. Principally this work of neutralization is done by

salts of phosphoric and carbonic acids, with aid from the amphoteric proteins. In simplified form the process may be represented by the two reactions,

$$M_2HPO_4 + HA = MA + MH_2PO_4;$$
$$MHCO_3 + HA = MA + H_2CO_3,$$

where M stands for any basic radical, A for any acid radical. Other less important simultaneous reactions are of the same type, except perhaps the union of the weak acids with basic proteins like globine, and the union of bases with more acid proteins. Through the remarkable circumstance that phosphates and carbonates possess, among all known chemical substances, the highest power to preserve neutrality in solution, this function is so well performed that the alkaline reaction of the body scarcely varies, even when the load upon the mechanism is heavy.

Regularly, as they form, the acid products are afforded alkali by blood and protoplasm, for every molecule of carbonic acid about 0.9 molecule of alkali, for every molecule of phosphoric acid 1.8 molecules of alkali, and for every molecule of sulphuric acid 2 molecules of alkali, in accordance with chemical laws and the normal reaction of the body.

The conditions are closely parallel in all organisms, and there is reason to believe that constancy of alkalinity is quite the earliest and most universal physicochemical regulation of active protoplasm. In fact, as the investigations of Palitzsch show, the ocean itself is likewise constant in its alkalinity. It is worthy of note that this is due to the simultaneous presence of carbonic acid and bicarbonates in the sea water, a fact which lends some support to Macallum's ideas about the derivation of the body fluids. Thus active protoplasm everywhere, as well as that which surrounds it—the environment and the *milieu intérieur*—appear to be and to have been always of stable reaction.

According to the modern theory of solution water itself, like the dissolved electrolytes, is dissociated into ions, though only to a very slight degree.

If the water be pure the concentrations of hydrogen and hydroxyl ions are necessarily equal, for water is electrically

neutral. A variety of independent methods of estimation have shown that at 25° this concentration amounts almost precisely to N/10,000,000. This corresponds to 0.0000001 gram of ionized hydrogen and 0.0000017 gram of ionized hydroxyl in 1,000 grammes of water. Further, the theory of solution explains acidity by the presence of hydrogen ions, formed from dissolved electrolytes, in excess of hydroxyl ions; and alkalinity by a similar excess of hydroxyl over hydrogen ions. Neutrality is, accordingly, the condition when, as in pure water, the two concentrations are equal. In short, expressing the concentration of ionized hydrogen by $(\overset{+}{\mathrm{H}})$ and of ionized hydroxyl by $(\mathrm{O}\overset{-}{\mathrm{H}})$, if

$$(\overset{+}{\mathrm{H}}) = \frac{\mathrm{N}}{10{,}000{,}000} = (\mathrm{O}\overset{-}{\mathrm{H}})$$

the solution is neutral. If

$$(\overset{+}{\mathrm{H}}) > \frac{\mathrm{N}}{10{,}000{,}000} > (\mathrm{O}\overset{-}{\mathrm{H}})$$

the solution is acid. If

$$(\overset{+}{\mathrm{H}}) < \frac{\mathrm{N}}{10{,}000{,}000} < (\mathrm{O}\overset{-}{\mathrm{H}})$$

the solution is alkaline.

Thus the nature of acidity and alkalinity may readily be represented by a straight line with the neutral point at its centre, acidity increasing in one direction, and alkalinity in the other.

Whenever a weak acid is present in aqueous solution in company with such bases as sodium, potassium, calcium, magnesium, etc., which are invariable constituents of the ocean, blood, protoplasm, etc., *provided the acid be in excess,* it is a simple matter to determine the reaction, which can best be measured by the values of $(\overset{+}{\mathrm{H}})$ and $(\mathrm{O}\overset{-}{\mathrm{H}})$, following the considerations just set forth.

For this we possess a host of reliable data and a tried and well-seasoned theory—the mass law. There is, in connection with the application of the mass law to ionization, a certain characteristic property of an acid, its ionization constant, k, which measures its tendency to dissociate in aqueous solution, thereby to produce hydrogen ions, and hence to increase the

intensity of acidity. Strong acids have ionization constants which are of the order of magnitude of 1.0, weak acids of the order of magnitude of 0.0001, the weakest acids, 0.00000001, or less.

It has been discovered that in this general case of weak acid and salt, the concentration of ionized hydrogen is always almost exactly proportional to the ratio of free acid to salt and is approximately equal to the product of this ratio by the ionization constant of the acid. That is to say, representing free acid by HA and salt by BA.

$$(\mathrm{H}) = k \times \frac{HA}{BA},$$

whence, if $k = (\overset{+}{\mathrm{H}})$

$$\frac{HA}{BA} = 1.$$

From this relationship therefore follows the conclusion, fully established by experiment, that whenever in such a solution the excess of acid, HA, is chemically equivalent to the quantity of salt, BA, the hydrogen ion concentration is almost exactly equal to the ionization constant of the acid. But the ionization constant of carbonic acid (first hydrogen atom) at room temperature is 0.0000005. Hence, in a solution containing exactly equivalent quantities of free carbonic acid, sodium bicarbonate, the hydrogen ion concentration must be approximately 0.0000005 N, or five times the value at neutrality. Further, since

$$\frac{HA}{BA} = \frac{(\overset{+}{\mathrm{H}})}{k},$$

if the amount of acid be ten times the amount of salt

$$\left(\frac{HA}{BA} = 10\right)$$

the hydrogen ion concentration must be about 0.000005 N, or fifty times the value at neutrality, and if the reverse be the case

$$\left(\frac{HA}{BA} = \frac{1}{10}\right)$$

the value must be nearly 0.00000005 N, or one-half the value at neutrality.

The range of variation of concentration of hydrogen ions in the usual solutions of the chemical laboratory considerably surpasses the limits of 1.0 N and 0.00000000000001 N. In comparison with such enormous differences those between 0.000005 N and 0.00000005 N are almost negligible (1/100 : 1/100,000,000,000,000). Hence ordinarily it is quite accurate enough to speak of any solution containing both free carbonic acid and a bicarbonate, when the disparity between the concentrations of the two substances is not very great, as of neutral reaction. For, obviously, the neutral point falls well within the narrow range of reaction of such solutions, being characterized by a ratio of carbonic acid to bicarbonate of about 1 : 5.

Thus carbonic acid, like the almost equally weak acid, phosphoric acid (after its first hydrogen has been neutralized by base), has the remarkable property of preserving a neutral reaction whenever it exists in solution with its salts, provided there be an excess of acid. All acids whose strength is even a little either greater or less than carbonic acid lack the property. There is nothing mysterious about this fact; any other weak acid will hold constant the reaction in its own range of reaction; *e.g.*, acetic acid in the neighborhood of a hydrogen ion concentration N/100,000.

This characteristic of carbonic acid is important, first to regulate one of the most fundamental of physicochemical conditions, and secondly, to preserve throughout nature the characteristic chemical inactivity of water, which disappears whenever the reaction becomes either appreciably acid or appreciably alkaline. Almost the only case of important geological action due to acidity or alkalinity of water is the action of fresh water, containing carbonic acid itself, to weather the rocks. This process is, however, self-limited, for the dissolved material forms bicarbonates, and thus at once provides permanently balanced solutions.

Elsewhere, within and without the organism, carbonic acid is almost always accompanied by bicarbonates, and a close approach

to neutrality is everywhere the result. In the organism the variation in ratio of phosphates is similar to the case of the carbonates, as may readily be illustrated by experiment. Thus a solution consisting of equal parts of monosodium phosphate and disodium phosphate will be found to give a neutral reaction with both methyl orange and phenol phthalein, and the neutrality, thus indicated, will not be disturbed by the addition of relatively large amounts of either acid or alkali.

We may next consider the equilibrium in the blood, where the concentration of ionized hydrogen can undoubtedly vary between 3 N/100,000,000 and N/10,000,000, but during life probably not much more widely.

In order to bring about this seemingly insignificant change in reaction, the relative quantities of acid and base in the body must undergo very great changes; or, otherwise stated, until very large quantitative changes in the amount of acid or base in the body have come about, there can be no appreciable change in the reaction. Thus it is that even in extreme acid intoxication, as for instance diabetic coma, almost the only large chemical change that can be detected, as a result of the action of enormous quantities of acid through long periods of time, is a large diminution in the bicarbonates of the blood. For the range just mentioned this would amount to a decrease of more than a half in the total carbonic acid. Meanwhile about a quarter of the phosphoric acid of the body will probably be changed from alkaline to acid phosphate, and the proteins will have given up a portion of the alkali with which they are combined.

The recognition of the fact that diminution of bicarbonates is the principal effect of acid intoxication upon the blood, involves important consequences. On the one hand it has become clear that the therapeutic use of sodium bicarbonate is desirable in a large variety of pathological conditions and, on the other hand, it seems to be certain that the evil effects of acidosis partly depend upon interference with the transport of carbonic acid and its excretion from the body. In truth this equilibrium is intimately associated with the respiratory function, and with

a great number of other fundamental physiological activities, and with the osmotic pressure of the cell.

Further, the profound influence of hydrogen and hydroxyl ions upon many enzymatic processes, and upon colloids in general has been established, and it is gradually becoming clear that all the physicochemical conditions in protoplasm—alkalinity, osmotic pressure, colloidal swelling, chemical equilibrium, temperature—are interdependent, and that carbonic acid and the acid-base equilibrium are among all these things quantitatively the most important variables.

These conclusions rest upon one of the immutable properties of matter. Phosphoric and carbonic acids in solution everywhere possess this characteristic, independent of the presence of everything else, just as they everywhere possess their characteristic chemical composition.

Such is the immediate regulation of the reaction of the body, which, though nothing but an ordinary physicochemical equilibrium, manifests the highest physiological efficiency.

However delicate and efficient the physicochemical regulation of neutrality within the body may be, it is absolutely dependent for its continuous existence upon excretion. Acid and basic substances must be removed from the field of reaction. They must be eliminated ceaselessly, in such relative and absolute amounts as shall exactly reverse the chemical reactions of the internal regulation, so as to preserve the latter within normal bounds. In the total this amounts in all circumstances to an excretion of acid.

The process of acid excretion is three-fold. It includes, first, the excretion of very large quantities of carbonic acid, a process which is under the accurate control of the excretory apparatus; secondly the excretion of ammonia, which appears to be subject to little or no regulatory control; and finally the production of an acid urine, resulting from a carefully balanced and regulated excretion of basic and acid substances in solution. There is, to be sure, an obscure and probably insignificant fourth factor; excretion of phosphates by the intestine.

The first of these factors of acid excretion is only regulatory

in a provisional manner, and in its importance resembles the intermediate or internal physicochemical regulation, in which it engages. A simple consideration will make this clear. Carbonic acid is the chief excretory product of the organism. As such it must be eliminated promptly and completely. This is always done. Moreover, in that this substance leaves the body of man not in aqueous solution and as an acid, but almost exclusively in the form of gaseous carbon dioxide free from all base, there is little or no possibility of any variation of the permanent effect produced upon the reaction of the body by the elimination of a definite amount of it. In the final regulation by excretion it is not, therefore, concerned. And yet it has, in the process of excretion, a very important rôle in regulating the reaction of the body. This depends upon the fact that carbonic acid is not only a waste product, but also a normal constituent of the blood, and, as such, a principal factor in the physicochemical regulation. Thus, if the ratio of carbonic acid to bicarbonates in a normal individual were 1:12, a large production of acid might cause a destruction of a fourth part of all the bicarbonates, producing in its place an equivalent amount of free carbonic acid. This, if nothing else occurred, would reduce the relative amount of bicarbonates from 12 to 9, and simultaneously increase the free carbonic acid from 1 to 4. The ratio would now be 4:9, and since the hydrogen ion concentration is proportional to this ratio, this ion would suffer a five-fold increase of concentration. But at this point, or, more strictly speaking, continuously during the process, the excretory function intervenes. There is a tendency for the respiratory process to hold the tension of carbondioxide in the blood nearly constant. This is the reason why carbonic acid has sometimes been thought the respiratory hormone. Assuming that the exact quantity of carbonic acid set free by the reaction of neutralization were thus eliminated, the ratio would be reduced to 1:9, and the hydrogen ion concentration would rise but one-third above its original value. More recent investigations, however, have shown that a tendency to acidity is accompanied by a lowering of the tension of carbon dioxide. Let us suppose that

in this case the tension was lowered one-fourth. The free carbonic acid of the blood would then become 0.75 instead of 1.00, and the ratio of acid to salt 0.75 : 9, which is exactly equal to 1 : 12, the original ratio. Accordingly, the hydrogen ion concentration would be restored exactly to its original value, and the regulation by excretion would be quite perfect. Now there is abundant evidence to show that something very much like this is always occurring in the body, and, on the whole, I believe that the most delicate of all means to regulate the reaction of the body is to be found in this variation of the tension of carbonic acid during its excretion. Such considerations have strengthened the hypothesis that the hydrogen ion is the true respiratory hormone. But I think that this view marks the opening rather than the closing of a chapter in physiology, for there are undoubtedly many facts which are hard to reconcile with it. Meanwhile it is to be observed that this interesting and unique regulatory process involves a disturbance of the chemical constitution of the organism, a depletion of its carbonic acid. Such a process can never be the final one. It must ultimately depend upon other changes operating to repair those which, as a temporary protection, it has wrought.

The second factor in the excretion of acid is ammonia. In this case, however, the regulation consists in the control of production rather than in excretion itself. This control seems to be located in the tissues. So far as one can see the excretory apparatus is not concerned with ammonia except, as completely as possible, to eliminate it. In short, ammonia is, strictly speaking, a regulatory factor in endogenous restitution rather than in excretion. So far as excretion is concerned, we have only to note that the urinary ammonia has been substituted for a chemically equivalent amount of fixed alkali, which is retained in the body. Here again there is, at least in all ordinary circumstances, no possibility of a variation in the permanent effect on the composition of the body produced by the elimination of a definite amount of the substance. So far as ammonia is concerned the task of the kidney is excretory and not regulatory.

Finally, there is the factor of urinary acidity. Here, at

length, we come upon the mechanism by which the physico-chemical equilibrium of neutrality is permanently maintained in its normal condition; the function which, in the long run, preserves life against the invasion of acid. There is no difficulty in understanding this process. All the principles involved have been developed above, and so far as chemical substances are concerned, it depends almost alone upon phosphoric acid. In blood the ratio of acid salts of phosphoric acid (MH_2PO_4) to alkaline salts or phosphoric acid (M_2HPO_4) is approximately 1:5, and of course almost constant. In urine, as the hydrogen ion concentration varies, this ratio varies. In other words, by varying the hydrogen ion concentration of the urine the body causes to vary the amount of alkali carried out of the body by a definite amount of phosphoric acid. In extreme cases this may amount to less than one molecule of base, or to more than 1.9 molecules of base, for every molecule of phosphoric acid. To effect such a process with maximum variation in the ratio of basic to acid substances and minimum variation in the hydrogen ion concentration, phosphoric acid is the best possible compound.

There is no more difficulty in measuring than in explaining the nature of this process. Let alkali be poured into the urine until the reaction of blood is attained. Then the quantity of alkali added represents the amount of alkali retained by the kidney when the urine was secreted. This quantity may be termed, in the strictest sense, the acid excretion. The magnitude of this quantity varies with three factors: first, the hydrogen ion concentration of the urine, which fixes the ratio of monosodium phosphate to disodium phosphate; secondly, the concentration of total phosphoric acid; and finally the volume. It must not be overlooked that dilution has almost no effect upon the hydrogen ion concentration of such balanced solutions. Thus, a high concentration of the hydrogen ion, indicating a high ratio of acid to alkaline phosphate, shows that the kidney has been working to save as much alkali as possible from the phosphoric acid which it had in hand. If a large quantity of phos-

phoric acid were being excreted, the same result would be accomplished by producing a much less acid solution.

There has never been an attempt to discover what may be the hormone of this process. But I think that in this case, at least, the established facts point very clearly to the hydrogen or hydroxyl ion as the active agent. Even in this case, however, the problem is not free from complications.

Such, abstractly considered, are the various features of this regulatory process. It may now be said, I think, that there are but two really significant variable factors which reverse the tendency of the organism to become acid. These are the varying production of ammonia and the varying excretion of acid in the urine. In order to characterize these two processes, which are additive in their effects, only three analyses are necessary. The urinary ammonia must be estimated, the hydrogen ion concentration must be determined, and the urine must be titrated back to the reaction of blood.

Of course it is clear that these processes vary with and because of variation in the basic and acid constituents of the food, and because of disturbances in the normal processes of metabolism. Especially the production of β-oxybutyric acid affects them. They are also dependent upon a host of other things, including disease of the kidney, as well as many other pathological conditions, blood-pressure, and the amount of diuresis, and they may perhaps also be influenced by the excretory activity of the intestine. These considerations at once raise the difficulty, characteristic of all biological research, that the problem cannot, strictly speaking, be studied independently. And yet somehow it has to be studied. In attacking it Palmer and I have believed that one needs to know the characteristic average values and ranges in health and in disease of the several factors of the excretion of acid, and of the relations that may exist between them. In order to obtain a sufficient number of observations to make possible a statistical analysis, we have not assumed the impossible task of controlling the many factors —diet is but one of them—which are involved. But, while making special tests and exercising great care in order to

eliminate gross errors, we have left it to the laws of probability to overcome the effects of chance. In a sense, therefore, our studies may be regarded as a test of the value of statistical methods in this field. I think that we may claim a certain measure of success for this enterprise.

In health the hydrogen ion concentration of the urine is very variable, ranging from about 0.000016 N to about 0.000000035 N, with a geometrical mean of almost exactly 0.000001 N. In disease the acidity may very rarely surpass the highest observed normal value, but nearly always falls within the normal range; the mean, however, is commonly high. In cardiorenal disease the mean acidity is very high, approximately 0.000005 N, or five times the normal value.

These facts suggest the not unfamiliar idea that acidosis may be a frequent accompaniment of nephritis. They have led us to a study of the effect produced by administering sodium bicarbonate to diminish the acidity of the urine. Unknown to us, Sellards had already devoted some attention to this subject. His results and our own prove that five grams or less of soda, administered by the mouth, always produce a prompt and large diminution in the hydrogen ion concentration of the urine of a normal individual. In pathological cases, however, it is often necessary to administer large quantities of alkali, sometimes more than 100 grammes, before the effect is produced. In all such cases, so far as our observations extend, the retention is at least not chiefly due to the kidney. For, upon stopping the treatment, the acidity of the urine soon rises, but then a single dose of alkali will at once produce its normal effect. I think there can be no doubt that this phenomenon depends partly, at least, upon a diminished amount of bicarbonate in the blood—in short on the principal chemical characteristic of acidosis. In this manner our belief that moderate degrees of acidosis are very common incidents in many chronic diseases, especially in certain forms of nephritis, has been confirmed. Like water, sodium bicarbonate is retained in the body when the normal store of it has been depleted, otherwise it is promptly excreted; and this is a task that almost any kidney can carry

out. This conclusion is supported by a large number of facts with which we are not now directly concerned, and there can be no doubt that the cautious administration of soda should often be practised in all sorts of diseases.

In normal individuals the urinary ammonia is, on the average, equivalent to about 370 c.c. of tenth-normal alkali daily, the acid excretion to about 278 c.c. On the whole these two quantities seem to vary together, and the correlation between them is unquestionably significant. Nevertheless, their fluctuations in particular instances are far from proportional. Thus, the normal ratio between acid excretion and ammonia, a valuable index, as we shall see, is about 0.75, but it may range as high as 1.6 and as low as 0.3.

In like manner the hydrogen ion concentration and acid excretion are highly correlated. Nevertheless, in normal individuals there is very frequently little or no correlation between the hydrogen ion concentration and ammonia, a fact which can only be explained by the independent control of the two factors. However, as Blatherwick has shown, when there is considerable pressure upon the mechanism to remove either acid or alkali, correlation is very conspicuous, as I have no doubt that it may be in many other circumstances. For the two factors, though independently controlled, are liable to the same influences. It is perhaps not superfluous to point out that, statistically, two variables are often both highly dependent upon a third, yet nearly independent of each other.

Having regard to the great variability of physiological activities in general, and the many complicating influences which, in particular, have been revealed in the analogous regulation of body temperature, it is probably too much to regard these results as providing more than a very rough description of the process in the normal individual. They have, nevertheless, seemed to us not inadequate as a basis for pathological comparisons, and, taking them as such, we have extended our observations to the same factors in nephritis. These now include 44 cases and involve analyses of the regulatory process for 311 days. They are to be compared with earlier observations

upon 16 normal individuals, including a total of 122 days. The most conspicuous result of our pathological studies is the discovery that our cases fall into two quite distinct groups. In one of these groups the average values of the ratios of acid excretion to ammonia are altogether abnormal, in fact, never less than 1.6. In the other group of cases the average values are all within the normal range, and never greater than 1.3. The unavoidable impression that this is no mere chance is greatly strengthened by the invariable highness of the daily values of the ratio in all the cases of the first group, whereas, on the other hand, the majority of the daily values of the ratio are always low in the cases of the second group. This separation of our cases of nephritis into two discrete groups, though it need not necessarily depend upon the difference between two quite separate and distinct nephropathies, must have a substantial cause. Accordingly it will be well to consider the characteristics of the two groups of cases. In the cases of the first group the acid excretion is, on the average, a little greater than normal. This, however, indicates a perfectly normal amount of phosphoric acid in the excretion. The ammonia is very greatly diminished and amounts, on the average, to less than half the normal quantity. This diminution, therefore, alone is to be regarded as the cause of interference with the normal condition of the blood, an interference easily demonstrated by the test of feeding soda.

These cases, therefore, reveal the hitherto unknown phenomenon of a condition of acidosis with a constant diminution of urinary ammonia below the normal amount. As a further sign of the gaining acidity of the body, the hydrogen ion concentration of the urine is very high indeed.

It will be convenient to divide the cases in which the ratio of acid excretion to ammonia is not abnormally high into two groups, those in which the ratio is of medium value, and those in which it is low. In the former group there is at once a very high hydrogen ion concentration and an abnormally low total of the elimination of acid through acid excretion plus ammonia. In these cases, however, while the excretion of ammonia is much

nearer its normal value than in the cases first considered, there appears to be a real diminution in the true acid excretion. This depends upon an abnormally small amount of phosphoric acid in the urine.

Those cases in which the value of the ratio is low appear to resemble the median cases, but to be marked by less intense disturbances of function. The acidity is moderately elevated, the acid excretion slightly diminished, the ammonia almost unchanged, in comparison with the normal quantity.

In view of the necessity of very large numbers of observations in order that averages like these may be accepted with complete confidence, I do not feel disposed to regard all these fluctuations as significant. But, at least, of the great and constant diminution of ammonia in the first group of cases there can be no doubt. It is rare indeed to find any pathological factor so constant as this one.

Taking all facts into consideration we feel justified in drawing the conclusion that our cases of nephritis really divide themselves into two groups possessing the following characteristics: (1) Cases in which the volume of urine is abnormally great, its acidity abnormally intense, and the total acid excretion much diminished. These are signs of a condition of acidosis which may be of renal origin. The existence of this condition has been confirmed by independent observations. The diminished total excretion of acid is due exclusively to a very large and very constant deficit in the urinary ammonia. (2) Cases in which the urinary volume appears to be not far from normal, the acidity high, and often very high, the total elimination of acid often low but not infrequently normal, while the variation in this last quantity is again chiefly dependent upon the fluctuation of ammonia, which, however, is never very low for a long time. These cases suggest the idea that they involve varying degrees of a milder condition of acidosis than is to be found among the cases of the first group.

Group 1 appears to consist of a sharply defined class of cases which, functionally at least, are of one type. Group 2

might well consist of either one or more classes of disturbed renal functions, including, perhaps, among others, mild forms of the condition represented by Group 1.

I have called your attention to these results, which, apart from the incidental discoveries that I have specially emphasized, possess very little interest in themselves, only because they show what manner of information concerning excretion of acid may be acquired by studying that process alone, and without regard to the diet or the metabolism. Such results, however, can only acquire their real significance when treated with the help of modern statistical methods. But that is a matter hardly suitable for our present purpose. And yet you can readily see, in any event, that we now possess a quantitative description of what kidneys commonly do in eliminating acid from the body, and thereby closing the account of the acid-base metabolism.

Will you now return with me for a moment to our original question—the fundamental one of regulation? I think it may be truly said that neutrality regulation is, of all forms of organic regulation, that which, quantitatively and in *all* its phases, we now most clearly understand. What then is the hope of reaching a true description of it as a completely mechanistic process, and thereby overwhelming the vitalists on their own chosen field of battle. Very little, I fear, for the present. Because it must be confessed that we have no account of what happens when the cells of the respiratory centre are stimulated by the hydrogen ion or other substance, until we perceive the heightened respiratory activity. And, of course, the same remark applies to the activity of the renal epithelium. This, in a very simple instance, is Haldane's objection to the whole mechanistic standpoint. Nevertheless there is a clue. A change in the acid-base equilibrium of the blood necessarily affects that of the cells, which are nourished by the blood, and it is easy to see how, if the delivery of respiratory stimuli depended upon the velocity of some chemical process in the cells of the respiratory centre, and this, in turn, depended upon the hydrogen ion concentration, the activity might be automatic. And so, though it is

unreasonable to hope soon for a clear conception of even this one protoplasmic activity as a mechanism to be put beside those we know in the inorganic world, yet an achievement of this kind is decidedly not beyond our imagination. This is true just because the equilibrium of neutrality exists in protoplasm, and even there is not unknown to us.

Finally I think you will agree with me that, respiratory centre and renal epithelium apart, the whole intricate process is at once a genuine automatic mechanism and a typical organic regulatory activity. For the very process which creates the need relieves it, and operates meanwhile according to the mathematical laws of physical science.

IMMUNITY IN TUBERCULOSIS WITH SPECIAL REFERENCE TO RACIAL AND CLINICAL MANIFESTATIONS *

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NINE years ago Professor Theobald Smith [1] delivered an address before this body on the subject of tuberculosis with especial reference to the parasitic properties of the tubercle bacillus. He gave a most illuminating view of the bacillus from the biologist's point of view, as well as a working theory of the reciprocal action of the tissues and parasite.

I refer to his hypothesis that the bacillus forms a protective envelope or capsule which permits it to retain vitality in a latent focus. He further explained the mechanism of infection and immunity, and presented the problem for clinical medicine as developed up to that time.

I fear that my selection for a similar task is inadequate; certainly I am deeply conscious of the responsibility and honor in offering the Harvey Society this effort to supplement that of our most distinguished American worker in tuberculosis.

It appeared to me better suited and more useful at the present time to treat the subject of immunity in its clinical aspect; and, while discussing some of the recent laboratory researches, to take also a broad survey of tuberculosis from the racial and epidemiologic viewpoint. We may in this way discover a proper attitude toward the complex problem of prevention and treatment of tuberculosis, a matter fraught with frequent misunderstandings and wide differences of opinion. Only by this combination of experimental and clinical experience have we any claim to judicious decisions. Too often we encounter confusion and perplexity in the face of the frequent contradictions met with in tuberculosis from the clinical side. The deceptions and fallacies of laboratory experiments have

* Delivered January 16, 1915.

also led us astray to the discouragement of many. It must be admitted to be a difficult field, yet not barren of encouraging results to the patient worker.

To begin the theme of immunity, one must at once discard the meaning of absolute protection usually conveyed by it and employ the expression "relative immunity," or, better, "heightened resistance." We know, so far as tuberculosis is concerned, that *in man no absolute immunity has been observed,* that is, no *effective resistance throughout life.* The actuality is quite otherwise; yet many individuals unquestionably attain it in a practical sense, in that no disease follows infection with tubercle bacilli.

From the pathologist's viewpoint few escape the formation of tubercles from the introduction of the bacilli. Even the nearly refractory animals like the goat, cat or dog form primary foci. The immunity is thus not against infection but against its further spread. On the other hand, by the extensive observations of Harbitz,[2] Bartel [3] and many others, we know that living tubercle bacilli may lodge in the lymph-glands for a considerable time without producing infection or tuberculous tissue. Doubtless many are disposed of before they have a chance to multiply. Nevertheless, given the proper conditions for multiplication and a bacillus of sufficient virulence or adaptability, it is perfectly conceivable that a single bacillus may suffice for infection. We must keep in mind the reciprocal relations between host and parasite, so well emphasized by Professor Smith, to understand why an infection may be balanced by the defenses and fail to make headway beyond the primary focus or port of entry.

Some of the conditions favorable to immunity are fairly defined; others elude our search or can only be stated in vague terms. Inoculated bacilli will at least act as irritant foreign bodies, unlike most bacteria, owing to their waxy substance; yet we can be certain that small numbers of bacilli, for example, or those of low virulence for the species, will hardly produce a focus at all, or if such is formed it will soon be encapsulated or absorbed.

On the part of the body, maturity of tissues, freedom from trauma (including such as is produced by inoculation), normal nutrition and the absence of any exhausting malady or other toxic influence constitute factors of safety of first importance.

On the other hand, right here we must remember that whatever balance or adjustment may take place *after* tubercle bacilli have established a lodging place in the tissues, no *specific* natural defenses existed so far as we know when they entered. The bacillus undoubtedly has an advantage here, because, given virgin soil and a race of bacilli already adapted to the species, an initial infection takes place with little hindrance from the non-specific defensive powers. The further history of such infections depends in part at least upon the amount of specific resistance aroused.

NATURAL IMMUNITY

It has always been easier to define disposition to tuberculosis than immunity. Volumes have been written and an enormous literature has accumulated on the subject of disposition, yet something can conceivably be said for natural or acquired immunity in the qualified sense here used.

Certain species are relatively immune to strains of bacilli occasionally pathogenic for them. The horse and mule, for example, are rarely infected by the bovine bacillus that occasionally may produce disease in them under constant exposure and sufficient dosage. Likewise swine and sheep are ordinarily resistant to human infection. It does not seem difficult to explain these instances on the ground of non-adaptability of the parasite to races other than those forming its usual habitat.

The tubercle bacillus, especially the mammalian type, has, however, shown a rather wide-spread parasitic power as compared with other pathogenic organisms. It adapts itself also to many kinds of culture media in the laboratory, and there is practical agreement as to the existence of modifications of the two main divisions, human and bovine. Hence when any individuals of a race can be infected we can hardly speak of a natural immunity to all strains of tubercle bacilli.

Some ingenious explanations of the immunity of certain races and species have been propounded. As an example, a plausible theory for the immunity of a certain caterpillar of the bee moth is given by Metalnikoff,[4] who suggested that the immunity was due to the digestive power for wax with which the insect is endowed. Considerable experimental work is adduced by the author to support this idea of the mechanism of protection.

That cellular ferments of lytic character play a prominent part in natural resistance to bacterial infections is a convenient assumption. Otherwise there are temperature conditions, chemical and physiological differences, that can conceivably interfere with the growth of tubercle bacilli in highly resistant animals. We are obliged to leave the question unanswered by any formula capable of demonstration.

So far as man is concerned, no race has been discovered that exhibits high natural resistance. The true explanation of any observed differences is quite different, as will be referred to later.

Some observations have been made tending to show a natural resistance in the Zulu natives of Natal, South Africa; [5] also of certain French families.[6] Such instances may be dismissed on the ground that opportunities for infection were limited.

INHERITED AND ACQUIRED IMMUNITY

A consideration of inherited and acquired immunity is more important in its practical results.

Some experimental work has been directed to the solution of the problem as to whether a susceptibility or immunity can be transmitted to offspring of tuberculous animals. No conclusion favorable to immunity to my knowledge has so far been made. The universal observation is that during an active stage of the disease a tuberculous mother has borne weak offspring, which, if not as a rule tuberculous, often succumb to marasmus or are maldeveloped. If any form of specific resistance is conveyed from mother to young it is too much masked in these cases to be discovered.[7]

The toxic influences on the fœtus from maternal disease are added to those affecting the germinal cells before fertilization. Consequently, the anti-infectious or antitoxic principles, if present, are overshadowed.

In point of fact, as stated by Adami,[8] an actual specific immunity acquired by inheritance must be proved through the male parent. He says: "It is essential to immunize the male

parent alone, and that through a series of successive generations; and what is to be expected under these conditions is a Mendelian inheritance, certain of the progeny being immune, others not.''

From the maternal side we know that substances derived directly from the bacilli can pass through the placenta in one form or another, together with antibodies produced in the mother. The results are disastrous to the young as in the experiments of Carrière,[9] and Maffuci,[10] who injected healthy guinea pigs with extracts of tubercle bacilli. More than two-thirds of the offspring were either still-born or died in two weeks. Schenk[11] claims to have demonstrated antibody by means of complement fixation in the offspring of normal and tuberculous guinea pigs treated with bacillen emulsion. In my own experiments, in association with Dr. A. K. Krause,[12] the inheritance of anaphylactic sensitiveness was demonstrated by injecting pure tuberculoprotein into young guinea pigs.

These experiments do not, of course, directly relate to the problem of most interest, and that is, whether individuals who *recover* from tuberculosis can transmit any specific resistant quality, humoral or cellular. Several authors have assumed the presence of antibodies in the milk of vaccinated cows, which may fairly be considered as recovered animals while their immunity lasts. Von Behring in 1903 suggested this, and because of the known secretion in the milk of antitoxins for other diseases (tetanus) considered the possibility of a transient passive immunity obtainable in this way. No experiments were published to my knowledge, nor has it been claimed that the calves born of the vaccinated cattle have shown unusual resistance to natural infection.

The only published work that I have found showing either an active or passive resistance in tuberculosis conveyed by milk is that of W. L. Moss[13] and S. H. Gilliland.[14]

The former reported on a few calves fed on milk from vaccinated cows. There was a slightly favorable influence. Gilliland mentions a similar experiment with pigs fed on the milk of immunized cows in 1904–5, also suggestive of some protection. Neither experiment was very satisfactorily controlled.

So far, no evidence of an experimental nature has shown any form of active immunity to be transmissible. It is quite true, too, that a truly *specific* disposition, acquired by inheritance, lacks an experimental basis. Here again the bovine race gives a negative to the assertion that tuberculous infection necessarily involves a transmitted weakness or susceptibility. On the contrary, breeding from tuberculin-reacting cows is actually practised as of eugenic value in preserving the best stocks. The well-known Bang system has been on trial long enough in Denmark to have demonstrated its value, and is, I believe, the approved method of procedure in valuable dairies where tuberculosis is a serious menace.*

The observations on cattle here mentioned will not be conclusive that either resistance or susceptibility are stamped on the calves in one generation, but after the lapse of time the future generations should give an answer. They seem to me of great potential value at least in deciding whether the future generations will continue to be affected with the mild form of tuberculosis now prevalent and only discoverable by the tuberculin test.

Then, too, the non-inheritance of tuberculin sensitiveness as such leaves an opportunity for the discovery of some other hereditable quality, specifically adapted to favor or oppose the bacillus. (I shall again refer to the relation of tuberculin reactivity to immunity in connection with the clinical aspect of the problem.)

To make the assertion, finally, that there is no experimental basis for an inherited immunity or disposition does not mean that it is wholly impracticable to reach results of value, though admittedly difficult and wearisome to carry on such work successfully.

We must turn for the present to the biologist, the hygienist or the statistician, as well as the historian, for further informa-

* A recent contribution by Dr. Harlow Brooks (Amer. Journ. Med. Sci., 1914, cxlviii, 718) testifies that no defects were inherited from a tuberculous cow of unusual milk capacity.

tion. Immediately we find opposing views and many facts adduced to support each position. The matter is complex when inquiry is applied to families and races, and not less so in individuals.

Martius [15] took this view, and considers the statistical method inadequate to decide for or against a specific inherited predisposition or immunity. Hueppe [16] believes that both may be inherited, also certain organs show inherited predilection or the opposite for the tubercle bacillus. The latter, at least, has clinical observations to support it.

Turban [17] showed what he considered an inherited *locus minoris resistentiæ* in the large proportion of pulmonary cases where the morbid process began in the corresponding lung in parents and children. I was able to corroborate this observation in sixty-three families.[18]

I can only mention here some more recent noteworthy works on the subject of heredity in tuberculosis.

Cornet,[19] as is well known, has always scouted any belief in disposition.

Schlüter [20] in a monograph of considerable size and value concludes that there is no well-defined specific disposition.

Boeg's [21] studies on the Faroe Islanders give no support to hereditary disposition.

Grunberg,[22] in a study of 568 families, also found no specific heredity.

On the opposite side stands Karl Pearson,[23] the statistician, who by means of strictly mathematical rules applied to 384 families proves a clearly marked family inheritance of diathesis. It is impliedly a specific inheritance, though this appears an unnecessary assumption to me, considering that physical defects from other causes probably form an equally weak resistance and should be considered in estimating the specific element. Practically I think we must accept his conclusion that tuberculous families carry along more of the factors regarded as predispositions, and comprehended under the vague term "diathesis," than the average non-tuberculous family. Insurance interests must necessarily make their rules on the basis of such methods of study. But for the purpose of this lecture I have only considered evidences of *specific* resistance, inherited or acquired, and therefore we can hardly confine the study to family groups and a few generations.

In passing, however, it may be of interest to note that such an eminent clinician as Herman Weber [24] who believed in the heredity of tuberculosis, accepted insurance risks among the fibrous and so-called gouty tuberculous persons, thus recognizing their staying powers in the wrestle with the bacillus.

Leaving the question of family and individual inheritance for the moment, I will briefly mention the racial question. Reibmayr [25] in an interesting monograph twenty years ago made a fair argument that a gradual process of immunization was taking place in the white race under civilized conditions. His reasoning was that owing to greater care, more weakly children survived to become tuberculous. The disease was consequently more widely spread, but it developed chronicity as a sign of increasing resistance. Uncivilized races by contrast exhibited only acute forms of the disease of short duration. The infection is thus limited in extent. This theory admitted the paradox of a disposition and immunity (only relative, to be sure) in the same individual, and in my opinion has much to support it in clinical observation.

Many reports about tuberculosis in late years establish the fact that uncivilized races and those isolated for centuries from the chance of infection develop acute forms of the disease when exposed. An excellent review of this subject was recently made by Maurice Fishberg,[26] whose well-known studies on the epidemology of tuberculosis in the Jewish race have shown a decreasing mortality for many years in spite of their ample opportunities for infection. No doubt other factors—indeed, almost countless reasons other than a specifically acquired resistance—may tend to minimize the important conclusion that past generations of Jews have passed on an increasing resistance.

Enough evidence remains, I think, to accept the principle involved which Fishberg elucidates in his recent article: namely, that "the fatality of tuberculous infection depends on the length of time the ancestors of the affected people have been acquainted with the disease."

This principle is accepted with reference to other diseases,

such as syphilis, malaria and yellow fever, and is a rational inference from the known facts about tuberculosis.

There are, however, such marked exceptions to such a generalization that one must consider large groups rather than individuals. There are also other explanations of the relative mildness of epidemic diseases (as mentioned by Professor Theobald Smith) due to the disappearance or weeding-out process of the weaker individuals and races, both of host and bacillus.

I am more impressed, however, with the variability in resistance shown by man than by that of the bacillus. We may leave the subject of racial immunity, in my judgment, with a feeling of hopefulness that some progress has been made in the history of mankind, however slow, toward a self-protection. In this epoch, at least, the bacillus has not appeared to keep pace as an increasingly dangerous parasite.

ARTIFICIAL IMMUNITY

If the question of inherited predisposition or immunity is so difficult of answer, we yet have evidences of an artificially acquired immunity in the life of the individual. An endless amount of work has been done in laboratories and clinics to demonstrate this. A review of even the most important work would not be appropriate here;** it is enough to declare that up to now no satisfactory method of active or passive protection has been discovered which applies to man. Nevertheless I should like to discuss some of the recent contributions to the subject, and especially the reasons for failure as well as partial success. Finally, the application of the knowledge is of value in interpreting clinical tuberculosis.

Since the disappointment with the vaccination of bovines and serotherapy for tuberculosis, the conviction was borne in upon us that only transient protection could be attained, and this chiefly by a vaccine in which the vital spark was not quenched.

**A most satisfactory review may be found in the Kolle-Wassermann Handbuch, 1913, 2d Auf., Bd. v.

Since the time human bacilli were ruled out for use in vaccinating cattle, owing to the short duration of immunity, and their ability to get into the milk, other races of acid-fast bacilli have been put forward for protective inoculation. The feeble resistance obtained in this way merely confirmed previous experiments with them.[27] [28] [29]

McFadyean,[30] of Edinburgh, has more recently advocated avian bacilli for cattle vaccination because non-infectious for human kind, a doubtful assumption considering the occasional presence of such bacilli in tuberculous patients.

Webb[31] even ventured living human bacilli, trusting to small numbers gradually increased in children to avoid infection. This was found impracticable.

The results also with bacilli killed with very gentle heat[32] or devitalized by soap,[33] [34] glycerin,[35] sugar,[35] etc., have been but slightly encouraging thus far.

Vaccines made from the different elements of tubercle bacilli (von Ruck)[36] or from solutions of their substances (Deycke and Much)[37] have also failed to show any advantages over previous tuberculins used for immunization.

The significance of a living vaccine as compared with all substitutes has at present a close parallel in the now much-discussed *immunity from infection.*

The opinion is justified that mild infections are responsible for the considerable immunity hitherto observed in animals inoculated with weak-virulent living bacilli. These mild infections are closely comparable to those spontaneous ones from which the large proportion of our urban population recover. The results of bovine vaccination further justify the opinion that such protection as is conferred depends on the presence of living bacilli or tuberculous tissue in the animal. This protection gradually wanes or disappears with the absorption of the bacilli or the tuberculous tissue.

The above statement being granted as based on experimental grounds, we may first observe that a spontaneous vaccination of human beings is in progress owing to wide-spread opportunities for infection. Many slight infections must be effective if we will interpret the healed tubercles in that light when found

at autopsy. Unfortunately, neither the virulence nor the dose is controlled; hence progressive infections will occur in a considerable proportion of people. If both factors were under control and repetitions of mild infections obtainable at proper intervals, it is imaginable that much good would result. Since we cannot assure a stability of virulence for inoculated bacilli, such a dream is far from realization. But we may again inquire, why is the living bacillus necessary? Cannot the dead bacillus persist a long time in the body and produce tubercles (as we know to be true)? If tuberculous tissue is needed why not use dead bacilli?

The answer is difficult, but it is not likely that the dead bacilli persist long (except when shut off in the form of encapsulated nodules where large clumps may lodge). The tissue changes are also probably less pronounced and certainly more transient. I will also venture the theory that ferments in the living bacilli play a part. Until recently the presence of any function of ferment nature in the tubercle bacillus failed of demonstration. Through the studies of Wells and Corper [38] and those very recently published of Kendall [39] and his associates, the presence of a lipase or fat-splitting ferment in the bacillus appears certain. Reciprocal and antagonistic ferment activity on the part of the bacillus and tissues is doubtless a better means of producing an immune reaction than when the ferment action is one-sided.

The studies of Opie [40] in this country, and later those of Manwaring,[41] support the belief that cell ferments combat the tubercle bacilli. Furthermore the antibodies, such as specific lysins, are not readily demonstrable in the blood, yet their presence both in the leucocytes and fixed cells cannot be questioned. During a short period following inoculation the blood may contain some specific lysin, though it has not been fully recognized *in vitro*.

Roemer [42] was able to protect two sheep with the serum of another inoculated sheep; later this transferred immunity failed, probably because no serum lysin was left in the vaccinated sheep (some months after inoculation).

The "partial" serum antibody theories of Much[43] and others, while plausible in theory, seem to lack well-controlled experimental demonstration. In truth no serum antibody † in tuberculosis has been definitely associated with immunity; in all experiments the cellular influences appear predominant.

A most interesting confirmation of this view was obtained by Manwaring and Bronfenbrenner,[44] who demonstrated lytic properties in the fixed peritoneal cells of animals. On the other hand, with the serum of vaccinated horses, Ruppel[45] obtained evidence of protective substance of antitoxic nature.

HYPERSENSITIVENESS OR ALLERGY

There is another phenomenon intimately associated with immunity from tuberculous infection with which much speculation and many experiments have been made. This is the hypersensitiveness to tuberculin and especially to reinoculated tubercle bacilli, to which the term "allergy" was given by von Pirquet. It appears to be an important protective mechanism and probably a specific ferment action called forth from the leucocytes and fixed tissue cells, by the presence of the invading bacillus or its derived substance.

The similarity and differences between the tuberculin reaction and the tissue reactions following inoculation are being much discussed at the present time; also the analogy to the anaphylactic reactions.

Reviewing the work on the artificial immunization of animals and the attempts to transfer anaphylaxis in tuberculosis, I think we can conclude that the mechanism is the same for all these phenomena. The differences in the manifestation of the reactions are chiefly explained by the presence of focal deposits of tubercle bacilli which can be regarded for comparison in the light of solid particles of foreign protein peculiarly difficult for digestion by the cellular ferments.

† The "anticutines" of Loewenstein and Rappoport (Deutsche med. Woch., 1904, xxx, 835) and the hypothetical antitoxins of Sahli (Tuberculin Treatment, 1912) are too uncertain for demonstration if indeed such exist.

It is difficult to understand the cutaneous reactions, and most authors do not consider them anaphylactic manifestations. Unlike the majority of the latter, the skin sensitiveness as well as that of other tissues in a tuberculous animal is dependent upon the presence of tuberculous tissue or tubercle bacilli. Remove the bacilli or the tubercles and one finds that this form of sensitiveness disappears, and with it apparently all specific immunity to the disease is soon lost. The same thing happens when tubercles become thoroughly fibrous or calcified. In the changed reactivity, or the allergic inflammation, therefore, we have the essential features of the relative immunity of whatever grade, acquired in tuberculosis.

It is plain from these facts that a special form of sensitization is produced in tuberculosis by the cellular activities about the bacilli, which radiates gradually to distant tissues not necessarily touched by the bacilli as such. Dead bacilli will act temporarily in this way, but pulverized preparations and extracts appear too fleeting in their effects to arouse it. They do nevertheless cause a true anaphylactic sensitiveness without skin manifestations, but unaccompanied by any recognizable immunity. The inflammatory reaction following inoculations of immune animals is the prominent feature, and is conspicuously absent in the others. Hence, we are justly entitled to the opinion that the cell accumulations about the bacilli are performing the duty of dissolving them or restricting their growth. The lymphocytes and endothelial cells have thus received much attention in connection with the tubercle bacillus, inasmuch as they show lipolytic activity. The former are especially the reacting cells about the actively spreading foci of disease. Bartel [46] was one of the first to recognize the import of the lymphocyte reaction, and many facts have since tended to confirm it. (Opie,[47] Manwaring,[48] Kling,[49] Bergel,[50] Webb.[51])

No less important are the endothelial cells in connection with the fixation and absorption of the bacilli, as histological studies show, by vital staining methods.

Some recent contributions are noteworthy. Lewis [52] found by the removal of the spleen in white mice that a considerable

increase of resistance was created to bovine bacilli. A rapid increase of lymphocytes following the removal of the spleen, due to hypertrophy of lymphoid tissue, is a possible explanation. On the other hand, Murphy and Ellis [53] of this city have exposed white mice to Röntgen radiation to destroy their lymphoid tissue and cells with the result that a tuberculous septicæmia could be produced as compared with focal disease in control animals.

Much more may be said in relation to cell reactions and hypersensitiveness as a measure of resistance as well as the participation of humoral antibodies or ferments in the reactions.

These may be mentioned in connection with clinical observations.

CLINICAL EVIDENCES OF IMMUNITY

I will now ask your attention to a somewhat modern, possibly to some a novel, interpretation of tuberculosis in the symptomatology of the patient.

If our knowledge by experimental methods and observations is to be of value, we should expect to find signs of specific resistance under natural conditions of infection. This term "immunity" is seemingly too strong when used in describing such signs, but when we can discern that life is possibly conserved, and surely prolonged, by the defensive functions, it may not be amiss to employ that word.

Tuberculosis from the standpoint of the immunologist is possibly the best illustration of auto-immunity to an infectious agent. From this position we may divide tuberculous infections into four classes: (1) those which recover without spreading or metastasis of the bacilli; (2) those which continue to spread acutely—unresisted; (3) those which progress in a slow chronic form; (4) those which intermittently progress, becoming arrested in the intervals. Each form may pass into another in all possible variations. The types of each are recognized by symptoms except the first. This may only reveal itself by the various tuberculin tests or at post mortem.

It is most important from the point of view above taken,

i.e., from that of immunology, that the white race, at least, has for many centuries found a way to recover from repeated infections. We know that the chance of recovery increases from the second year of life to puberty in childhood infection,‡ depending on numerous factors such as frequency of exposure, amount and virulence of the bacilli, intercurrent diseases, trauma, etc.

We also know that the majority of first infections involve the deeper lymphatic glands, not being resisted at the path of entrance. The course of events from this time forth is of exceeding interest from every standpoint.

We can then trace the path of infection thus far with fair certainty whatever the path of entry. But owing to the allergic state now induced, and which is known to be almost universal in ages over fifteen (in greater or less degree with exposed families), we find it difficult to associate future disease with a new, exogenous infection. This is a problem about which discussion continues, and which is an important hygienic question.

Exposure to danger of massive infection without disease development in some instances can be ascribed to auto-immunity from earlier slight infections. In other instances it appears to be the cause of later pulmonary tuberculosis of the open ulcerated form occasioned by surface infection.

Both experimental and statistical studies have been brought to bear on this problem of re-infection, the first notably by Hamburger [54] and Roemer.[55] Pathological studies have seemed to give rather uncertain results with reference to the question of new exogenous infections. Recently, however, a few cases have been found where the bovine type of bacillus has been isolated from one part of the body, usually a healed gland, and the human type from a fresh lung focus (Rabinowitsch).[56]

‡ Hamburger, F. (Wien. med. Woch., No. 15, 1914), considers that all infections in infants under one year develop into progressive diseases and 50 per cent. of those in the second year. In ages over four infection is rarely followed by progressive disease. Only 2 per cent. are found between eleven and fourteen with an active form of tuberculosis.

Orth,[57] therefore, considers that such early bovine infections predispose to later human infections, yet the link is difficult to fit between them. Naturally, the question of changed type or virulence also comes into this problem.

Since old foci can break down and cause *autogenous* re-infection, there is reason to doubt the possibility of new *exogenous* infection in most cases. The acquired resistance to such a subsequent infection in adult life may be feeble after the complete healing of the old lesions, which are usually confined to the lymph-glands at the lung hilus or the near vicinity. While in these individuals the specific changes in reaction to tubercle bacilli seem to have been lost, when measured by a tuberculin test, we know the old allergy is latent, because in animal experiments reinoculations will arouse it even though no tuberculin reaction can be obtained. This is experimentally shown by intravenous inoculations, a rather extreme method, though we have a human parallel to it in the outbreak of an old focus suddenly rupturing into the blood stream, which is not uncommon in clinical experience. It is generally regarded inevitable that progressive miliary tuberculosis must follow, and yet it is not necessarily true.

No doubt many of these cases occur, but the small number of bacilli in the blood stream, and their low virulence, may serve to confine the disease to the upper lobes of the lungs, where complete encapsulation may soon follow the acute febrile reaction. This is not usually regarded as a bacillæmia, but we may readily consider it as such in the onset.

The commonly observed exacerbations or relapses in the course of tuberculosis follow the well-known "immune period" of two weeks in so many cases, that we may readily compare them to animals who overcome intravenous inoculations after a protective vaccination. In these it has been found that clumps of agglutinated bacilli are likely to lodge in some other organs, normally resistant to the germ (see Roemer).[58] The parallel may be carried yet further, for, in the case of man, some bacilli are apt to survive and form new sources of danger in other

organs, later emerging as open ulcerations in a soil of less resistance, that is, the lung.

Experience with cattle, sheep and goats has demonstrated this in various ways, yet in cattle, adult pulmonary tuberculosis is regarded as a new infection from without, obtained in adult life for the most part.[59]

The grafting of new exogenous bacilli in the human lung in adult life nevertheless requires especially lowered health or enormous doses of bacilli. Specific resistance may run down to zero and yet the adult tissues seem naturally prepared for ordinary exposure to infection. Therefore, it is difficult to ascribe the immunity to any allergic state. This seems especially true after the lapse of tuberculin allergy due to complete healing of former foci.

Some inquiries have been made to determine the frequency of adult infection from new sources. In Breslau an investigation of sources of infection by Bruck and Steinberg [60] led to the opinion that both autogenous and exogenous infections caused the adult disease. Hillenberg,[61] in a thousand tuberculous cases in the city of Leitz agreed with the above named authors. He thinks it safe to say that those patients who develop the disease after the age of forty-five are due to later infection than childhood, unless the disease was in the family. The relative infrequency of the disease in married partners he suggests as being due to a continuous process of immunization due to increasing exposure. The proportion of new infections among sanatorium and hospital staffs is so small that we may use the same explanation to account for it.

The facts anyway appear to show that frequent contact with the bacillus can occur without harm in adult life, and this is a comforting thought. This idea may be new to many who have become phthisiophobic, but a little reflection must arouse curiosity as to the vital resisting powers of many persons in constant contact with tuberculous patients!

As already noted, the absence of resistance in non-infected races ("virgin soil") is of some significance. Nor is it confined to uncivilized peoples, as many instances of acute tuber-

culosis are seen among healthy adults, particularly from rural districts.

I am not aware that the virulence of the bacillus in these cases is unusual—except for the individual involved. This is shown by the many instances of family infection of all grades of severity. In these, the age and amount of infection at the time of primary infection are of great importance.

Whatever the immediate exciting cause of metastatic or fresh re-infection, the result is a higher degree of allergy, unless miliary or acute pneumonic disease supervenes. We may note the symptoms of such resistance by the fever, stronger cutaneous reactions and hyperæmia about the foci, as, *e.g.*, in the larynx. That the disease now continues to progress is not necessarily because of failure of the immunizing mechanism to act. There may be mechanical reasons, such as trauma and over-exercise, that cause the bacilli to spread, bringing new foci to the lungs, or elsewhere into the system of tuberculous tissue, thereby heightening the allergy until it reaches a maximum point. From this time a paradoxical state may prevail. The hypersensitiveness now causes a chronic poisoning by its constant activity, or rapid exhaustion occurs from the toxæmia. On the other hand, we have reason to believe that but for this protective function miliary tuberculosis might occur oftener in a fatal form.

In recent years we have learned that tubercle bacilli wash into the blood stream more frequently than was formerly considered possible, unless an acute miliary disease was present. It is highly probable that many such bacilli are weakened and dissolved by lysis without producing lesions—causing only anaphylactic fever. They may even aid in immunization if intermittently received, so that the dose at any given time can be overcome readily. For example, it is alleged that a tuberculin reaction can produce a bacillæmia—which seems to me quite probable to a small extent—yet such a violent reaction as would lead assuredly to loosening bacilli from their moorings has been known to promote a rapid healing. It is not unknown to have other causes lead to strong reactions followed by improvement in the course of a progressive tuberculosis. Such

stimulations to the immune reactions are obviously dangerous and unsafe when repeated often, and no experience is so disastrous as an "autoinoculation" pushed too far; whether by natural means or by tuberculin treatment, artificially applied. It is significant, however, that physical and nervous exhaustion are the most dangerous sources of autoinoculation.

Moreover, we are familiar with the healing of sluggish ulcers as the result of irritants directly applied, and even intercurrent disease causing the absorption and disappearance of tuberculous foci in the skin, glands, and occasionally elsewhere. The method of Bier, by producing alternate passive and active hyperæmia, has much value and a correct rationale in the light of the immunology of tuberculosis.

Stimulation of foci in the lungs, however, requires clinical experience and judgment, for a highly vascular moving organ with, as we have seen, a possible selective affinity for cultivating the tubercle bacillus, is to be treated cautiously.

There is often a delicate adjustment or balance in tuberculous disease after it has become arrested. Regarded as a problem in maintaining immunity, the tuberculous individual must be taught to patiently await the time when cicatrization can change the highly sensitive foci to fibrous tissue, before danger of relapse is lessened for those who retain the two-edged sword of hypersensitiveness in an active form.

Here we may refer to the very strong contrast between a recently active tuberculosis and one of chronic, fibroid type. In the first there is need of strong lytic action to prevent dissemination of the bacilli. In the fibrous form the bacilli may be abundant in the sputum but so shut off from the blood that little allergy is present. These latter patients are true "carriers" of tuberculosis, but have no longer any symptoms of absorption from the focus, and consequently low capacity for reaction. They recover very slowly, if at all, but bear stimulation of all kinds more safely. I mention these contrasts for their suggestiveness in treatment.

Interesting examples of the healing of a tuberculous focus in one part of the body while an acute outbreak is in progress

elsewhere can be mentioned as auto-immunity phenomena. This is occasionally observed in the larynx during a pulmonary exacerbation, and *vice versa.* The influence of pleural effusion in arresting intrapulmonary disease of the opposite lung is another illustration. The laryngeal swellings often diminish without reactive indications. Likewise pulmonary ulceration may heal in the course of pleurisy, apparently with no increased inflammation. Immune bodies circulating in the blood best account for these cases, but I cannot offer a satisfactory explanation.§ The use of autoserotherapy from pleural effusions has been considered rational, on the presumption that immune substances are present, but with what experimental proof I am uninformed.

Many other manifestations of auto-immunity could be cited, including all forms of surface ulceration in advanced tuberculosis, also the various cutaneous affections. These can only be grouped as consequences of the life-protecting functions but not happy in their effects.

SUMMARY

It remains to emphasize some of the points from which I may draw conclusions:

1. There is no natural immunity to tuberculosis in warm-blooded animals to the types of bacilli found in the bovine or human race.

2. There is a considerable variation in resistance in some species, probably due to the chemical effect of their secretions or physiological differences in the animals, but chiefly to the fact that the bacillus has not become adapted to long-continued parasitic existence in them.

3. In the human species no natural immunity is found in

§ A few cases of completely arrested advanced tuberculosis following artificial pneumothorax with marked purulent pleurisy have suggested anew to me the importance of the cellular reactions in tuberculosis. The pus which contained tubercle bacilli alone was repeatedly withdrawn, and striking improvement in the intrapulmonary disease accompanied the effusion after it became full of leucocytes.

any race. All uncivilized races long removed from infection are very susceptible, but the white races, especially the European Jews, have acquired a certain degree of immunity by inheritance and almost universal infection. The rapid increase of intercourse between all lands and races facilitates the universal spread of tuberculosis, which is certain to occur through the medium of numerous bacillus "carriers."

4. The ultimate survival of those who acquire a relative immunity will tend to diminish the severity of the disease, but many generations may be required to accomplish this.

5. The opportunities for infection, now universal in cities, will diminish gradually in civilized lands by lessening the danger from advanced cases, also from bovine sources. For many years, however, the number of "carriers" will increase owing to improved care, longer life and higher standards of living among the people.

6. The best degree of resistance against tuberculosis that has been attained by experiments on the lower animals involves inoculation of living bacilli. This is of little value because of short duration of the protection and the danger of sequestered bacilli.

7. The natural infection of human beings takes place largely in childhood and increases the resistance to subsequent disease in a large measure. Under improved care of the tuberculous and better hygiene the amount and frequency of severe infections should diminish, while the number of those with slight, relatively harmless infections should relatively increase.

8. Adults withstand exogenous re-infection under extreme exposure, partly on account of slight infections in earlier life and favorable occupations, environment and nutrition.

9. The specific immunity acquired from natural infection is largely due to cellular reactions of bacteriolytic nature, which take place outside the blood stream for the most part.

10. The interaction between the ferments of the body cells and those of the bacillus lead to heightened activity of the lytic power, both lipolysis and proteolysis.

11. The tuberculin sensitiveness or "allergy" is the chief

indication of specific resistance. In the patient most of the inflammatory symptoms are due to the actively-working immunity functions.

12. In the therapy of tuberculosis this principle should be applied: Avoid interference with Nature's powers of resistance when she is attempting to localize the infection with apparent success.

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THE MORE RECENT DEVELOPMENTS IN THE STUDY OF ANAPHYLACTIC PHENOMENA*

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I

IT is a fundamental biological truth that the systematic treatment of an animal with a foreign protein, if this is administered by any route other than that of the alimentary canal, induces profound physiological changes. These changes are primarily recognizable by the appearance in the circulating blood of substances which superficially react with the injected protein. For convenience of discussion we speak of these reaction products as antibodies, and of the injected substances, which possess this power of inducing their formation, as antigens.

Antigens, then, are all substances which, injected into the animal body, induce specific antibody formation. They form a large group in nature and are chemically proteins; indeed, we may say that all known proteins may act as antigens. Whether or not this term may also include lipoid-protein combinations, lipoids or the higher protein derivatives is as yet uncertain and need not in the present connection concern us.

We may divide antigenic substances into two main classes. One of these comprises all of those substances of bacterial, animal or vegetable origin which, injected into the animal body, give rise to specific *neutralizing* or *antitoxic* properties in the blood of the injected animal. These are the bacterial exotoxins, the snake venoms, some powerful vegetable poisons and proteolytic and other enzymes of animals and plants. They are all substances which are powerfully active—some of them strongly toxic to the living animal, others true enzymes or ferments. Indeed, all of them possess properties which at least suggest our placing them into the class of enzymes in general. The number

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of such substances known is limited. The reaction they call forth in the animal body seems aimed directly at the specific neutralization of their respective activities, and is so unique and different from that induced by other antigens that it would be convenient had we another term like "antitoxinogen" to set them apart by themselves.

The other class of antigens comprises all proteins which are inactive, showing in themselves neither toxic nor enzyme-like properties. Introduced into the animal body parenterally, they call forth a response of a nature entirely unlike that of the antitoxins, and which as far as we can fathom its purpose seems aimed merely at the assimilation or the removal of the injected substance. For the cells of the animal cannot utilize the foreign protein as such, and thus it is only foreign proteins injected into an animal that act antigenically and no antibodies are formed when homologous material is injected.

This large group composed of all formed and unformed substances in nature in which a protein structure is involved, does not induce the formation of anything like the neutralizing antitoxins spoken of above. The antibodies appearing in animals treated with such substances have been spoken of as cytolysins or cytotoxins—precipitins—and in the case of formed antigens like bacteria or blood-cells—agglutinins and opsonins. It is our opinion that all these various antibodies are identical in structure and significance. The probable identity of agglutinins and precipitins was suggested long ago by Paltauf, and the identity of precipitins with the antibodies which sensitize foreign proteins to the action of alexin or complement has been rendered more probable, we believe, by our own experiments. The terms agglutinin—lysin—precipitin and opsonin are all descriptive of effects produced when an antigen meets its specific antibody. These effects will differ according to the physical condition of the antigen. We believe that it is most likely, both from a study of the work of others and our own experiments aimed at this point directly, that the visible agglutination or precipitation are secondary effects incidental to the colloidal nature of the reacting bodies and to the quantitative proportions

in which the reactions occur, the essential process being the union of antigen and antibody, by which the former is rendered amenable to the action of complement (alexin) or leucocyte, as the case may be. It is not necessary, at any rate, to assume that functionally there is more than one variety of antibody, this one being the specific sensitizer. However this may be, the definite fact remains that the injection of antigens of this second class into animals induces specific reaction bodies or antibodies in the plasma of the treated animal which can be shown to unite with the homologous antigen in vitro, and which probably do so in the body of the animal when the antigen is reinjected into a subject in which antibody formation has taken place.

We must not forget, however, that the observation of antibodies in the circulating blood is but one of the changes that have taken place in the treated animal. Much has been made of this phase of the problem because serum antibodies are readily studied in vitro; but their origin of course should be sought in the body cell, in which the original and most profound changes must necessarily have taken place during such treatment, changes the nature of which are to a large extent still a mystery, but on which ultimately depend the important physiological differences between treated and untreated animals. For such changes—whether we refer to those immediately under discussion, namely, those of allergy or anaphylaxis, or whether we think of the so-called immunity remaining after attacks of many diseases—remain present long after the circulating antibodies have disappeared and must therefore be regarded as associated with profound alterations in the ultimate tissue unit, the body cell.

Pasteur's observation that animals systematically treated with sublethal doses of bacteria became specifically more resistant to subsequent infection, carries in it all the principles of the process of which we speak as "active immunization," and all the modifications and adaptations to special cases which we now recognize are based on this simple truth. The successful transference of such increased powers of resistance to normal animals with the serum of the immunized individual, by Behring

and his collaborators, gave us "passive immunization," and these two discoveries are the pillars on which all our complicated subsequent development of details has rested. Since with bacteria and their poisons the process implied the protection of the body against disease or death, we have, rather unfortunately, come to speak of these procedures as "immunization," although the reactions of the animal body to injections of bacteria, reactions on which incidentally the protection depends, are in principle identical with similar reactions resulting from the injection of entirely innocuous substances, such as egg albumin, blood-serum or blood-corpuscles. It is, therefore, misleading when we speak of the immunization of an animal to, for instance, sheep cells or horse serum. A physiological change takes place in such animals entirely analogous with that which occurs in those receiving bacterial protein, but the substances injected are in the former entirely harmless; and indeed, as we shall see, the animal, while entirely immune to large quantities at the first injection, may be severely injured or even killed by subsequent administrations of the same substance. Thus the animal most "immune" to horse serum is the one that has never received an injection of horse serum. It is necessary, therefore, to emancipate ourselves from the misleading elements in the habitual terminology so that we may avoid confusion in grasping underlying principles.

The essential feature common to all antigen injections, therefore, is that of specific antibody formation. That their production in the case of living or dead bacteria—harmful in themselves—protects the animal from invasion and prevents development and multiplication of the organisms once admitted, though of the greatest practical importance, is purely incidental.

It is not impossible that the physiological reaction, indicated among other things by the circulating antibodies, denotes a mechanism aimed at the more effective assimilation and elimination of the body-foreign antigens that have been injected, and this, in the case of the bacterial cell, which of course represents a foreign protein, has the effect of protection against invasion. This point of view of the significance of antibodies is the so-called

theory of "parenteral digestion" of which we will have more to say directly. We must remember at any rate that in all cases in which, clinically or experimentally, we are confronted with the presence of foreign antigens in the blood and tissues, we are dealing with abnormal conditions in which the mechanism available under normal circumstances for the disposal of foreign proteins, which may gain entrance accidentally in extremely minute quantities, is under a strain and abnormally active. The extreme quantitative increase of the antibodies is alone sufficient testimony for this, and under the special conditions which we are about to discuss the repeated introduction of such antigens into the body of an animal in which specific reaction bodies have been induced, whether these are freely circulating or still parts of the cells which produced them, may have illness or even death as a consequence. This is anaphylaxis.

To approach this subject logically without allowing secondary factors to divert our attention from the fundamental principles involved, we should not limit this term to any arbitrarily stated train of symptoms, nor should we attempt too rigidly to limit the definition of what we call anaphylaxis. Indeed, from the point of view of human pathology, it is of quite as much, if not more, importance to study the effects of slow and slight injuries of this category, than it is to observe them only in the extreme and stormy manifestations of acute anaphylactic death. The former are the types of reactions occurring in the ordinary incidents of life. The latter are extreme results of experimental procedures and are for this reason of course more likely to reveal the underlying principles. But it would lead to false logic in our deductions were we to mistake a difference in degree for a difference in principle.

In the light of our present understanding, therefore, we should broadly define the term as the injury, acute or slow, severe or slight, which under manifold circumstances may follow on the meeting of an antigen with its specific antibody within the animal body. When such injury fails to result in the case of the spontaneous entrance or the experimental injection of bacteria into an immunized subject it is probably because the

organisms are disposed of before the amount of foreign protein is sufficient to permit such a harmful reaction. What these circumstances are is the problem before us. In the case of innocuous foreign proteins such as blood serum or cells incapable of multiplication, it is doubtful whether immunity—that is, ability to escape harm on reinjection—ever exists. However, we do know that the animal may be non-sensitive, as on first injection when practically no specific antibodies are present, or it may be hypersusceptible, anaphylactic or (the most comprehensive term) allergic.

II

We may discuss briefly the conditions under which so-called anaphylactic shock may be experimentally elicited in animals. Although of relatively recent development, in their details, the observations which underlie the phenomena took root in the early history of serum investigation. Morgenroth [1] speaks of an observation by Magendie as early as in 1839 in which he describes the sudden death of dogs when repeatedly injected with egg albumin. Flexner reported similar deaths in rabbits repeatedly injected with dog serum. Richet and Hericourt [2] in 1898 showed that toxic eel serum injected into dogs would kill at the second injection in far smaller doses than were necessary to kill at the first injection. Similar significance attaches to the work done by Portier and Richet on actinocongestine. Properly belonging in this group of phenomena are the early observations on hypersensitiveness to toxins in repeatedly injected animals made by Behring and his collaborators. The problem was brought into particular prominence by the observations of Arthus [3] in 1903, who found that horse serum injected into rabbits at intervals of several days would eventually, in the latter injections, give rise to severe infiltration and œdema, and almost at the same time Theobald Smith noticed the great susceptibility to horse serum acquired by guinea-pigs that had been used for diphtheria antitoxin standardization. Independently and with great clearness of vision von Pirquet [4] had made similar investigations on clinical material, and in his work on

serum sickness appears to have grasped the fundamental significance of the phenomena with a thoroughness not shared by most of his contemporaries. The historical development of this subject and the experimental conditions under which hypersusceptibility may appear were the subject of a paper read before this society some years ago by two of the pioneer workers in this subject, Rosenau and Anderson.[5] The fundamental facts concerning the anaphylactic reaction were worked out almost immediately under the observations of Theobald Smith and Arthus by these workers and by Otto[6] in Germany. I may be permitted to summarize this early work and the fundamental principles of anaphylaxis very briefly in order that we may not spend our time in detailed consideration of facts entirely familiar to most of us.

It is now certain that hypersusceptibility may be produced in human beings, in guinea pigs, in rabbits, in dogs, in sheep and probably in all mammals, if we were to investigate them carefully.

The condition may be produced by treatment with any of the substances known to us which have the property of antibody production; in other words, with all substances in nature of which we speak as antigens.

The condition is like other antigen-antibody reactions, specific within the limits of specificity recognized for all such reactions. It is certain that in so-called active sensitization, hypersusceptibility develops only after lapse of a definite interval, and this interval depends to a certain extent on the amount administered at the first injection.

An animal once sensitized if not reinjected may remain sensitive for a long period; its sensitiveness will disappear immediately after recovery from a non-fatal reinjection or the animal may temporarily be desensitized by reinjection of the antigen at a period before hypersusceptibility has developed.

Of the greatest theoretical importance, furthermore, is the fact that a normal animal may be rendered sensitive passively, by the injection of blood-serum from an actively sensitized animal, or by the blood-serum of any animal which has been once or repeatedly injected with the antigen; and according to Doerr and Russ and others there is a definite parallelism between the capacity of a serum passively to sensitive an animal, and its contents in specific antibodies.

There are many other facts which are of importance, but which for the present we will neglect, since these are the funda-

mental phenomena on which we may build our discussion. We may also dismiss very briefly the earlier theories of anaphylaxis, like those of Gay and Southard [7] and Besredka,[8] in which attempts were made to show that the substance which sensitizes is not identical with that which is responsible for the development of shock in the reinjected animal. We may, indeed, disregard as premature theories all those in which the anaphylactic reaction is removed from the sphere of true antigen-antibody reaction. Indeed, von Pirquet and Rosenau and Anderson from the beginning regarded anaphylaxis as the result of the reaction between the reinjected antigen and the antibody formed in response to the first administration; and indeed, this is the essential premise of the still earlier view of Vaughan. We may accept it at present, identifying the anaphylactic antigen with antigens in general, and the anaphylactic antibody with the protein antibody, not distinguishing for this purpose between agglutinins, precipitins, or cytolysins.

The symptoms which follow on the reinjection of antigen into sensitive animals may show a wide range of variation according to the degree of sensitiveness and amounts injected. In acute anaphylaxis of guinea pigs, which as you know has been the most thoroughly studied, there is a rapid and severe death which may not occupy more than a fraction of a minute or at most five to ten minutes. The animals repeatedly show restlessness, cough, pass urine and fæces, develop severe dyspnœa, with infrequent respiration in which there seems to be almost complete immobilization of the chest wall and in which finally only shallow, irregular, spasmodic efforts take place. This, as Auer and Lewis have shown, is due to tetanic contraction of the small bronchioles, with occlusion of the air passages, practically no air entering the lungs. As the dyspnœa develops, there may be at the same time spasmodic twitching of the limbs, retraction of the head and general convulsions.

When for some reason or other the reaction is not so severe the animal may show merely general signs of illness, ruffling of the fur, twitching and restlessness, with respiratory difficulty of varying degree, coughing, and evacuation of urine and fæces. In rabbits the symptoms are often less rapid in development, but in general principles are similar; in rabbits there is more frequently in the moderate cases a gradual muscular weakness in which the animal lies flat on the ground unable to support itself on its

legs, a condition which may proceed for long periods. Death is largely respiratory and the heart may continue to beat for a long time after respiration has completely stopped.

There is a sinking of blood-pressure and a depression of temperature.

The coagulation time of the blood is lengthened, there is apparently a depression of the leucocytes, and according to a number of investigators, who have been recently confirmed by Behring, there is a disappearance of blood platelets and an increased flow of lymph.

Pathologically in an animal dead of anaphylaxis there may be petechial hemorrhages, according to Gay and Southard, in the heart muscle, pleura and intestinal wall and there may be fatty degeneration of the vascular endothelium. In guinea pigs especially there is a marked emphysematous dilatation of the lungs which is very constant, although according to Doerr it is not absolutely characteristic of this condition. Apart from the anatomical changes following acute anaphylaxis, frequently repeated injections of small doses of horse serum or egg white in dogs, cats, rabbits and guinea pigs have been shown by Longcope to produce cell injury in various organs, especially in the liver, myocardium and kidneys.

The sudden onset, the nature of the reaction in the animal and the pathological lesions seem to indicate that the injury as occurring in anaphylaxis is due to a poison. It appears, then, that an animal is sensitive to a protein at certain stages at which specific antibodies to the sensitizing protein have been formed, and that under special circumstances the meeting of antigen with antibody within the animal results in a reaction in consequence of which the poisonous substance is liberated. This being the logical point of view on the basis of available knowledge, it was quite natural that many investigators were attracted by the theory of parenteral digestion.

The curious changes in the coagulation of the blood during the anaphylaxis have led to an interesting and important theoretical conception, namely, that the meeting of antigen and antibody may not, as otherwise believed, lead directly to the formation of a poison, but that in some way the results of such a union may influence the coagulation processes and that these alterations are the direct cause of shock. The first to give serious attention to such a train of reasoning was probably Nolf, and Doerr has recently called attention to the work of a number of investigators (recently confirmed by Moldevan) who observed that freshly defibrinated blood, *i.e.*, blood in which the normal coagulation has been interrupted, may be toxic even when reinjected into the same animal.

The same is true of serum taken from rapidly defibrinated blood. There is at least a possibility, then, that the anaphylactic injury is the result of an alteration in the blood indirectly brought by the union of antigen and antibody. However, the premises for such reasoning are still very vague, and moreover, any view which introduces the various elements which participate in blood coagulating processes can have no part in such manifestations as those observed on isolated and washed tissues, as in the experiments of Schultz and Dale.

III

It is one of the earliest premises of Pfeiffer's conception of bacteriolysis that the cell-dissolving action of immune serum liberates a preformed poisonous substance or endotoxin from the bacterial cell. It may be remembered that early in the history of such researches Pfeiffer and some of his pupils showed that an immune animal could be killed more quickly by large doses of dead bacteria than could a normal animal, an experiment from which the conclusion was drawn that the more rapid bacteriolysis in the immunized animal resulted in a more rapid liberation of the endocellular poisons. This point of view has been many times brought forward, and of recent years most clearly by Wolf-Eisner.

It is also a point of view represented by the theory of Nicolle, who similarly tried to explain anaphylaxis by the liberation of poisonous substances from the antigen through the action of the cytolysins or "albuminolysins."

As the investigation of antibody formation against foreign proteins of inherently harmless nature progressed, the belief gained strength that antibodies in facilitating the chemical disintegration of the injected foreign protein represented a sort of emergency apparatus for parenteral digestion and consequent assimilation. Throughout the development of Metchnikoff's ideas of immunity it is plain that he had tended toward such an interpretation, looking on the process of phagocytosis as a method of facilitating the removal of undissolved foreign substances from the tissues and blood, while the serum antibodies were conceived as more particularly concerned with the unformed foreign proteins which in the accidents of ordinary life

gained entrance. The most clear and thorough exposition of such a point is that which since 1907 has been carefully worked out by Vaughan, and to him belongs the credit for the development of many of the ideas underlying prevalent opinions on anaphylaxis. Vaughan, as you well know, subjected many different proteins, bacterial and others, to hydrolytic cleavage in absolute alcohol containing 2 per cent. of hydroxid.

The protein is covered in flasks with 25 to 30 times its weight of this alkaline alcohol and the mixture boiled at 78° C. for an hour or more. In this way he has succeeded in splitting off from a large number of different proteins the toxic fraction.

Since Professor Vaughan[9] himself has but recently embodied his views in a concise treatise, it is quite unnecessary to go into it more than to review briefly his views. He believes that all true proteins contain a poisonous group which is practically the same in all of them. This poison can become free and active when proteins are submitted to various methods of decomposition. Protein sensitization, in other words, is due to the fact that there is developed after the first injection a specific proteolytic ferment, and this on second injection so acts on the reinjected antigen that the toxin fraction is set free and poisoning results. This, in brief, is Vaughan's point of view and is supported, first, by the fact that such poisons can be formed by his chemical methods from many different kinds of protein; and second, that these poisons, whatever the antigen from which they are derived, may produce symptoms which are in many ways identical with those characteristic of anaphylactic shock. Since, as Vaughan states, proteolysis consists in a gradual breaking up of the protein molecule into simpler and simpler groups, there is an increase of poison liberation up to a certain point in the process; but when it has proceeded beyond this the poison itself is decomposed and ceases to have toxic action. Vaughan believes that anaphylaxis in all its manifestations, whether acute or chronic, is merely an incident in parenteral protein digestion. In the course of this when the relation between circulating antigen and the specific enzyme is such that large amounts of the toxic fraction are suddenly liberated, acute shock follows.

It is hardly necessary to call attention to the attractiveness of such a theory, which so simply explains the apparently mysterious conditions prevailing in anaphylaxis, and there seemed to be very little doubt as to its correctness when Friedemann [10] some years later showed that the action of fresh unheated serum (*i.e.*, alexin or complement) on sensitized red blood-cells will produce a poison that, injected into rabbits, gives rise to anaphylaxis-like shock. Following him Friedberger [11] succeeded in producing a similar poison by allowing fresh guinea-pig serum (*i.e.*, complement) to act on both precipitates formed by the union of the serum with its antiserum and on sensitized and unsensitized bacteria. These investigations clearly suggested that the action of the alexin present in the circulating blood, on an antigen sensitized with its specific antibody, might produce protein cleavage in which there was liberated a toxic fraction similar to that produced by Vaughan with his chemical hydrolytic methods. It is but natural, therefore, that Friedberger, to whom the greatest credit in the more recent development of this point of view belongs, should assume that the poison liberated in this way is the toxic factor concerned in anaphylaxis, and name it "anaphylatoxin." For reasons which will appear directly, we think that a preferable term would be "proteotoxins."

The technic developed by Friedberger consists, in the case of dissolved proteins, in allowing the antiserum to act on the serum until a precipitate is formed, then subjecting this precipitate to the action of fresh guinea pig serum or complement. After a variable number of hours, the length of time depending on secondary factors, which need not be discussed in describing the process, the centrifugation removes the precipitate, the supernatant guinea pig serum is found to be strongly poisonous, and injected into guinea pigs intravenously in quantities of from 2 to 4 c.c. produces symptoms typical of acute anaphylaxis. With bacteria his technic is similar. At first bacteria sensitized with inactive immune serum were subjected to the action of fresh guinea pig complement for from one to two hours at 37° C. to as long as twelve to twenty-four hours at refrigerator temperature. At the end of this time the bacteria is removed by rapid centrifugation, and the supernatant fluid injected into guinea pigs produces again typical symptoms of acute anaphylaxis.

The first interpretation applied to these experiments by Friedberger was an entirely natural one if we consider the general views held before this concerning bacteriolytic and bactericidal processes. He assumed that the complement acting on the sensitized bacteria or on the sensitized protein in the precipitate experiment (or later on the unsensitized bacteria), produced proteolytic changes in the course of which the toxic split product was formed. It seemed that the poison was pharmacologically the same whatever the antigen used, and experiments also seemed to show that the poison could be produced more rapidly from strongly sensitized than from unsensitized bacteria, and that an excess of sensitization or a too prolonged interaction resulted in non-toxic supernatant fluids, which was taken to indicate that the protein had been split beyond the toxic stage by too energetic hæmolytic action.

Here, then, we have a simple and apparently logical explanation of anaphylaxis, entirely in accord with Vaughan's views of parenteral digestion. An antigen is injected into an animal, specific antibodies and enzymes against it develop in the animal; reinjection of this antigen results in relatively rapid proteolysis in the course of which poisonous substances, the anaphylatoxis, are produced and anaphylaxis is the result. This hypothesis, although very attractive, does not entirely meet with the facts as they have been developed since Friedberger's first work. The premises on which it is based assume in the first place that the poison or "anaphylatoxin" is formed out of the matrix of the antigen; further, it is definitely assumed that in the production of the poison after the antigen and antibody have met, the complement or alexin plays an active part. Friedberger's hypothesis as stated by him, moreover, seems to assume that the entire process takes place intravascularly, a matter which we will discuss at considerable length in a short time. It is important to note also that Friedberger, with Nathan, was able to show that this anaphylatoxin production could take place within the animal body; that is, within the peritoneum of a guinea pig into which bacteria had been injected.

The simplicity of Friedberger's explanation and the correct-

ness of his experimental data soon persuaded many investigators that, in essence, his hypothesis probably contained the nucleus of the solution of this difficult problem. However, even his own early experiments aroused some misgivings concerning the matrix of the poisons produced, for he found that the poisons could be obtained as well when boiled antigen was used as when the fresh, unheated substances were employed, and the poisons were easily obtained from such organisms as the tubercle bacillus, which is extremely insoluble and unamenable to serum influence. It was also doubted whether one could truly assume the participation of this specific antibody or sensitizer in the production of Friedberger's poisons, since it soon developed that from bacteria, at least, the poison could be produced when the organisms were directly exposed to the action of fresh guinea-pig serum without the presence of any immune serum.

Experiments which soon threw a definite doubt on the assumption that the poisons were produced by a decomposition of the antigen were reported by Keysser and Wassermann.[12] These workers substituted insoluble substances like barium sulphate and kaolin for the antigen; that is, the precipitates or bacteria used in Friedberger's experiments. They found that if kaolin were treated with horse serum and then exposed to the action of guinea-pig serum or complement, poisons were produced identical in every respect to those produced by Friedberger's method. The conclusions they drew were that the poisons were produced, not by action of the complement on the antigen, but by its action on the horse serum absorbed by the kaolin. In other words, they transferred the matrix of the poison from the antigen to constituents in the serum itself, possibly the sensitizer or amboceptor. Bordet [13] also was able to show that poisons similar to those of Friedberger could be produced by the action of fresh guinea-pig serum on agar, and recently Bordet has further shown that this is the case even when the agar has been by special methods deprived entirely of its nitrogenous components and represents simply a complex of carbohydrates. Agar-guinea-pig serum mixtures of this kind show an increase in total non-protein nitrogen which would

prove that the proteolytic action of the guinea-pig serum must have been active against its own proteins.

An interesting further development of this work has recently appeared in the experiments of Jobling and Peterson.[14] They showed that when bacteria are mixed with fresh active serum they absorb the antienzymes normally present in blood. They have shown this experimentally and have proved that similar antienzyme removal can be accomplished by the addition of kaolin or agar, and by treatment with chloroform. Serums so treated become toxic, the actions of the poisons formed showing great similarity to that produced by Friedberger's anaphylatoxins. According to them, the poisons are formed because of the fact that antienzymes are absorbed by the antigen, thus setting the normal ferments in the fresh serum free to act on their own serum protein.

It should be recalled that Friedemann, who was really the first one to show that the toxic substances could result from the interaction of fresh serum and sensitized antigens, although he succeeded only in doing this with red blood-cells, suggested rather early that the success of such an experiment does not necessarily mean that the antigen furnishes the matrix entirely. He had studied the metabolism in anaphylactic poisoning and with Isaac has shown that the nitrogen output following reinjection in a sensitized animal is far in excess of that which could be derived solely from the injected antigen, and in this he has been confirmed by many other workers, notably by Vaughan.

It would seem to us our present knowledge of this phase of anaphylactic investigation permits us only to conclude that wherever proteolytic changes take place these "proteotoxins" may be formed. That they can be produced from a protein antigen has been shown beyond doubt by Vaughan and his collaborators for both formed and unformed antigens. Also this is evident from the experiments of many workers and has been confirmed in our own experience with poisons appearing during the autolysis of bacterial emulsions. On the other hand, it is also clear that the antigen need not represent the matrix which furnishes the poison, and that in the reactions as they are gener-

ally performed both in the test tube and in the animal body, it is more than likely that if an antigen participates at all in furnishing the substratum for the poison, this is probably less important than that furnished by the animal's own proteins. However, this does not weaken the importance of the knowledge that the antigen-antibody reaction in the presence of normal serum and certain antigens in the presence of normal serum alone, induce a reaction in the course of which such poisons are formed. And the fact that they can be produced experimentally in the peritoneal cavity of a living guinea pig renders their participation in such reactions in the animal body a likely assumption.

Our own work [15] on these substances induces us to believe that proteotoxins so formed are identical with Bail's aggressins, a point to which we will refer later.

Granted that such a poison, call it "proteotoxin" or "anaphylatoxin" or "serotoxin," as Jobling and Peterson have called it, is formed, it is important of course to determine as closely as possible its nature. Apparently the poison is the same as far as we can determine by pharmacological action when produced by the chemical methods of Vaughan or by the biological methods of Friedberger and others. As obtained by Vaughan it is water-soluble with slightly acid reaction, is freely soluble in alcohol and mineral acids. It is not diffused readily and contains no carbohydrates. In its crude state it gives a biuret reaction, although this may mean simply that the poison has not been completely derived. The fact that the injection of Witte peptone into animals may give rise to symptoms very similar to anaphylaxis has been taken by many workers to signify that the anaphylactic intoxication is produced by a poison which is very similar to, or possibly identical with, the active constituents found in this peptone. After peptone injection in normal animals there is a lowering of blood-pressure, a delay in the coagulation of blood and a development of subsequent tolerance, together with many clinical symptoms which emphasize this similarity. Biedl and Kraus, who have especially studied this condition in dogs, have felt emphatically that the

anaphylactic poison is probably very similar to peptone. Recently Dale has suggested that β-iminazolylethylamin or histamin may be the active principle concerned in anaphylactic shock. Intravenous injection of 0.5 mg. of this substance into large guinea pigs results in typical respiratory difficulties, convulsions with death and distention of the lungs typical of anaphylactic shock. Treatment with atropin diminishes this action, just as Auer and Lewis found this to be the case in true anaphylaxis, and fall in blood-pressure also occurs. It would seem then that substances representing cleavage products of native proteins of highly complex nature, the result of proteolytic cleavage not very far advanced, are probably concerned in the production of anaphylactic shock. The anaphylatoxins of Friedberger cannot of course be studied chemically by the methods to which Vaughan's poisons are amenable.

IV

A further problem which has arisen in connection with the conception of parenteral digestion is that which concerns the participation of complement or alexin in the cleavage process during which the anaphylactic noxious agent is liberated.

When bacteria or red blood-cells are sensitized, that is, have been combined with their specific antibodies, we have believed that it is the complement, or active constituents of fresh blood, which then acts on this sensitized complex, either producing hæmolysis in the case of sensitized red blood-cells, or the bactericidal or bacteriolytic effect in the case of sensitized bacteria. It is also well known to you that this substance, which we call complement or alexin, but about the true nature of which we know nothing, is fixed or bound by dissolved proteins when they have combined, with or without the formation of precipitates by their antibodies. We have ourselves [16] shown that such fixation of complement by precipitates (formed when an antigen and its precipitin have united) is bound in exactly the same way as this occurs in the case of sensitized red blood-cells; that it is not a non-specific physical complement fixation such as that which occurs when complement is fixed by kaolin, yeast cells or

other unsensitized emulsion. From this knowledge there has gradually grown the conception that the complement or alexin may be a necessary, active factor in the cleavage of the antigenic molecule. (This may or may not be so; we may say we think that we have no proof at present that the complement acts as a proteolytic enzyme; on the other hand, it is more than likely that in some way it is connected with such cleavage processes.) At any rate, since we know that the anaphylactic reaction is the result of the union of an antigen with its antibody, and this together with our knowledge of complement fixation, naturally suggests that the complement may be directly concerned in the mechanism of anaphylaxis.

The first method of approaching this problem naturally was the examination of animals with regard to quantitative changes in the complement contents of the blood during anaphylactic shock. It was found by Sleeswijk [17] that animals actively sensitized and reinjected showed a very definite diminution of complement. However, under such conditions the diminution was neither rapid nor very extreme, facts since confirmed by Friedberger and Hartoch,[18] who found the diminution very much greater in experiments with passive sensitization. In such cases there was a regular and considerable diminution, so that after shock four to eight times as much serum was necessary to produce the alexic effect as before shock. Friedberger even believed that there was a definite parallelism between the intensity of shock and the degree of complement diminution. The question immediately arises is the loss of complement, which we may now regard as a demonstrated fact, an incidental effect of shock or has it casual relationship to the development of shock? The latter seemed at first to be likely for a number of reasons. It was found, in the first place, that the addition of complement to the circulation of an animal during the anaphylactic experiment did not serve to prevent shock. Similar evidence seemed furnished by certain experiments on the complement of birds, by work of Loeffler [19] and by the observation of Hartoch,[20] that but slight shock could be produced in guinea-pigs suffering from trypanosomiasis in which, as is well known, complement is

greatly reduced. Loeffler also attempted to support this point of view by sensitizing guinea-pigs and then reducing their complement by the injection of sensitized beef blood intraperitoneally. Such animals showed diminution of reaction when reinjected with the sensitized antigen. Loeffler's experiments are not conclusive, since the action of the sensitized blood-cells in the peritoneum must surely have induced an intoxication not at all unlike that taking place in true anaphylaxis, and, as we have shown recently together with Dr. Dwyer, such intoxications are followed by non-specific tolerance to the anaphylactic poison.

However, another method of approaching this problem was attempted by Friedberger in his well-known salt experiment. It had been shown by a number of workers, among whom we may mention especially Nolf [21] and Hektoen,[22] that complement is not bound by sensitized complexes in the presence of hypertonic salt solution. In fact, hypertonicity seems to inactivate complement, and indeed it is a method of many laboratories to preserve complement for considerable periods by adding hypertonic salt solution, in which condition it will last a considerable time and is easily reactivated on dilution to isotonicity with distilled water. Friedberger [23] injected concentrated salt solution into sensitized guinea pigs just before reinjection. It is possible, as he found and as we have found since, to inject 0.3 c.c. or even more of saturated salt solution intravenously into guinea pigs of about 200 grammes weight without killing them. When sensitized guinea pigs were injected in this way and immediately afterwards received a toxic antigen injection, shock was definitely diminished and death averted. This has been one of the strongest bulwarks of those who have believed in the participation of complement in serum anaphylaxis. And it was assumed that the mechanism of the salt experiment consisted in a prevention of complement action. Recently doubt has been thrown on this because Ritz [24] has shown that salt injection not only prevents anaphylactic shock but will prevent the toxic effects of Witte peptone and of the so-called "anaphylatoxins." Recently with Dr. Dwyer [25] we have carefully repeated this work and have

found that when the dose is carefully adjusted there is no question about the fact that an immediately preceding injection of concentrated salt solution will prevent death or even symptoms in animals injected with proteotoxins. This tends very strongly to diminish the weight of Dr. Friedberger's interpretation of the salt experiment; it means either that the salt in diminishing anaphylactic shock does so by a mechanism not concerned with the prevention of complement, or else it signifies that the so-called proteotoxin itself is not a finished poison as it has been thought to be but must still be acted on by the active constituents of serum before it becomes active.[1]

It is true, indeed, that heating serum to a temperature of 56° C. renders it impotent to lead to proteotoxin production when added to antigen in vitro and that this same inactivation destroys the complementary effect on sensitized red cells or bacteria. This, after all, does not prove identity of the substances carrying those activities, but merely establishes an interesting parallelism.

We must not forget that the substance of which we speak as "alexin" or "complement" is not very well understood. We know little of its nature. It has been successfully shown that globulin participation will divide it into two parts, that it will spontaneously reactivate to a slight degree after heat inactivation, that its activity is influenced by concentration, and that it can be inactivated by shaking. We are aware of the fact that we are here, possibly, dealing not with a single substance, but with one of the effects of a complex serum constituent. As to its relation to anaphylaxis we can only say that the diminution of complement during anaphylaxis is perfectly definite. However, we cannot claim with certainty, in spite of the evidence so far advanced, that it plays an active part in the production of anaphylactic shock.

[1] With Lieb and Dwyer (Proc. Soc. Exper. Biol. & Med., 1915, xii), we have been able to show that the hypertonicity produced by salt injections protects by decreasing the irritability of smooth muscle.

V

The fact that the hypersusceptible condition can be transferred from a treated to a perfectly normal animal with the blood-serum of the former, was in itself one of the first strong arguments in favor of the antigen-antibody conception of anaphylaxis. And this point of view was still more clearly defined when Doerr and Russ [26] subsequently showed that the power of a serum to convey hypersusceptibility was directly proportionate to its contents of specific antibodies. A serum which was strongly precipitating for the antigen would passively sensitize in quantities far smaller than those necessary for the same purpose in the case of a weakly precipitating serum. The principle that anaphylaxis depended directly on the meeting of the antigen with its specific antibody has never been seriously questioned since this time. However, from the very beginning of experimentation on passive sensitization it has seemed unlikely that the acute reaction, as seen especially in guinea pigs, could be attributed entirely to the meeting of these two elements in the blood-stream. It was observed by Nicolle, Otto, Friedemann, Gay and Southard and by many others since then, that sharp reactions can be produced with regularity only when a distinct interval was allowed to elapse between the administration of the sensitizing serum and the injection of the antigen. When the two are injected together, mixed or simultaneously, symptoms may be and usually are entirely absent, whereas severe and unfailing shock results when the antigen injection is deferred from twelve to twenty-four hours after that of the sensitizing serum. According to Doerr and Russ the interval may be shortened to four hours, but if lessened beyond this, the reaction may fail to appear, or if present at all is weak and indistinct.

This observation alone would tend to persuade us that mere contact within the blood-stream of antigen cannot account for the entire train of phenomena and suggests that the characteristic anaphylactic reaction takes place only after the injected antibody has become attached to the body cells in the same manner.

The idea in itself is not new. Wassermann had first sug-

gested it in an attempt to explain the peculiar hypersusceptibility to toxin possessed by some of Behring's toxin-immunized animals. He assumed that in such animals the formation of antitoxins may indeed have been stimulated, but that much of it might still be attached to the generating cells themselves, thereby rendering these proportionately more vulnerable to the injected toxin.

Such a conception of "sessile receptors" was applied by Friedberger [27] to anaphylaxis in his first attempts to formulate a hypothesis. He assumed that at the first or sensitizing injection the production of antibodies (precipitins) was stimulated. These, however, were not produced in great quantity and were not discharged into the circulation, possibly owing to the small single dose given for sensitization. They were present at the end of the anaphylactic incubation time as sessile receptors or sessile antibodies (precipitins). On the second injection a reaction occurred between the injection antigen and these sessile precipitins and the cell was injured because the reaction occurred on its substance, a reaction which, it is suggested, might have been harmless had it taken place in the blood-stream. In passive sensitization, conversely, no injury could result until considerable quantities of the antibody had become united to body cells in the course of several hours. That the antibody injected into passively sensitized animals indeed disappears from the circulation with relative speed, has been shown by Doerr and again recently by Weil.

Besredka's [8] early hypothesis, too, though incorrect in most of its premises, assumed the necessity of the intravention of the body cell in anaphylaxis—an opinion here again largely based on the observed interval in passive sensitization; and the same idea occurs at about this time in the work of Doerr and Russ, who likewise conceived the process as taking place directly on the body cell.

It is true as Doerr [28] has pointed out in a recent summary of anaphylaxis, that these early hypotheses were for a time relegated to the background, yielding the prominent central position to opinions which held that anaphylactic shock was the result

of intravascular parenteral digestion. To some degree this is due to the fact that Vaughan's work on the toxic protein split products and Friedemann and Friedberger's experiments on the production of similar poisons by purely biological methods, seemed to offer for the time being a field of work promising logical solution of this difficult problem. At the same time there was much evidence in the published work of such investigators as Friedemann, Scott, Briot, Biedl and Kraus, and Doerr himself, which seemed to show clearly that the interval in passive sensitization was not an invariable necessity. Consequently and very naturally the early purely cellular conceptions were not accepted as telling the whole story, and a few observers allowed the pendulum to swing completely away from this point of view. Nevertheless it is not fair to say that during this time the cellular theories were entirely neglected. We do not believe that von Pirquet ever entirely abandoned his original opinion that there was involved in certain phases of anaphylaxis an "allergie" of the tissues. Moreover, it was during this period that those methods of research were first applied to anaphylaxis which furnished in principle and fact all the important premises for the present almost universal cellular point of view. I refer to the transfusion method as employed in anaphylactic dogs by Pearce and Eisenbrey [29] and the method of observing isolated tissues from anaphylactic animals as used by Schultz [30]—work which appeared as early as 1910. Pearce and Eisenbrey working with two normal and one sensitized dog, transfused the blood of one of the normal animals into the sensitized one, transferring the blood of the latter to the normal dog. "At the proper moment the normal dog containing the blood of the sensitized animal and the latter containing the blood of the normal dog, each received intravenously the toxic dose of horse serum." The normal dog having the sensitized blood did not react, the sensitized dog having the normal blood showed typical fall of blood-pressure. Pearce and Eisenbrey drew the conclusion "that the phenomenon of anaphylaxis is due to a reaction in the fixed cells and not either primarily or secondarily in the blood."

In the same year Schultz began to work with what is now

spoken of as the physiological method. He determined that smooth muscle—freshly excised from various animals—will react with contraction when brought into contact with serum. When such muscle was taken from anaphylactic animals after being thoroughly washed free of blood, it would react more energetically and to smaller amounts of the homologous serum. There are many interesting by-products of Schultz's work, such as the differences between fresh arterial blood and blood-serum in their abilities to stimulate contraction, but this and other points will not be discussed at present. The important and incontrovertible fact established by Schultz is the changed reaction-energy or, in truth, "allergie" of the smooth muscle of anaphylactic animals to the stimulus of the sensitizing antigen. Dale [31] has confirmed and extended these observations of Schultz. He removed the uteri from guinea pigs after thoroughly perfusing them with Ringer's solution to remove all blood. He then suspended them in baths of Ringer's solution and by the customary physiological methods measured the contractions following the addition of various amounts of foreign protein in the form of—among other things—horse serum and beef serum. He found that the uterus of an animal sensitized to horse serum would react to this substance in dilutions of 1 to 2000 or even 10,000, while the organ taken from a normal guinea pig reached its limit of reaction ability at dilutions often less than 1 to 200. A uterus that had reacted strongly was found to be subsequently desensitized. A normal uterus could not—strangely—be passively sensitized by immersion into a solution containing serum antibodies. This method of investigation has recently, also, been taken up by Richard Weil [32] who has fully confirmed the principles laid down by Schultz and Dale. He has incidentally also answered an objection to the conclusions of Dale and Schultz (never indeed a very valid objection), namely, that the reaction of the muscle tissue of a sensitized animal might be in part due to the fact that the blood, *i.e.*, the antibodies, had not been entirely washed out of the tissue spaces by perfusion. Weil performed the very simple and ingenious experiment of injecting a normal guinea pig with large amounts of immune serum

(antihorse serum) and after a few minutes killing the animal. He then suspended the uterus in Ringer's solution in the usual manner without washing it completely free of blood. Contact with the homologous antigen produced no response. We may accept as definitely established by these researches of Schultz, Dale and Weil that the fixed cells of anaphylactic animals possess an increased reactionability toward the antigen which is in no sense secondary to processes involving the circulating antibodies. Moreover, the work of Weil seems to indicate that desensitization of a passively prepared guinea pig deprives the uterus of its power to respond and that the gradual spontaneous diminution of hypersusceptibility on the part of the guinea pig is accompanied by an entirely parallel loss of reaction-capacity on the part of the isolated uterus.

The recent work of Coca,[33] too, has further fortified the cellular point of view by a method which in principle is similar to that employed by Pearce and Eisenbrey. Coca succeeded in perfusing activity and passively sensitized guinea pigs with the defibrinated blood of normal guinea pigs in such a way that the original blood of the sensitized animals was reduced to a necessarily slight residue. Animals so treated could be kept alive for as long as six hours after the transfusions and remained delicately hypersusceptible in spite of the blood substitution.

Limiting ourselves for the present to the phenomena of anaphylaxis in which non-cellular antigens are employed, we may safely say that the evidence furnished by the incubation time necessary in passive anaphylaxis by the transfusion experiments of Pearce and Eisenbrey and of Coca, and most conclusively by the work on isolated tissue of Schultz, of Dale and of Weil, shows conclusively that the hypersusceptible state is largely determined by a changed reaction-capacity to the specific antigen on the part of the fixed tissue cells—an "allergie" which is probably due to the presence of specific antibodies in the substance of the cell protoplasm, and incidentally accounts for such effects as the skin reactions. It is probable that the acute symptoms and death of anaphylactic guinea pigs (and indeed of other animals) is in most cases of experimental anaphylaxis

due to the reaction which takes place between the injected antigen and these sessile receptors.

So much we must logically accept. However, are we justified in denying all possibility of injury to the animal when antigen and antibody meet in the circulation? This is indeed the claim of a number of workers who are inclined to regard the presence of circulating antibodies not only as incapable of leading to injury, but in fact as a protection, in that the antigen is deflected by them from the antibodies united with the cells. Personally we believe that this radical cellular interpretation of all phases of the phenomena of anaphylaxis goes too far. It was shown by Friedemann as early as 1909 that typical anaphylactic reactions could be produced in rabbits when the antigen (beef serum) was injected simultaneously with or mixed with the serum of passively sensitized rabbits. Indeed Friedemann claimed that by this method severe and fatal reactions could be produced in rabbits more regularly than when an interval was observed. Richet in the same year reported experiments in which immediate symptoms were elicited in dogs injected with mixtures of crepitin and the serum of a crepitin-treated dog, the crepitin in quantities far below that necessary to elicit symptoms in itself. (In this experiment of Richet the crepitin and the serum were left in contact in vitro for 20 minutes, a fact which somewhat detracts from the direct bearing of this work on our present discussion.)

In 1910 Biedl and Kraus [34] obtained immediate and severe symptoms in guinea pigs when they injected intravenously mixtures of horse serum together with the serum of sensitized guinea pigs. Briot [35] in the same year obtained reactions in young rabbits into which he had injected mixtures of horse serum and antihorse serum. Gurd [36] in a recent publication obtained reactions in guinea pigs when he injected intravenously immune rabbit serum (antisheep serum) and immediately thereafter sheep serum. We ourselves have been able to obtain occasional and distinct results in rabbits and guinea pigs both by simultaneous and immediately consecutive intravenous injections of antigen and antibody, though we did not succeed in attempts to dupli-

cate exactly the experiments of Friedemann and of Biedl and Kraus.

We have here a not inconsiderable mass of evidence which points to the conclusion that the whole story of the anaphylactic phenomena cannot be told by the cellular conception alone, and that probably—as in immunity—both cellular and intravascular processes are involved. Few thoughtful workers on hypersusceptibility would think of denying at present the probably predominating cellular factor in the ordinary anaphylactic type-experiment. I may say that many of us have never doubted that this element was an undeniably important one in serum anaphylaxis since the time when the experiments of Pearce and Eisenbrey and those of Schultz confirmed the suggestion of this conception forced on us by the incubation time in passive sensitization and the studies of von Pirquet. We do not share, however, the exclusively cellular view recently advocated in a recent summary and apparently accepted by Doerr, one of the most capable students of this subject.

It is true that almost all of the workers cited above as having obtained passive sensitization, without the interval, admit the irregularity of such results, and Friedemann, Gurd and others call particular attention to the great importance of the relative amounts of antigen and antibody when these are injected together or in rapid sequence. This has been our own experience and although we have obtained very definite reactions in this way, we feel that in any given experiment success or failure cannot be as regularly foretold as in the experiments in which the interval is allowed. Moreover, the reactions obtained by these methods are often mild—delayed—and are rarely violent or rapidly fatal. We ourselves have never obtained a fatal result. Yet it is idle to say—as has been said—that the reactions so obtained are accidents, probably due to secondary factors, negligible in formulating a conception of anaphylaxis. There is no such thing as an accident in nature, and the observation, though irregular and depending on elements in the experimental procedures not easily amenable to control, has been made too often and independently by a number of different trained

observers to be thrown out of consideration in a theoretical scheme which is to be just to all the facts.

Since we cannot, therefore, deny that under certain circumstances injury to the animal may result from the meeting of the antigen and antibody within the circulation, how are we going to account for the fact that such reactions are difficult to obtain and cannot be obtained with regularity? This question is not a simple one but it is our own opinion that a possible explanation may be found in the failure of rapid union of antigen and antibody in the blood-stream. We have already mentioned that all observers who have experimented along these lines have found that very definite proportions between antigen and antibody govern the success of such attempts and that with each lot of serum and antiserum the optimum proportions must be determined by experiment. In Friedemann's work on rabbits he found that the relative amounts of antigen and antibody which produce reactions in his rabbits if injected together corresponded roughly to the proportions which in vitro gave precipitates. An excess of one or the other substance would prevent reaction or at least result in a negative experiment. Now it is well known to all who have worked with antiprotein serums that the precipitin reaction can be inhibited by an excess of one or the other reagent.

When a constant amount of precipitating serum is used, the most prompt and voluminous precipitation may, for instance, occur when the antigen dilution is 1 : 50, and both the speed and the amount of precipitate may diminish not only as this dilution is increased, but also as the concentration is increased. This is a phenomenon which is common to all colloidal reactions and the mutual precipitation of the two colloids is to a large extent dependent on relative proportions.

It is a well-known fact (also familiar to many of you) that Linossier and Lemoine,[37] Eisenberg,[38] Ascoli,[39] von Dungern [40] and others have frequently noticed that animals treated with a foreign protein such as horse serum, for instance, may contain in their blood-serum, as late as six, seven, eight or more days after injection, both the antigen and its antibody ununited

and separated. Thus we have often seen ourselves, if we bleed an animal that has been rapidly treated with such a foreign protein, that its serum will precipitate horse serum, and will at the same time be precipitated by antihorse serum taken from another rabbit. It is thus plain that the serum in the case mentioned contains not only horse serum as such (a remnant of that injected) but also antibodies against horse serum which have been formed in response to the injection. It is unquestionable from the experiments of others and from our own extensive confirmations, that the serum of such an animal may contain side by side free antigen and free antibody. Why have these failed to unite? If such a serum is allowed to stand at room temperature or in the ice-box there will take place a very slow precipitation and a concomitant diminution in the amount of precipitin present. The precipitate thus formed has slight and distinct complement-fixing properties. Slow union, therefore, is taking place.

Another strange fact about such serums is that if two such rabbits are prepared, in each of which both free antigen and antibody can be determined, these serums when mixed will promptly precipitate each other.

A number of explanations have been advanced for the simultaneous presence of antigen and antibody in the same serum without union. Eisenberg and Volk have attempted to explain it by dissociation—that is the antigen and antibody are present united and also dissociated, reacting according to the laws of mass action. This has seemed to us unlikely. For, were this the case, the serum, as taken, should in itself exert definite complement-binding properties, since on the basis of this explanation it must contain not only the two reagents separate but a rather large proportion of the antigen-antibody complex united. This is not the case according to our own observations and according to similar ones made by Gay and Rusk.

Von Dungern [41] has assumed that the state of affairs described was due to the fact that the antigen might contain a number of different substances, alpha, beta, etc., each of which produces its own specific *Teil-präzipitin*. He believes it possible

that at certain stages in the immunization the free antigen present might be, say, an alpha fraction, the free antibody, let us say, a beta precipitin, the two not fitting and therefore unable to react.

This has not seemed likely to us although they are clear when taken and remain so for considerable periods, but do eventually precipitate slowly and in the course of days, an observation made not only by us but by Merckel.

It has seemed to us most likely that there might be in the circulation of animals an inhibiting agent, somewhat in the nature of a protective colloid, which prevented the union of antigen and antibody, or at least tended to make it an extremely slow process.

We may assume in the light of our present knowledge that both the antigen and the antibody are colloidal in nature, and together with Stuart W. Young, we [42] have been able to produce an analogy to the condition found in the serums just described by using three colloidal suspensions, that is, arsenic trisulphid, gelatin and gum arabic. Emulsions of gelatin flocculate suspensions of arsenic trisulphid; if small amounts of gum arabic are added flocculation is prevented. In order that a protected suspension shall be produced in which no precipitation will occur, very definite proportions between the three suspensions must be arrived at, but a number of quantitatively varying mixtures of the three can be produced which will hold up without precipitating for a considerable period. Like the serums described above, two such suspensions in which the relative proportions of the three are not the same will precipitate each other when by rapid mixing the quantitative relationship necessary for protection is suddenly disturbed.

We have here, then, a complex analogy to the conditions in the serums. Two substances, mutually flocculable, do not precipitate. They are prevented from precipitating by the presence of a third substance which "protects" when certain definite proportions between the three are maintained. Many quantitatively different mixtures of this kind may be made in which flocculation is in this way prevented. Mix two such protected

mixtures, disturb these proportions and flocculation occurs, faster or slower according to the relations arrived at in the mixtures.

Moreover Porges [43] has shown that the factor of colloidal protection may well play a part in the occurrences taking place in a medium of blood plasma or serum. He has found that fresh native serum will precipitate mastic emulsions. The same serum heated, if used in very small quantities, will protect mastic emulsions against precipitation of the fresh serum. This alone shows what delicate physical changes in the body fluids may make for fundamental changes of reactions.

In our own experience these experiments of Porges were in principle confirmed; small quantities of heated dog serum added to arsenic trisulphid precipitated this suspension; slightly greater quantities again dispersed it. Of similar significance are experiments by Streng on the so-called conglutinins, substances in serum which are supposed to produce an agglutination of blood-corpuscles or bacteria which have been previously treated with fresh serum or alexin. The addition of minute quantities of alexin to typhoid bacilli and agglutinin prevents agglutination.

Friedemann,[44] furthermore, a pioneer in this branch of serum investigation, in studies on the serum reactions has come to the conclusion that certain anticomplementary activities of the serum globulins may be inhibited by the albumins of the same serum. Schmidt [45] speaks of a similar *Schutzwirkung* on the albumin of normal serum. When lues serum was mixed with certain lipoid extracts (of human heart, used for Wassermann antigen) precipitation resulted. Such precipitation was brought about also by the globulins of normal serum—but was prevented or "protected against" when the albumin of normal serum was added to the mixtures. Friedemann himself (and Schmidt agrees with him on the main points) thinks that the globulins and albumins of normal serum are in antagonism, the albumins preventing certain reactions (such as complement fixation) in which the former become active as soon as the albumins are removed or diminished.

We do not have to force analogy to look on such serum reactions as essentially following laws similar to those observed in the case of chemically definable colloids. Apart from the protein character of serum constituents, we know that serum reactions follow quantitative laws analogous to those observed in colloidal reactions (inhibition zones, etc.). We know the importance of the electrolytes in the phenomena, we know that the immune bodies like the colloids diffuse but slowly, and we know from the work of Landsteiner and Pauli [46] especially that certain serum hæmagglutinins will wander, like other colloidal substances, to one pole or the other when a direct electric current is passing through solutions containing them, like amphoteric substances changing the direction of wandering according to the alkalinity or acidity of the menstruum. The points of similarity are too numerous to be exhaustively reviewed in this connection. They are so many and so striking, however, that we should hesitate to apply any explanation to serum phenomena of any kind which is not in accord with the general behavior of colloids.

In recent experiments of our own, moreover, we have been able to show that when precipitin reactions are set up in comparative series, in one case using the globulins of normal rabbit serum, in salt solution, as the diluent for the antigen, and in another series the albumins of the same serum, the reactions in the latter are noticeably slower than in the former—than similar reactions in salt solution or in active or inactive serum. There is apparent inhibition of the reaction by the serum-albumin.

Enough has been said to show the justification of any theory which utilizes as a major premise the possibility of the participation of protective colloids in reactions taking place within the vessels of an animal. We suggested some years ago in a paper on this subject that it was such a protective colloidal action in the plasma of animals which prevented the rapid union of antigen and antibody in the blood-stream, and we thought at the time that such an arrangement would indeed constitute an automatic protection of animals against sudden and severe

injury when a foreign protein gained entrance to the blood-stream. Our conception of the whole process would therefore be something as follows: The injection of a foreign antigen into the animal body leads it to antibody formation by the tissue cells. These antibodies are in part discharged in the blood-stream and in part sessile on the cells. There is a gradual union between the circulating antigen and antibody and probably between the circulating antigen and the sessile antibodies. Under conditions apt to occur in the course of normal conditions the quantity of nitrogen which gains entrance is small and no injury results from such union by which probably a gradual parenteral digestion of the foreign substances is obtained. When in the course of abnormal states, infectious disease, etc., a situation arises in which considerable amounts of antibody have been formed and relatively large amounts of antigen are also present, all the conditions are furnished for what we call anaphylactic injury, unless there were some efforts to prevent the rapid union in these animals. In the anaphylactic experiment we see that the rapid union of antigen and antibody on the cell will kill. But it is likely that in most cases during immunization the circulating antibodies are far in excess of those still sessile on the cells, and were rapid union between these and the antigen not inhibited in the circulation, the animal would be constantly and severely ill during all processes of immunization. However, we know that in highly immunized animals antigen and antibody may be present side by side ununited. Is it not necessary to assume that this is evidence of a protective inhibition of union? For the colloidal protection would lead to a very slow union, in which, because of the gradual nature of the process, practically no severe injury of the individual could result. According to this conception we can quite easily explain why the simultaneous injection of antigen and antibody into the normal animal would result ordinarily in slight and delayed symptoms. Accidental success in so balancing the proportions that complete elimination of protection results would account for the occasional acute symptoms and death observed in such procedures. It is quite clear

that such an ideal experiment cannot be regularly obtained, for the simple reason that the protective element may be subject to variation, and since there are so many secondary factors even in test tube experiments on precipitation which influence such reactions.

When the animal is sensitized by the methods of the classical anaphylactic experiment, the union in the cells, violent and stormy, results in death after anaphylactic shock, and whatever symptoms might have resulted from the union of the two substances in the blood-serum are overshadowed and secondary.

It is perfectly clear that there are many gaps in the absolute experimental proof of such a conception. We know, however, that slow, gradual and acute injury may follow on the simultaneous interaction of antigen and antibody in the animal body. We know from the many experiments of Vaughan, Friedberger and others that *in vitro* such a meeting in the presence of active serum can result in the production of injurious substances which produce anaphylaxis-like symptoms when injected into the animal. We know from the experiments of Doerr that the injection of formed precipitates will injure. Whatever we may think about the nature of the poison and its mechanism of production there is little reason to doubt that the noxious agent can be produced without reference to the body cells. And we believe from this, together with the premises on which we have developed our idea of colloidal protection, that such a conception may form a perfectly legitimate explanation for the scattered and yet definite observations made since Friedemann by many others and by ourselves, of immediate symptoms after simultaneous injection of antigen and antibody.

VI

In discussing the probable localization of anaphylactic reactions in the preceding paragraphs, we limited ourselves entirely to the phenomena occurring when sensitization is carried out with non-cellular substances such as blood-serum, egg albumin, etc.

When the antigen employed is cellular, consisting of bacteria

or red blood-cells, we are confronted with a problem of considerably greater complexity. As morphologically compact structures these cells cannot enter into direct chemical relations with the fixed tissue cells until they have been either disintegrated or at least have given up constituents to solution in the blood plasma. In consequence we must assume two separate phases of all such reactions—one the occurrences within the circulating blood in which the injected cells come in contact with the solvent elements of the plasma and during which the solution of antigenic constituents is brought about, the other the subsequent reactions entered into by these dissolved substances, either within the circulation or on the fixed tissue cells with their respective receptors or antibodies.

If therefore Doerr [29] and others (Denzer and Weil) claim that anaphylaxis with cellular antigens is entirely similar in principle to that produced with dissolved, unformed antigens, they may well be perfectly right in so far as the second phase of these phenomena is concerned. They found that guinea pigs injected with hæmolytic serums reacted to the injection of the blood-cells when, as in passive serum anaphylaxis, a latent period or interval was allowed to elapse between the administration of the antibodies and that of the nitrogen. This means simply that they failed to obtain acute or marked symptoms (for quantitative reasons possibly) when the cells and antibodies met in the blood-stream.

Analyzing the phenomena in this way it becomes clear that when we inject cellular material we are merely injecting an antigen—or more probably a group of antigens—enclosed in the morphological structures of the cell, and amenable to reaction only after liberation. After this has taken place, subsequent occurrences should in no important principle differ from those following on the injection of an unformed substance like serum, or we may say for the sake of clearness, a predissolved antigen; and all that we have said about such conditions in our preceding discussion should apply here.

Added to this, however, we have in the case of cellular antigens a process unnecessary when unformed antigens are

injected, namely, the cytolytic or cytotoxic reaction which precedes the liberation of the cell-constituents, and in the course of which the formed elements are broken up. And we need only compare the slow autolytic disintegration of cells in sterile inactive serum or salt solution with the rapid changes occurring in active hæmolytic or, in certain cases, in bacteriolytic serums, to be convinced that such disintegration is due to reaction with active serum constituents.

We may logically accept, then, that by injecting cells, we are for one thing injecting substances which will, in part, soon be liberated and which will call forth all the changes and enter into all the reactions which are associated with the injection of dissolved antigens. In addition to this, however, we are confronted with a further problem. Is there injury to the animal body, comparable in broad principles with anaphylaxis, during this intravascular reaction between whole cell and cytolytic antibodies which precedes the liberation of the soluble constituents? Is there, in other words, a true "cell anaphylaxis"?

Since it is probable that the principles of cellular anaphylaxis are the same whatever the variety of cell employed, we may take red cell hypersusceptibility as a basis for discussion. It is a well-known fact, long recognized, that a serum which is capable of hæmolysing the red cells of any species is toxic when injected into an animal of this species. This is true not only of hæmolytic serums but also of such normal serums which like, let us say, goat serum and rabbit cells, can hæmolyze normally the red cells of another animal. Since occasionally the serum of an individual of one species can so act on the red cells of another individual of the same species, our surgeons call for careful investigation of receptor and donor before performing transfusion. The injection of such a serum intravenously may kill with symptoms not unlike anaphylactic shock. Here it is often difficult, as we shall see (or indeed it may be impossible), to determine, whether such death is truly anaphylactic in nature or whether it is due to clumping of red cells or hæmagglutination, a property which is very often an accompaniment of hæmolytic power. However, hæmagglutinating properties can-

not be held responsible for the œdema and localized injury which, as Uhlenhuth and Haendel have shown, may follow the subcutaneous injection of such serums. It thus appears as though the process of hæmolysis were accompanied by the liberation of injurious products.

The first systematic investigation of red cell anaphylaxis was undertaken by Ulrich Friedemann.[11] Friedemann injected washed beef cells into rabbits and followed this by a second injection after from seven days to three weeks. Rabbits so treated showed the symptoms ordinarily associated with anaphylaxis in these animals. Active sensitization seems thus to have been accomplished with beef cells. Schiff and Moore have recently suggested that Friedemann really obtained serum anaphylaxis, but since Friedemann explicitly states that he worked with washed cells, we can see no just reason for such an assumption. Another objection to Friedemann's results, however, is possible—one which is far less easy to controvert—namely, that the illness of his rabbits may have been due to hæmagglutination, which by itself may produce serious illness or even death by mechanical obstruction of blood-vessels. Friedemann, indeed, takes cognizance of this possibility but makes no attempts to rule it out in his experiments. As a matter of fact we think it unlikely that hæmagglutination played a part in his rabbits, but the possibility cannot be excluded. We will revert to this particular question.

Passive sensitization was produced by Friedemann against beef cells in rabbits by injecting the specific hæmolytic serum. He obtained his best results when he injected serum and cells together, mixed *in vitro*. However, he also obtained positive experiments when the two were simultaneously injected into opposite veins. His results were inconstant when he allowed an interval to elapse between serum and cell injection—a fact which argued for the direct occurrence of the reaction within the circulation.

Most important of all, Friedemann mixed hæmolytic serum and cells in test tubes, letting them stand for five minutes in a water bath and then, before any considerable degree of hæmol-

ysis had taken place, he centrifugalized and injected the faintly red supernatant fluid into rabbits. A rabbit so injected became extremely ill and many of them died after shorter or longer intervals, with symptoms typical of anaphylaxis in rabbits. Friedemann concluded that when red cells came in contact with hæmolytic antiserum, poisonous substances were liberated, even before actual hæmolysis had taken place, and that these toxic products were responsible for the subsequent injury to the animal. He identified the anaphylactic antibody with the hæmolysin. This view, therefore, is identical in principle with the one we have discussed as the conception of parenteral digestion. Indeed Friedemann's experiments furnished the point of departure for Friedberger's subsequent work on the so-called "anaphylatoxins."

Doerr and Moldovan,[47] a little later (1910), studied the effects of the injection of serums hæmolytic for guinea pig erythrocytes into guinea pigs, and drew conclusions which substantiated those of Friedemann. They found that the toxic effect was due to the action of the hæmolytic serums on the guinea pig erythrocytes. Toxicity could be removed from such serums by absorption with these cells, and the toxic products could be produced by contact of serum and cells *in vitro*. From these experiments, again, it seemed that the liberation of a toxic substance followed on contact between erythrocytes and specific antibodies, whether this contact took place within the circulation or in the test tube. That the antibodies concerned need not necessarily be identical with hæmolysins themselves follows, we think, from the work of Doerr and Moldovan as well as from work of our own on the toxicity of certain normal serums [48]—experiments which could not be discussed in detail without taking more space than seems justified.

Although much irregularity of result has been obtained in the production of active erythrocyte anaphylaxis in both guinea pig and rabbit experiments, nevertheless, it seems clearly established that acute death does follow the repeated injection of such cells when dosage and interval are properly observed. The recent experiments of Schiff and Moore,[49] though they

clearly illustrate the difficulties of such procedure in guinea pigs, still record a sufficient number of positive results to reconfirm its actual occurrence. From one of these experiments, indeed, as well as from the experience of Friedemann and others with passive sensitization by antierythrocyte serums, it would appear that with red cells the phenomenon requires a procedure differing from that successful with serum anaphylaxis, in that a considerable concentration of antibodies is needed, *i.e.,* a condition calling in the active experiment for more than one preparatory injection, or, in the passive sensitization, for the injection of a serum of high potency. This, as we know, is the case, also, in bacterial anaphylaxis, in which experiments are usually successful only if many and repeated preparatory injections are made. It is this factor, possibly, which may account for the failure of so many workers to obtain true cell anaphylaxis when they have followed the technic successful in the serum experiments—*i.e.,* that of only one preliminary sensitizing dose—or that, in the passive experiment, many have failed to duplicate Friedemann's success when both antigen and sensitizer were simultaneously injected. It is more than likely that a weak sensitization and consequently a slow reaction between the cells and the antiserum may be interrupted by prompt phagocytosis of the injected cells, with consequent protection against the further developments of the process.

It is true that in many cases of erythrocyte anaphylaxis it may be impossible to say with certainty whether death was due to true shock or whether it was caused by embolic processes due to hæmagglutination. This possibility has not been ruled out in many otherwise complete investigations—though in experiments like those of Friedemann and Amako [50] it seems but a remote possibility. However, in individual instances, such as our own experiments with normally toxic serum, it has been shown that the toxicity may disappear with inactivation, though hæmagglutinating properties are retained, and it seems that, to kill acutely hæmagglutination must be rapid, powerful and extensive. Moreover, the speed and completeness of recovery showing non-lethal degrees of erythrocyte anaphylaxis argues

at least against the frequent occurrence of hæmagglutinative death by embolism in experiments carried out in this way. The local injury following the subcutaneous injections of normal and immune hæmolytic serums must of course occur entirely independent of hæmagglutination. Finally, the fact that contact of the cells with active serum—as first carried out by Friedemann—produces a poison *in vitro* which kills acutely with symptoms of anaphylaxis, seems to render fairly certain the assumption that similar contact in the circulation may lead to like result. For we know that the entire process of hæmolysis can take place intravascularly.

Whether the antibodies that so react with the cells are the hæmolysins themselves, is a question that we hardly have the time to discuss and which moreover is merely an incidental one. After all, hæmolysis itself is merely one visible result of a reaction which probably affects profoundly the entire cell structure. About bacterial anaphylaxis our knowledge is still more defective than is that occurring when erythrocytes are used. We *do* know, however, that active sensitization with bacterial proteins and while whole bacteria is possible—though many injections are apparently necessary—the exact procedure being subject to so many fortuitous influences that so far no regularly successful method can be outlined. We also know that, as with red cells, contact between the bacteria and active serums will result in the production of acutely toxic substances—which we have discussed above as "proteotoxins."

We may summarize our views on cell anaphylaxis, briefly, as follows: When whole cells are injected into an animal two distinct processes are set in motion. First, the formed cells come into relation with circulating antibodies. During this contact toxic substances—"proteotoxins"—may be set free if quantitative relations are suitable and cells sufficiently sensitized. Where the matrix of the poison is found and to what an extent the complement participates—these are in many respects still open questions. This reaction alone, if sufficiently vigorous, may cause acute symptoms and even death.

During this reaction antigenic cell constituents are set free

to solution and these then enter into reaction with their respective antibodies or receptors in the blood or on the fixed cells. The last-named reactions are entirely comparable to those of serum anaphylaxis and have been sufficiently discussed.

Whether in the first-named process, when the whole cell meets its antibody in the blood-stream, we regard the poison as originating from the matrix of the antigen or from the serum itself by the withdrawal of antienzymes is immaterial. The reaction is subject to so many modifying factors that experimental control is made difficult and results cannot at present be so regularly foretold as is the case in serum anaphylaxis. It seems probable from the work of Friedberger and others that a delicately balanced optimum proportion between antigenic cells and antibodies must be obtained. Moreover, unless the process is rapid and harmful effects very sudden, prompt phagocytosis of the cellular elements may remove the antigen from further reaction possibility.

It is plain that such a conception has the greatest importance in the understanding of infectious diseases. When bacteria form the antigen which gains entrance to the animal body, the gradual stimulation of specific antibodies in the animal may eventually lead to such a two-phase reaction. Specific sensitizers or amboceptors (cytotoxins) are gradually formed and these may react with the dead and the living micro-organisms. There may be a direct formation of proteotoxins and at the same time a liberation of soluble antigen from the bacteria. It may be, as von Pirquet has suggested, that the sufficient establishment of such reactions between cell and antibody may mark the end of what we speak of as "incubation time," no noticeable time accruing to the animal body until the antigen-antibody reaction has been initiated. The "proteotoxins" so formed, whatever their matrix, may then, as we have shown with Dr. Dwyer, act as aggressins, lead to a leukopenia, as in typhoid fever, and thereby increase indirectly the invasive capacity of the micro-organisms. The antigenic substances which have gone into solution may at the same time react both on the fixed cells with sessile receptors, and, to a merely incidental degree,

with their receptive circulating antibodies, adding thereby to the injury sustained by the host.

True immunity against dissolved antigens, we have stated in the beginning, probably does not exist, for animals having high antibody contents in their serum may still die suddenly with convulsions after a fourth or fifth injection with foreign serum. In the case of cellular antigens, however, and especially bacteria, true immunity may exist in two forms. On the one hand if the animal possesses a high concentration of antibodies before the micro-organisms have gained entrance, an immediate bactericidal effect may prevent their multiplication, the harmful effects resulting from the union of the small initial amounts of antigen and antibody being so slight as to be unnoticeable. Again, after the bacteria have gained entrance, if the quantitative relations between antigen and antibody are such that the reaction is either slight or for purely quantitative reasons results in little injury for the time being, then sensitization of the bacteria or other cells by the antibodies leads to rapid phagocytosis. And this process of phagocytosis represents true immunity, a removal of bacteria incidental to which there is, as far as we know, no injury to the host. It is in the process of phagocytic removal, chiefly, in which the reaction to cell injection differs from that taking place in response to the administration of unformed protein. It may be this element which renders it so difficult to obtain sharp anaphylactic reactions with cellular antigens. And it is the absence of phagocytosis in the latter case which probably prevents the existence of a true immunity.

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[41] Von Dungern: Centralbl. f. Bakteriol., I Abt, Orig., 1913, xxxiv, 355. Auch hier handelt es sich nicht um zwei reaktionsfähige Körper, deren Verbindung aus irgend Grunden unterbleibt, sondern um Substanzen,

welche keiner Affinität zueinander besitzen. Die betreffenden Kaninchen haben zu dieser Zeit noch nicht alle möglichen Teilpräzipitine gebildet, sondern nur einzelner derselben. Diese zunächst produzierten, nur auf bestimmte Gruppen der präzipitablen Eiweisskörper passenden Partial-präzipitine sind es, welche nach der Absättigung aller zur Verfügung stehenden zugehörigen Gruppen der präzipitablen Substanz im serum nachweissbar werden. Daneben bleit aber ein anderer Teil der präzipitablen Substanz, der keiner Affinität zu dem gebildeten Präzipitin besitzt, bestehen, solange bis ein anderes Partial-präzipitine von den Kaninchenzellen geliefert wird welches sich mit Gruppen der in Lösung gebliebenen Eiseisskörper vereinigen kann.

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SOME PROBLEMS IN THE PATHOLOGY OF SYPHILIS*

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IT is proposed in the present paper to discuss some of the phases of syphilis which have resulted from the intensive investigations of the past ten years. These problems include the question of immunity, a few of the most striking anatomico-pathological changes met with in the disease, as well as its effects on the cardiovascular and nervous systems, and the lessons to be deduced from the knowledge which has been gained.

IMMUNITY

One of the most interesting and perhaps least understood problems in the pathology and biology of lues is the question of immunity. Formerly it was taught that like many of the other infections one attack of syphilis conferred a lifelong immunity, that a syphilitic mother might transmit an immunity to her child, while, conversely, the mother of a syphilitic child was rendered insusceptible to the disease, as postulated in the well-known laws of Profeta and Colles-Baumès. Since the work of Neisser, Finger and Landsteiner, Levaditi, Uhlenhuth and Mulzer, and others, however, our conception of immunity in syphilis has undergone considerable modification.

Investigations along these lines and in microbiology have shown that syphilis is closely allied with the protozoal affections, especially those caused by trypanosomes and piroplasomes. These diseases differ from those of bacterial origin in the manner in which they respond to the introduction of organisms into the system. While in the great majority of infectious processes acquired immunity is expressed by a protection more or less absolute against a new attack and the disappearance of the causal agent from the body, or its innocuousness to the host

* Delivered February 13, 1915.

if it persists, diseases of protozoal origin behave in a different way. Levaditi has demonstrated, for instance, that animals suffering with a spirillary infection are immune to a new inoculation. Their serum has a high antibody content, but the blood still harbors parasites and is capable of producing a fresh infection in healthy animals. So with the serum of guinea pigs inoculated with Nagana or Surra trypanosomes. This is trypanocidal for these organisms *in vitro,* but *in vivo* they have acquired an insensibility to the trypanolytic antibodies, for the blood and tissues of the animals still contain parasites. The same is true of human subjects suffering from sleeping sickness in whose serum trypanolytic, agglutinating and other protective bodies have been demonstrated. Carrying the analogy to syphilis we find that an individual may harbor spirochætes for forty or fifty years, while his skin and mucous membranes exhibit an insusceptibility to re-inoculation under natural exposure. However, as soon as he is freed from his infection he is again in as susceptible a state as he was prior to his first attack.

It is a well-known clinical observation that after the development of the initial lesion a syphilitic is immune to a fresh infection. Why have the integument and mucous membranes ceased to react to organisms introduced from without when they are susceptible to their action from within? The opinion has been generally held that for infection to take place one of the first laws of its requirements is fulfilled by a surface covered by squamous epithelium. The earlier experiments on animals seemingly corroborated this view, for inoculations were only successful where scarification of a squamous-celled area was performed. Improved laboratory technic has, however, overcome the difficulty, positive results now being obtained in animals by the introduction of the virus into the testicle, peritoneal cavity, under the skin, or directly into the circulation, especially the heart.

In human syphilis, while it is more usual for the general infection to be preceded by the development of a primary lesion, somewhere on the cutaneous or mucous surface, it is not rare for the local reaction to be altogether absent. To illustrate:

a male nurse in my hospital service pricked himself with a needle used for obtaining blood for the Wassermann test directly after he had withdrawn it from the vein of a patient with florid syphilis. He was carefully watched for the development of a chancre at the site of injury, but the first macroscopic manifestation was a late secondary papule on his foot. Similar cases are reported in the literature. Another instance is exemplified in a personal communication by Dr. Dade, in which he related the infection of a young girl by blood transfusion. Her brother, whose blood was used for the treatment of her anæmia, was at the time of the operation in the second incubation period of the disease. She in due course of time developed a secondary rash. Congenital lues is a further example of syphilis without the development of an initial lesion. Neisser came to the opinion, from his many experimental observations, that in certain cases infection might take place through the integument without visible or even microscopic changes, the organisms gaining access to the lymphatic or blood-stream direct.

When spirochætes are successfully implanted, what takes place? As soon as they have become acclimated to changed nutritional conditions at the site of inoculation, as shown by Levaditi and Yamanouchi, they multiply, call forth microscopic changes in the cutaneous vessels, and enter the lymph- and blood-stream. This is known as the first incubation period and varies in length in different subjects. Just how far the organisms penetrate beyond the local sore and satellite lymph-nodes in human subjects in this stage is not known, but it has been demonstrated by Neisser that the spleen and marrow of inoculated monkeys contain virus thus early in the infection. Now, as the chancre is forming, a peculiar reaction on the part of the host is also developing. At first it is so feeble that the individual is for some time susceptible to re-inoculation. In animals it has been found that superinfection is possible if made eight days before the evolution of the primary lesion. Not rarely we see patients with multiple chancres. In some instances they develop simultaneously as the result of multiple inoculations; in others they appear successively either from

auto-inoculation or contamination with a fresh virus. Human and animal experiments are at one in showing that superinfection lesions do not develop in the typical manner of those implanted on a virgin soil. Their incubation period is shorter and their clinical characteristics simulate those of the stage the patient is in at the time; that is, papular if close to the secondary period and ulcerative if in the tertiary stage. In other words, they are the expression of an altered tissue reaction. The hypothesis has been advanced by Krause and Volk that this immunity has a regional development, beginning at the site of the primary sore and progressively involving the mucous and cutaneous covering. Neisser has suggested that the susceptibility to re-infection returns in an inverse manner.

During the second incubation period the refractory state continues to develop until it attains its maximum with the outbreak of the secondary rash. Even now, however, it is only relative, for laboratory experiments have yielded positive results if special methods are employed, namely, larger amounts of active virus introduced subepidermically. From a clinical stand-point, however, this refractory state, or anergy—the term usually applied—is complete as far as ordinary infection is concerned, disappearing only with the cure of the disease, when the patient is again in a receptive condition.

It has been demonstrated further that the anergy is a specific one, the attempts of Neisser, Baermann, and Halberstädter to induce it by inoculation with *Spirochæta pertenuis,* of Hidaka with *Spirochæta duttoni,* and of Uhlenhuth with *Trypanosoma lewisii* having proved futile. None of these infections protected against that of syphilis, while, on the other hand, luetic animals were all susceptible to frambesia, relapsing fever, and dourine. Little is known of the nature of this state of resistance or how it is brought about, whether by the tissues, cells, the serum, or intermediary organs. Organisms or their toxins when introduced into the body behave as antigens and stimulate the formation of antibodies which are specific for that particular bacterium. These antibodies are of the nature of antitoxins which neutralize the toxins; opsonins which lead

to the ingestion and destruction of the organisms by the leucocytes; agglutinins which cause the organisms to adhere together and probably also bring about their destruction; and bacteriolysins which cause their dissolution. From the contributions to this branch of research in lues the conclusions are that substances of a parasiticide nature do not develop in the course of the disease. At the most, says Neisser, we can reckon with complement binding and agglutinating substances of a specific nature. Levaditi, however, argues that since the spirochætes even before the evolution of the primary lesion may penetrate to the hæmatopoietic organs, they act like true antigens and their products of secretion stimulate the formation of antibodies. He assumes an analogy between these immune bodies and those of related diseases, where bacteriotropic substances and opsonins ensure destruction by phagocytosis. *Pari passu* with this antibody formation the skin acquires a refractory state to exogenous spirochætes, the biological changes being sufficiently advanced at the close of the second incubation period to determine the character of the reaction. He has offered two attractive hypotheses to explain why the skin which is resistant to exogenous spirochætes reacts to endogenous organisms, (1) by a generalized eruption, and (2) after a varying latency, by local and destructive lesions. His assumption is that for a time the immune bodies hinder the multiplication of the parasite, but with the enfeeblement or disappearance of the former the virus gains the ascendency, generalizes itself, and produces the secondary eruption. Or the spirochætes are vaccinated, so to speak, against the immune bodies, and, becoming resistant to these defensive substances, they succeed in multiplying and provoking the exanthem.

It is a well-established clinical fact that the early cutaneous lesions are superficial and generalized, that with each relapse they show a tendency to localization and grouping with involvement of the deeper structures until we reach the tertiary stage with extensive destruction. Since it has been shown that these differences are not inherent in the specific virus but in the tissues of the host brought about by prolonged contact with the causal

agent, the term allergy or, according to Neisser, Umstimmung has been proposed to explain the biological change which has taken place in the tissues. In the primary and secondary stages, when the organisms are numerous, the host is refractory; in the tertiary stage when they are few in number an anaphylactic state exists, the tissues are frail, and more readily undergo necrosis. This is also seen in superinfection in tertiary syphilitics when the lesion corresponds to that type, and in the luetin reaction, which is especially applicable in congenital and tertiary syphilis, depending for its result on the hypersusceptibility of the tissues. In tuberculosis a similar condition is encountered. Erythema induratum, for instance, is caused by few organisms, and yet clinically presents extensive destructive lesions. Another clinical type is seen in large and deep ulcerations of the extremities in tuberculous subjects, all treatment, excepting surgical, being of little avail. The writer has recently had under his care a young man with glandular tuberculosis in whom the entire right leg was involved, necrosis extending through the musculature and laying bare the bone.

The immunity processes as outlined in the foregoing considerations are subject to modification under treatment. Where treatment is intensive and yet not sufficient to effect a complete cure it would appear that the anaphylactic stage is hastened, for it has been repeatedly claimed that specific remedies given early in the disease tend to produce relapses of severe local intensity. Herein we find the basis for the contention that salvarsan has changed the course of the disease, and, when not given in sufficient doses to sterilize the patient, tertiary lesions appear much earlier than when the affection is permitted to run its course with the gradual establishment of a defensive mechanism, and consequently a longer refractory state. In so-called malignant syphilis we must also seek the cause in a precocious anaphylactic condition of the tissues rather than in the type of the invading organism, the reason usually evoked for this form of the infection. It has repeatedly been reported that spirochætes are numerically few in such lesions and that successful inoculations are relatively rare.

As accumulated evidence has shown that individual immunity is only relative, so racial immunity in the strict sense of the word does not exist. Lesser has said that neither race nor climate makes any difference in the receptivity of the virus of syphilis. Furthermore, it has been demonstrated that the children of syphilitic parents do not develop an immunity. Such children may be frankly syphilitic at the time of birth or they may be in the latent stage for years (syphilis hereditaria tardiva). Or if they escape infection and acquire the disease later on, it has never been observed to have a milder course. Gluck reports that although syphilization is almost general in Bosnia, extra-uterine acquired lues is not rare. He noted 10 per cent. of cases of recent syphilis in children under fifteen years, one-third of whom were only five or six months old.

While complete statistics and comparative studies on the course of the disease in the different races are not available, the investigations made have revealed some interesting facts. Neisser found that in the Malays primary and secondary syphilis was insignificant. Treatment is therefore deferred and the percentage of cutaneous tertiary manifestations is proportionately large. He seldom met with visceral or nerve manifestations, and never encountered tabes or paresis. In Java and the tropics generally Europeans suffer more severely than the natives, which is attributable to unfavorable climatic conditions and changed mode of living. According to Quennac, Europeans, Hindoos, and Arabs in Africa suffer severely, while the negroes only have mild attacks. In Central America, Rutschuh reported severe syphilis in the whites and negroes in contradistinction to the Indians and half-breeds who only suffered mildly. Among the Indians in the United States the infection is very destructive in some localities, as cited by Dr. Hrdlicka, while it runs a mild course in certain other tribes. This authority also states that he has never seen tabes or paresis among the Indians, although they suffer from other forms of mental disease. As to the negroes in our country, the writer has a personal communication from Dr. Green, of Milledgeville, Georgia, in which the statistics collected by him show that 5.27 per cent.

of negroes suffer from paresis as against 2.12 per cent. of whites. Tabes is rare in this race.

It is often said that the European pandemic at the end of the fifteenth century was an exceedingly malignant one. This claim is now challenged by syphilographers that the disease was malignant in the present sense of the word, that is, ulcerating lesions in the early secondary period, but rather that many of the so-called cases were ordinary ulcerating tertiary forms. Instead of malignancy *per se* and a racial susceptibility, other factors are called into account, as the undeveloped treatment, wars, famines, etc. Neisser suggests that spirochætes in their continual passage through human beings lose in virulence, or that the treatment which the patient receives so modifies the virulence that when such modified spirochætes are transmitted they produce a modified disease.

HEREDITY

The question of the transmission of syphilis to the offspring is a very complicated one, and still far from clear. While the accession of serological knowledge has presented a partial solution, there still remain certain clinical phenomena for which no satisfactory explanation can be given.

The opinion is gaining on almost every hand to-day that germinal infection on the side of the father does not take place, but that the mother, infected by the father either before or at the time of conception, transmits the disease to the fœtus through the placenta. Is this view tenable on the ground of our clinical and laboratory observations?

Disease of the testicles during the secondary stage is not common, although it has been demonstrated by animal experiments that this organ is one of the seats of election of the syphilitic virus. Later a diffuse orchitis is more frequent, and during the tertiary stage gummata are not uncommon. As to other lesions of the genito-urinary tract which might harbor spirochætes little is known.

In the transmission of syphilis the results depend upon the intensity of the infection, which intensity weakens with time.

In the secondary stage it is most active, so that a man is almost certain to infect his wife. If she becomes pregnant early abortion will result. Such a woman, if untreated, may abort later, then have a stillborn child or a living child with active syphilis, and finally, after eight to twelve to twenty years, healthy children. Such women are obviously syphilitic. On the other hand, there is the group of women who have been free from the clinical manifestations of the disease but who have had several miscarriages or children with unmistakable signs of the infection. These women, according to the Colles-Baumès law, have not syphilis but are immune to syphilis. What has taken place that prevents such a woman from developing a primary sore or a secondary rash and still has rendered her insusceptible to the disease? Is it because she has the infection or because Wassermann substances or antibodies are passed through the placenta? In other words, must we conclude in view of the positive Wassermann reaction that her tissues are harboring living spirochætes which, without treatment, are overcome by the defensive mechanism of the patient, or does she simply receive Wassermann-producing substances?

Serological research has in the main overthrown Colles's and Profeta's laws. It has been demonstrated by a host of investigators that the majority of women apparently healthy who give birth to syphilitic children have a positive Wassermann reaction and are therefore in the latent stage of the disease. Furthermore, Baisch, Trinchese, and Weber constantly found spirochætes in the maternal portion of the placenta and in the intervillous spaces even in negatively reacting women. The claim then that Wassermann-producing substances pass over from the fœtus to the mother had to be dismissed as the persistence of the reaction after birth was strong presumptive evidence of the presence of living spirochætes, their demonstration in the placenta supporting the contention. The transmission of organisms to the fœtus by way of the placenta finds its analogy in other diseases, such as smallpox, relapsing fever, and tuberculosis. In tuberculosis this takes place only in advanced and florid cases in contradistinction to lues, where no

clinical evidence of the disease may be present, multiplication of the spirochætes probably taking place through the impetus given by pregnancy. It is interesting to note in passing that Uhlenhuth found typical testicular lesions in the young of a syphilitic mother rabbit, illustrating that the organisms pass through the placenta. Varying degrees of latency are met with. In some women a negative Wassermann in the blood is accompanied by a positive reaction in the colostrum of the breast, or the placental blood may be stronger than the rest of the serum. The evidence submitted in favor of maternal transmission is convincing, but the question as to how the mother received her infection is not so easy of solution. Is she inoculated prior to or at the time of conception or later? Do the organisms penetrate the ovum or do they enter the tissues of the mother without the production of the usual sequence of symptoms? It has been shown experimentally that the semen of latent syphilitic men may from time to time contain spirochætes. As many of these patients have had more or less treatment, it has been suggested that an attenuated form of the disease is transmitted to the wife. There are two ways in which paternal transmission could take place. In the one we may assume that the parasite is carried in the head of the spermatozoön and with it penetrates the ovum. As the organism is three times the length of the spermhead this possibility is dismissed by the majority of syphilographers. The other alternative is that the spirochætes are free in the semen and with it enter the egg cell. Such an advent, it is believed, would have an untoward effect on the fertilized ovum, as it has been shown that the disturbance of a single blastomere in lower vertebrates is sufficient to arrest development or cause malformation, and the regenerative powers are greater in these animals than in the higher vertebrates (Weber).

LATENCY

What is our present conception of latency in syphilis? During these periods of apparent quiescence does the defensive mechanism hold the pathogenic agent in abeyance or is some

pathological process insidiously undermining tissue in some part of the body? The application of the Wassermann reaction has materially changed our views since the demonstration in a large number of so-called latent syphilities of involvement of the aorta and the central nervous system. It has been shown that such patients may have a chronic meningitis for years without producing obtrusive symptoms, and a limited aortitis may persist for a long time without making its presence known either subjectively or objectively. Aside from gummatous involvement of the viscera, little is known of the effects of the infection on the various organs. Where must we seek the explanation of persistent Wassermann reactions in cases intensively treated in whom involvement of the central nervous system and the heart and aorta can be excluded? A solution will probably be reached when the nature of the Wassermann reaction is fathomed, but on *a priori* grounds if a focus in the aorta or nervous system is capable of keeping up a positive reaction indefinitely, it is reasonable to assume that a similar process in one of the viscera may be provocative of a like result.

It is often asked where the spirochætes are lodged during the latent period. A number of investigators have urged that, during this stage, resting forms of the organisms are harbored by the lymph-nodes or blood-forming organs. Present knowledge does not support the theory of a cycle of evolution with the power to produce lesions according to the stage of its development. Spirochætes recovered from any source whatever—chancre, mucous patch, or gumma—the blood, nervous system, or viscera have all shown similar morphological and practically the same biological characteristics.

The relation of trauma to the localization of specific lesions has been so well appreciated by the medical profession that it is given a prominent place as an etiological factor. The query naturally arises as to whether the spirochætes in such cases have remained *in loco* since their primary deposition or are carried there by the blood after injury to the tissue. Pasini found spirochætes in an atropic and pigmented spot two years after the involution of a papular syphilide, while Hoffman

demonstrated them in scar tissue of chancres long after their regression. Levaditi and Yamanouchi were able to recover them from the cornea of a rabbit 113 days after the keratitis had healed and at which time a recurrence took place. The so-called chancre redux is interpreted by many as the result of organisms which have escaped destruction rather than as a superinfection with a new strain. Neisser has demonstrated that spirochætes may be present in the skin without producing any macroscopic changes, and more recently Whartin has called attention to their presence in the heart without exciting tissue reaction.

Studies on the infectiousness of the blood in the various stages of syphilis have supplied us with the following data: In 19 cases of primary syphilis, Uhlenhuth and Mulzer obtained positive results in 16 rabbits injected with 2 c.c. of defibrinated blood into the testicle; in 36 cases of early secondary syphilis, 27 positive results, and in 15 cases of latent syphilis, 2 successful inoculations. One of the latter was with the blood from a woman whose infection was four years old and who eighteen days before had given birth to a syphilitic child. Lieberman also succeeded with the blood from a woman whose primary lesion dated back four years and who six weeks before had given birth to a luetic child. Frühwald reports 2 cases: In one the disease had existed for one year, the Wassermann was positive, and the patient had had two doses of neosalvarsan, each 0.75 Gm.; in the other the infection was one and a half years old, the Wassermann was positive, and the patient had received two doses of neosalvarsan of 0.6 Gm. each.

Numerous attempts have been made with the blood of paretics and tabetics, but with little success. Levaditi demonstrated spirochætes in the blood of one patient with paresis, and Graves reported positive results with blood from two paretics. Ellis's experiments to confirm these findings gave constantly negative results.

The above facts are in accord with the clinical teaching that the blood of luetic individuals is infectious during the active primary and secondary stage, and that this diminishes with the

age of the disease. Exceptions to the innocuousness of the blood of latent syphilitics are seen in the occasional infection of a surgeon in his operative work on an individual in this period of the disease. It is to be inferred therefore from clinical and laboratory experience that, under conditions of which we are at present ignorant, spirochætes may reach the circulation from some focus in the body. In certain cases this may precede the cutaneous relapse, in others it is accompanied by no visible manifestations. Kraus has called attention to febrile attacks as the only symptom of latent lues, and suggests the spirochetæmia as a possible explanation. An analogy is found in trypanosomiasis where the parasites may disappear spontaneously from the blood and, reappearing, give rise to an eruption and fever. Moreover, a latent period of three years may ensue before the nervous symptoms appear.

PATHOLOGICAL ANATOMY

The predilection of syphilis for the vascular system is noted almost from the inception of the disease in the involvement of the cutaneous vessels at the point of inoculation. Here the pathological process consists of an endarteritis, later a panarteritis, with a characteristic inflammatory infiltration. Excepting the macule, all the lesions succeeding the chancre show a marked affection of the vessels, especially those of the late secondary and tertiary stages. The formation of giant-cells so frequently encountered in the secondary papule, the nodular syphilide and gumma may be traced to a vascular genesis, while the extension of serpigenous lesions may be explained by the progressive thrombosis of the vessels.

A study of the pathological anatomy of syphilis shows that fundamentally the reaction is on the part of the fixed connective-tissue elements, the labile constituents coming into play locally only secondarily. The chief cells are the lymphocyte and the plasma cell, the latter believed to be a derivative of the former and the antecedent of the fibroblast. It has been shown by Hazen that a circulatory leucocytosis is present in untreated secondary cases, sometimes as high as 20,000 white cells. The

neutrophiles are absolutely and relatively increased and the percentage of eosinophiles is higher. Under treatment there is a slight drop in the total count, with a marked relative increase in lymphocytes which under treatment may run as high as 65 per cent.

The syphilitic process is essentially a granuloma having its origin in the perivascular lymphatic spaces. In the primary lesion the main changes are found in the cutis, the very earliest of which are in the new formation of capillaries and the grouping about these and pre-existing vessels of lymphocytes and plasma cells. At first they mantle the vascular structures as a "coat-sleeve" infiltration, but as the lesion grows older, spread out and become diffuse. The endothelium of the capillaries is swollen and proliferated so that the lumen is narrowed or altogether occluded, and in the larger vessels with an external coat there is in addition evidence of inflammation and an increase in thickness. In some instances giant cells are found. From the newly-formed granulation tissue, connective tissue is formed which later scleroses and leads to induration. Owing to interference with nutrition, regressive metamorphosis sets in. The epidermis presents a varied picture, depending on whether there is pressure from the infiltrate or retrograde changes with erosion and ulceration.

In the secondary stage the disease is characterized by a succession of eruptions and a general adenopathy. Ehrmann has said that the distribution of secondary syphilides is governed by the branching of the vascular stems. The roseola or macular syphilide under the microscope shows very few changes, being simply an erythema with dilatation of the vessels of the papillary body and adjoining corium and an infiltration of lymphocytes and plasma cells about them. In the papular or lenticular syphilide we find in the cutis a circumscribed lesion made up of lymphocytes, plasma cells, and proliferated fibroblasts, all in close relation with the vessels which show the characteristic changes. Lichen syphiliticus owes its peculiar features to localization, namely, distribution about the pilosebaceous apparatus. Here the process surrounds a hair follicle

and extends deeply into the corium. Its structure is like that of other lesions, with usually abundant giant cells of vascular origin. The epidermis in all lesions of this stage shows only secondary changes. It may be thinned from pressure of the granulomatous tissue beneath or there may be œdema with an increase in thickness and scaling. In condylomata papillomatous overgrowth is a marked feature. Pustular and suppurating syphilides usually signify extraneous inoculation with pus-producing organisms. The pigmentary syphilide, or leucoderma syphiliticum, according to Ehrmann, is due to chromatophores, the pigment passing from them to the basal layer of the epidermis.

As a rule, secondary syphilides undergo spontaneous absorption. Microscopic residua may, however, persist for a long time. Their connection with local relapses has been suggested.

The type of lesion of the tertiary period is the gumma. The process consists of an infiltration of lymphocytes, plasma cells, and proliferated connective-tissue cells about the vessels, newly-formed and old, which are the seat of an endarteritis and panarteritis, and give rise to giant cells. Caseous degeneration usually begins in the centre of the granuloma, or in some cases it may be fatty or mucoid in character. The last mentioned is most often found in bones. The necrotic areas liquefy and are absorbed or discharged, the usual procedure being gradual absorption, with formation of cicatricial tissue, the contraction of which leads to deformity. It is believed by many that the fibrosis is not purely a process of repair or due to the irritating action of necrotic tissue, but that there is also a syphilitic element. The plasma cell thought to be the precursor of the fibroblast has been credited with being more than a passive participant. The late nodular or tubercular syphilide is in reality a gumma, situated more superficially in the cutis. The serpigenous lesions which also belong to this stage are made up of groups of nodules situated about the cutaneous vessels, the thrombosis of which probably explains the progressive character of the lesion.

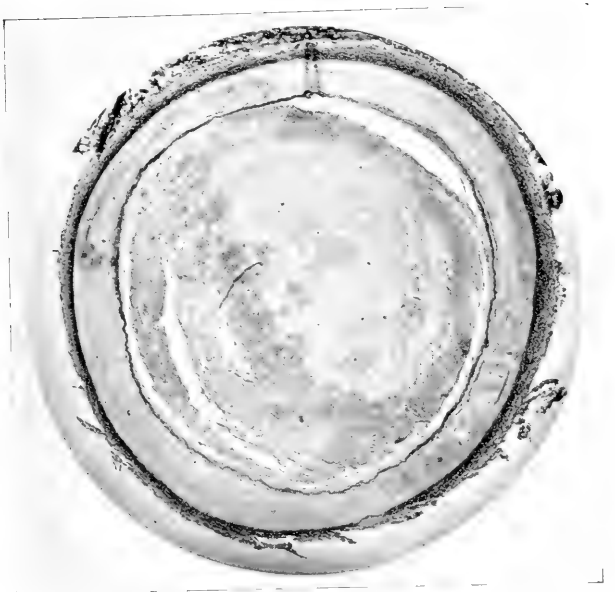
Syphilitic phlebitis is relatively infrequent, occurs usually

in the veins of the lower extremities, and is of minor importance. Arterial disease, however, is very common and of serious import, the vessels especially attacked being the aorta, the cerebral, pulmonary, subclavian, femoral, and popliteal arteries. Owing to disease of the walls, vasomotor response is impaired or lost, and where there are obliterative changes due to endothelial proliferation or secondary thrombosis, interference with the blood supply and impairment of nutrition. While the brain and the heart suffer more severely in this respect, other organs and the vessels of the extremities are not exempt. Several years ago the writer had under observation a case of gangrene of the leg consecutive to a syphilitic thrombosis of the femoral artery (Fig. 1). The patient was a colored girl, in the City Hospital, upon whom several amputations were made, after each of which the gangrenous process developed and spread. A picture closely simulating Raynaud's disease is also met with as the result of luetic involvement of the vascular supply of the extremities, and in several cases of symmetrical cutaneous atrophy which I have had under my care the syphilitic element was the predominant feature.

AORTITIS

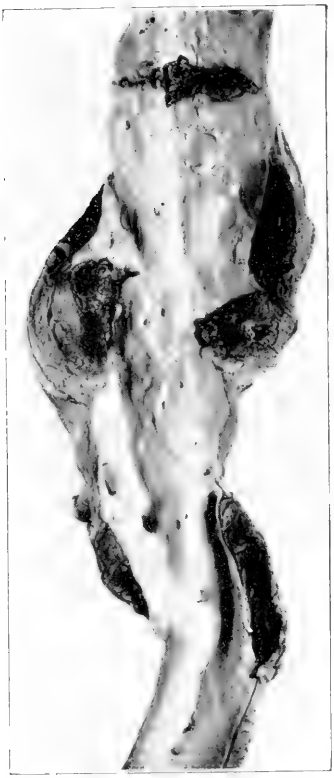
It is only within the last decennium that the syphilogenic nature of aortitis or mesaortitis has been generally recognized among internists and syphilographers. During this period the development of the Wassermann reaction and röntgenology, as supplementary to the physical examination, have shown us how widely prevalent the disease is among individuals who have had a syphilitic infection.

As to its frequency of incidence, Chiari found the condition present in 59 per cent., Fahr in 29 per cent., and Fraenkel 53 times in 102 cases of constitutional syphilis. Stadler, whose statistics cover 256 cases, demonstrated the disease in 82 per cent., and of 211 of this number it was the cause of death in 117. Staub found an aortitis in 82 per cent. of paretics which came to autopsy, Buder in 84.5 per cent., and Alzheimer in 74 per cent. The findings of Rach and Wiesner show that in



Courtesy, The American Journal of the Medical Sciences.

FIG. 1.—Syphilitic thrombosis of femoral artery. Transverse section of artery, showing thrombus and thickening of arterial coats.



Courtesy,
The American Journal of the Medical Sciences.

FIG. 2.—Syphilitic aortitis, showing characteristic changes in the walls of the vessels, transverse rupture, and dissecting aneurism into coats of the aorta. (Specimen of Dr. John H. Larkin.)

congenital syphilis, changes in the aorta or pulmonary artery are seen in 67.4 per cent. Lenz states that in large cities 25 per cent. of all syphilitics die from aortitis (angina pectoris, aortitic insufficiency, aneurism) as against 3 to 4 per cent. from paresis, 1 to 2 per cent. from tabes, and 10 per cent. from all other syphilitic affections, as of the brain, liver, kidneys, etc.

Mesaortitis has been defined by Doehle, Heller, and others as a specific inflammation of the adventitia and media which terminates in cicatricial deformity. As a rule, there is a combination of processes so that cicatrization may be present in one part while active inflammatory lesions are found in another. The disease shows a decided preference for the ascending portion of the aorta and the arch, the explanation being that the impact of the blood is greater here than in the rest of its extent, following the well-known law that syphilis localizes where trauma has produced a *locus minoris resistentiæ*. In contradistinction, atheroma selects the lower portion of the aorta where the mechanical element is of slighter moment. Stadler could find no relationship between an increased blood-pressure or marked variations in pressure. Strümpell favors a summation of injurious agencies, as the noxious influence of alcohol and tobacco, with consequent lowering of resistance of the vessels.

Grossly the aorta gives a characteristic picture (Fig. 2). The inner surface shows isolated or confluent elevated, wrinkled, grayish, and translucent sclerous areas, with puckering or cicatricial pitting, or again radiating ridges with depressed cicatrices between. The intima over the affected areas preserves its glistening appearance. The thickness of the artery varies in different portions, so that while indurated and sclerotic at one point it is thin and translucent at another. Depending on the extent of involvement, dilatation is present. This may be uniform throughout the length of the thoracic portion or appear as small aneurismal pouchings or a true saccular aneurism. All transitions are found. Ordinarily it is not difficult to differentiate this condition from arteriosclerosis in

the absence of fatty degeneration and calcification. However, in old subjects there may be a combination.

In the beginning the lesion is often confined to the vessel in proximity to the aortic valves. Later it extends to them and gives rise to insufficiency, which condition is very frequent. With the extension of the process the ascending aorta and the arch are involved. Not rarely changes are also noted about the mouths of the larger branches of the arch, causing narrowing of the left carotid, innominate, and subclavian arteries, which localization is significant from a clinical point of view. With involvement of the aortic ring the mouths of the coronary arteries are also considerably reduced.

The relation of syphilis to aortitis was for a long time disputed. On the one hand investigators classed it with parasyphilis because of the difficulty of demonstrating spirochætes, its indurative or fibrotic tendencies, and indifferent results from treatment. On the other, which has the support of recent examinations, it was classed with active syphilis. The demonstration of spirochætes in the aortic wall by Reuter, Schmorl, Wright, Richardson, and others in acquired syphilis, and by Wiesner and Rach in congenital, together with the microscopic picture of gummatous lesions, and a positive Wassermann reaction in about 80 per cent. of cases, definitely places the affection in the category of active syphilis. It is difficult to demonstrate the organisms even in comparatively recent and active lesions. This, however, does not militate against their syphilitic nature, as they are equally difficult to demonstrate in cutaneous gummata. In both, the tissue is probably in a state of altered reactivity, so that numerically few organisms are capable of bringing about the necrotic change. The striking changes are in the media, although the disease begins in the adventitia. Miliary gummata undergo necrosis and are replaced by cicatricial tissue. In the adventitia in recent processes an inflammatory infiltrate of lymphocytes and plasma cells is localized about the vasa vasorum (Fig. 3), while in older ones only fibrotic changes may be left (Fig. 4), the nutrient vessels themselves being the seat of an obliterating endarteritis, such as is found in syphilitic

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FIG. 3.—Early stage of aortitis, showing characteristic lymphocytic and plasma-cell infiltration about the small vessels in the adventitia.

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FIG. 4.—Later stage of aortitis, showing advanced sclerosis of the vessel walls.

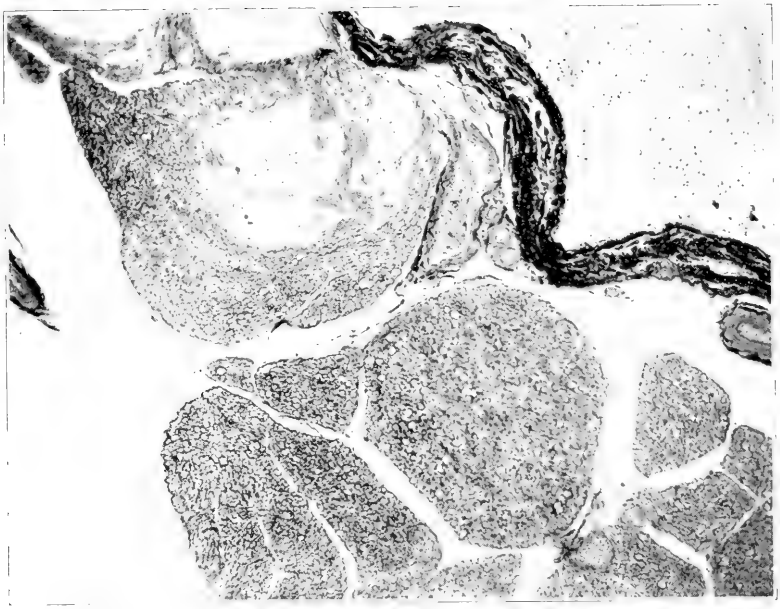

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FIG. 5.—Insular sclerosis, posterior nerve root, with accompanying meningitis. Posterior columns not involved.

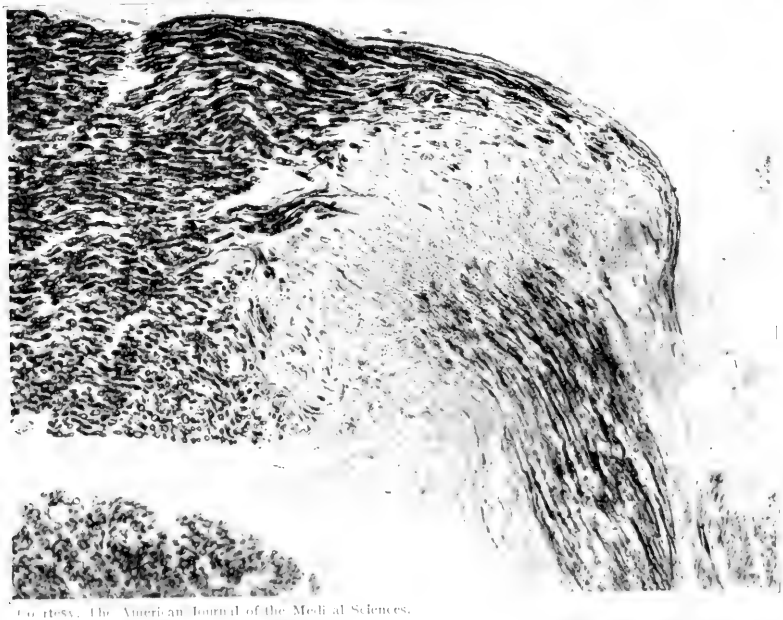

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FIG. 6.—Degenerated posterior nerve root in tabes. Posterior columns involved.

lesions elsewhere in the body. The elastic tissue, and this is characteristic of luetic disease of the larger vessels, is either fragmented or has entirely disappeared. The intima shows no change at all or various grades of a compensatory thickening. On the question of genesis of the early changes there is some dissonance of opinion. Doehle, Heller, Backhaus, Saathoff, and others trace the beginning of the disease to the vasa vasorum. They claim, primarily, a swelling of the endothelium of these vessels in the middle third of the media to large epitheloid cells, which lead to an obliteration with secondary regressive metamorphosis of the aortic wall. Marchand, Beneke, Arnsperger, and others not corroborating these findings incline to the view that the medial changes are primary, the virus so injuring the elastic lamellæ that they succumb to the pressure of the blood. The difficulty of determining the genesis with positiveness even early is apparent, for, as Stadler points out, a simple infiltration of the vasa vasorum may also occur under the influence of the toxins of alcohol and other infections. Benda describes two forms of aortic disease. In the one submiliary lesions without necrosis which cicatrize and leave none or only a very slight deformity of the vessel wall, in the other typical gummata, with central necrosis, the scarring of which leads to typical sclerotic changes in the wall.

The clinical diagnosis of aortitis is often extremely difficult. Limited and circumscribed changes of the ascending portion without involvement of the ring are, as a rule, not accompanied by subjective or objective manifestations. Such patients may be observed for a long time without eliciting signs pointing to the condition, Röntgen-ray examination also being negative. Only when a greater area is involved, especially more or less of the circumference, are there symptoms suggestive of disease, namely, pain, dyspnœa, and tachycardia on slight exertion, increased blood-pressure, weakness, and easily-induced fatigue.

The pain is often the primary and predominant symptom, although frequently it is lacking or indefinite. It is characterized as a dull aching or a feeling of pressure under the sternum, with pain radiating to the side of the chest, the back, and the

arms. Disease of the innominate, carotid, and subclavian may be attended by a condition simulating intermittent claudication of the lower extremities, with weakness, radiating pain, and sensory disturbance. Periodic or continuous headaches, often with vertigo, accompany involvement of the mouth of the left carotid, and where the coronary arteries are the seat of disease the attacks are anginal in character. There appears to be no satisfactory explanation for the origin of the pain. Thoma referred it to injury of the larger and smaller nerve trunks as well as irritation of the Pacinian bodies in the aortic wall. Huchard attributes it to a peri-aortitis, and the neuralgic pains in the chest and shoulder to the changed relation of the subclavian to the brachial plexus. Longcope came to the conclusion that the anginal attacks were due to reflex disturbances set up by the syphilitic process involving the root of the aorta, the paroxysmal dyspnœa being regarded as an acute bronchiospasm.

When does the aortic disease begin? Obviously this is difficult to affirm, as the disease in the majority of cases is insidious in its onset, the first symptoms appearing only when the affection has made some progress. In assuming that involvement takes place at the close of the primary stage when there is a general dissemination of the spirochætes, we are confronted with the same problem as when we endeavor to establish the time of infection of the central nervous system. Palpitation, arrhythmia, tachycardia, and disturbances in the pulse-rate frequently occur in early secondary syphilis, which has been adduced as evidence that involvement takes place at the time the disease is a spirochætemia. The publication of fatal cases shortly after infection also inclines to the view that involvement takes place early. Brooks recorded perforation of an aneurism before the secondary rash had fully appeared, and another case with a fatal aortic lesion within six months of infection. Longcope cites two patients who died from the effects of syphilitic aortitis four years after the appearance of the chancre. Stadler publishes two cases in whom death occurred five years after infection. The first patient, aged twenty-nine years, showed at autopsy a fibrous aortitis and aneurism. The second, aged twenty-seven

years, the objective and subjective signs of an aortitis. He further studied 12 post-mortem cases of severe secondary and tertiary syphilis in patients under thirty years of age. He assumed on account of the youth of the subjects that the infection could not have been of long standing, but in none were changes of syphilitic aortitis demonstrable.

The writer has recently investigated, in his private practice, all latent syphilitics, especially those with a persistent positive Wassermann, for the occurrence of cardiovascular disease, and in a large percentage has found the existence of such a lesion.

The average age at which the disease appears is forty-seven to forty-nine years, but it is not rare to find it in much younger patients. Aortic disease in general makes slow progress, for Weintraud, Stadler, and others give the average lapse of time as twenty years, the interval vacillating between five and forty years. The disease usually runs a fatal course in about two years after the development of the symptoms. The importance of early diagnosis is, therefore, apparent, especially in view of the fact that the affection is a manifestation of active syphilis and amenable to treatment. In 95 cases of aortitis collected by Benda the termination was as follows: in 9, coronary stenosis; in 22, aortic insufficiency; in 37, combined coronary stenosis and aortic insufficiency; in 27, aneurism. In a total of 248 cases he found aneurism forty-eight times.

The relation of cardiovascular affections to tabes has for a long time occupied the attention of clinicians. According to Lesser's statistics in 96 cases of tabes come to autopsy, aneurism of the aorta was found 18 times. A review of the literature shows that the coincidence of tabes and aortic insufficiency is most frequent. Rogge and Ruttner report the association in 6.5 per cent.; Rogge and Müller in 10 per cent.; Stintzing in 3.8 per cent., and Stadler in 6.2 per cent. of all cases of well-developed tabes. The latter did not include his cases of incipient tabes; in manifest tabes which came to autopsy, however, he found disease of the aorta in almost all. Rogge and Müller have called attention to the cause of sudden deaths in tabetics as not

being due to lesions referable to the central nervous system but to sudden cardiac insufficiency or rupture of an aneurism. The so-called cardiac crises are in reality attacks of angina pectoris, due to changes in the coronary arteries.

Strümpell has emphasized the surprisingly frequent presence of rudimentary tabes in patients who seek medical advice for symptoms referable to the heart. Many of these cases show only pupillary changes, such as narrowing, irregularity, and fixation to light. Only one pupil may be affected, as in a case cited by him with severe endocarditis, myocarditis, and aortic insufficiency. The reflexes are only slightly altered or the Achilles reflex alone lost and the patellar reflex exaggerated. Observing these cases over a long period of time, weakening and gradual disappearance of the reflex may be noted. In general, symptoms of aortic disease appear later than the earliest tabetic manifestations. Rogge and Müller are of the opinion that tabetic symptoms average four and a half years earlier than those of the circulatory apparatus. It is probable that it is not a later involvement, only that the condition has a longer latency and therefore manifests itself later.

The writer has under treatment at the present time several patients with tabes and concomitant aortitis. One of them, with optic atrophy, has an aneurism. Five others, in whom the tabetic symptoms have existed on an average of eight years, show a marked sclerosis of the aorta. In a paretic, Röntgen-ray examination shows a dilatation of the ascending aorta and in a case of cerebrospinal syphilis, with cardiac symptoms developing four months ago, a small aneurism. In another patient, aged thirty-one years, who had had an attack of hemiplegia four years ago, signs of aortitis have developed within the past few months. The Röntgen-ray plates also show a marked sclerosis.

For the following interesting post-mortem examination and also some of the illustrations which accompany this article I am indebted to Dr. John H. Larkin, Director of Pathological Laboratories, Department of Charities, New York City:

J. E., aged forty years. The patient was admitted to the City Hospital (service of Dr. Evan Evans) the latter part of 1913 for sciatica. After a few weeks he was discharged and five months later readmitted, at which time in addition to the sciatic pains he complained of girdle pains. Lumbar puncture showed 38 cells, an excess of globulin, and a strongly positive Wassermann. His serum was also positive. A diagnosis of tabes without clinical evidence, excepting the girdle pains, was made. Further examination, including Röntgen-rays, revealed an aneurism of the ascending aorta. He was placed on salvarsan and mercurial treatment, which reduced his cell count and reversed the Wassermann in the fluid, but had no effect on that of the blood. The patient died suddenly from a rupture of the aneurism into the thoracic cavity. Microscopically the aorta showed a characteristic picture with a round-celled infiltration in the adventitia. The interesting feature of the finding in this case was in the cord, which showed an involvement of the pia-arachnoid with a round-celled infiltration about the small vessels and an insular degeneration of the posterior nerve roots (Fig. 5). The posterior columns were unaffected.

NERVOUS SYSTEM

With the acquisition of more exact knowledge our ideas concerning syphilis of the nervous system have undergone considerable revision. The present views based on clinical and laboratory investigations may be formulated as follows: Infection of the central nervous system probably takes place in the early stage of the disease with the generalization of the virus. Accumulated clinical evidence points to meningeal involvement during the first few months, in some cases before the appearance of the cutaneous eruption. Lumbar puncture in the hands of different investigators has elicited varying results. Thus, Ravaut found the spinal fluid in secondary syphilis abnormal in 67 per cent. of cases, Altmann and Dreyfus in 78 per cent., Nonne in 40 per cent., and Swift and Ellis in 36 per cent. The writer's findings, based on the examination of cases in the secondary stage with and without cutaneous lesions, showed meningeal involvement in less than 20 per cent. Fournier's dictum that patients in whom secondary symptoms are overlooked or very mild later show involvement of the central nervous system has been reiterated again and again. This led Finger to divide syphilitics into two classes: those with manifest cutaneous

lesions, and those with absent or mild skin lesions and meningeal involvement, which division is too arbitrary, for many cases with severe cutaneous lesions as well as those which have been treated early later develop disease of the nervous system. The observation of patients over long periods of time has shown that a low-grade meningitis may exist for years without producing obtrusive nervous symptoms. Such lesions are comparable to the superficial lingual and palmar syphilides, which persist for years, produce little inconvenience, and are refractory to treatment. There has been a good deal of speculation as to whether the spirochætes producing lesions in the central nervous system are the remains of those deposited during the spirochætemia or whether they later reach the nervous system from another focus. Head's work is perhaps suggestive. In seeking to explain the more frequent involvement of some areas in root affections over others, he found that the roots most commonly subjected to irritation were those in connection by their visceral afferent fibres with certain organs known to be the seat of active spirochætosis in syphilis. Thus the second and third cervical contain afferent paths from the tonsil; the first, second, third, and fourth thoracic from the aorta, while those from the seventh thoracic to the first lumbar, the most frequently involved, carry afferent paths from the liver, kidney, suprarenal, and testicle, organs which are the sites of election of the parasite. Orr and Rows have shown in their investigations on the lymphogenous infection of the nervous system that organisms and their toxins travel along the perineural lymphatic space which surrounds every spinal nerve and extends along the roots to the pia mater. These spaces are not only in connection with the pia, but through the latter in communication with the adventitial lymph spaces of the perforating vessels. The rôle of these channels as distributors is therefore quite obvious.

The sharp distinction formerly drawn by clinicians and laboratory workers between cerebrospinal syphilis and parasyphilis is no longer tenable. Gradually the barrier has given way until we have come to regard these conditions as identical from an etiological stand-point, though differing in reaction,

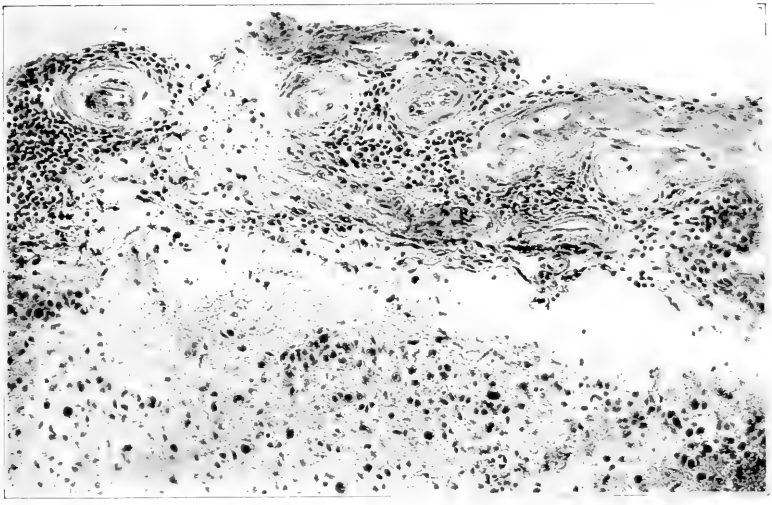

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FIG. 7.—Syphilitic meningitis, showing various stages of obliterating endarteritis.

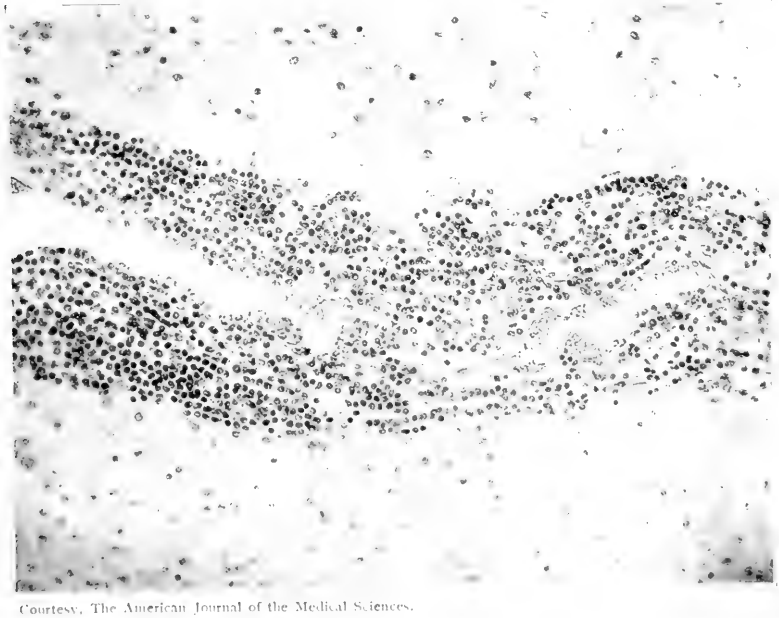

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FIG. 8.—Mantling infiltration of lymphocytes and plasma cells about pial vessel in brain cortex.

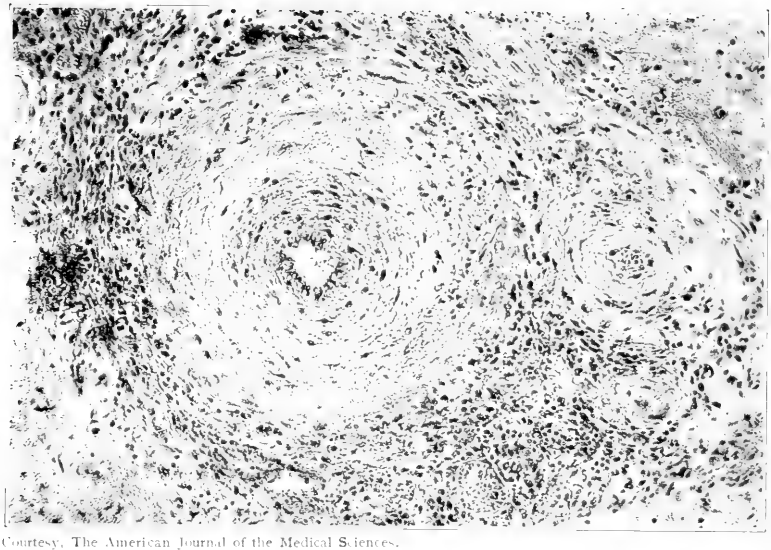

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FIG. 9.—Heubner type of obliterating endarteritis in syphilitic meningitis.

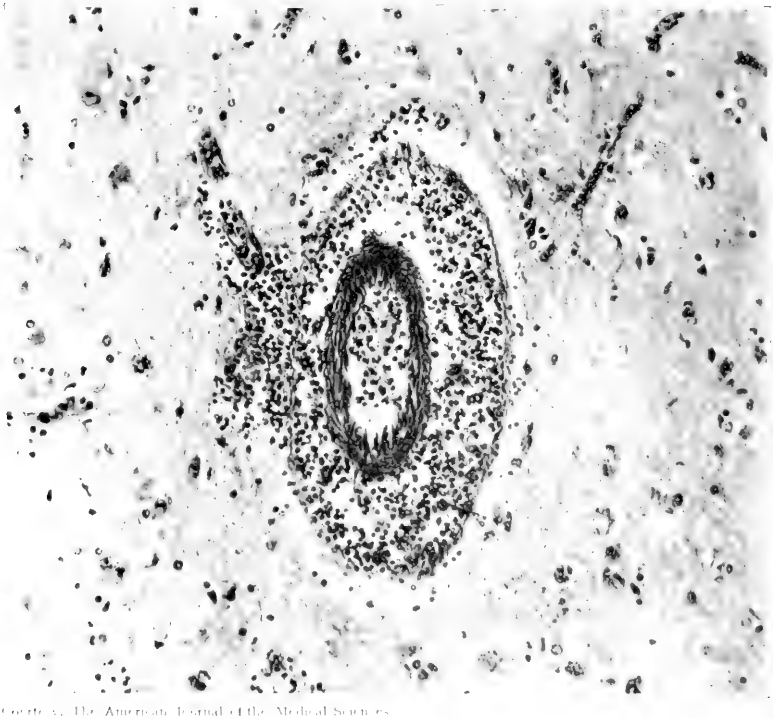

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FIG. 10.—Small vessel in cortex of the brain, showing infiltration of perivascular spaces with lymphocytes and plasma cells.

which difference depends on localization and the tissue involved. By cerebrospinal syphilis we understand the exudative, vascular, and gummatous processes which involve the coverings of the nervous system and the blood-vessels within them (Figs. 7, 8, 9 and 10). These processes in the great majority of cases remain superficial, but in some cases extend into the essential nervous structures along the pial or adventitial sheaths of the vessels, and give rise to the borderline cases of tabes and paresis.

It is proposed by Head and Fearnsides to divide luetic disease of the central nervous system into syphilis meningovascularis and syphilis centralis, the latter including all those cases where degeneration of nerve tracts or nuclei shows that the lesion must lie within the structure of the nervous system itself. As meningitis and endarteritis, however, are common to all these pathological states, this classification does not seem to the writer a tenable one.

What is the nature of paresis and tabes? In paresis there is a combined meningitis and encephalitis with a typical infiltration of lymphocytes and plasma cells in the adventitial lymph spaces (Figs. 11 and 12), the secondary degenerative changes probably depending on the primary vascular disease. Likewise we have a meningitis in tabes, but it is easier to comprehend the pathological condition and manner of invasion in paresis than the tract degeneration in the cord. The view that the genesis of both affections lies in a chronic meningitis now has a pretty wide acceptance. In tabes, Nonne believes it leads to disease of the roots and secondarily of the posterior columns. Nageotte was the first, in 1894, to describe a low-grade meningitis at the junction of the anterior and posterior roots on the proximal side of the ganglion, and in addition a neuritis, as shown by an inflammatory condition in the perineurium and a perivascular infiltration of plasma cells and lymphocytes in the posterior roots. This was confirmed by Dinkler, Dejerine, and others, but their views were not generally accepted, as the findings were interpreted as a combination. Again, in 1906, Schröder showed that collections of lymphocytes and plasma cells occur in the lymph spaces of the vessels in paresis and

tabes, and that in the latter they are found not only in the pia and connective tissue about the vessels in the peripheral parts of the cord, but in the intramedullary portion of the columns as well.

Stargardt's findings in optic neuritis also have an important bearing. In a number of cases of tabes and paresis he found a true inflammation of the sheath and endoneurium. He also described an active inflammatory process in the joint structures of tabetic arthropathy, as evidenced by plasma cells and lymphocytes, with endarteritis of the small vessels. Steiner's studies on peripheral nerves in tabes and paresis also brought him to the conclusion that the process is an inflammatory one. The older view of a primary neuron degeneration in the light of present evidence would seem to have to be abandoned.

According to McIntosh, Fildes, Head, and Fearnsides, parasyphilis "depends upon an anaphylactic reaction in the tissues of the central nervous system which have been rendered hypersensitive in accordance with their peculiar lymphatic distribution." Since it has been shown by Marie and Guillain, Orr and Rows, and others that the posterior columns are in direct lymphatic communication with the posterior roots, the aforementioned authors have evolved the theory that sensitization takes place during the secondary period by the spirochætes or their toxins ascending the afferent peripheral nerves. When spirochætes again become active in the posterior columns, a violent reaction may ensue, injuring the nerve fibres and producing proliferation of the neuroglia and wandering cells. The injury of the nerve fibres will lead to degeneration, which will continue as far as the reflection of the neurilemma. In the same way the cerebrum or isolated groups of cells in the anterior horns or other groups of fibres or cells alone or in combination may be affected, the particular region involved determining the clinical picture. Noguchi had previously shown by animal experiments that sensitization may be necessary before spirochætes can infect the brain. In both rabbits and monkeys he obtained negative results when the organisms were inoculated directly into the brain. He therefore injected rabbits with living and

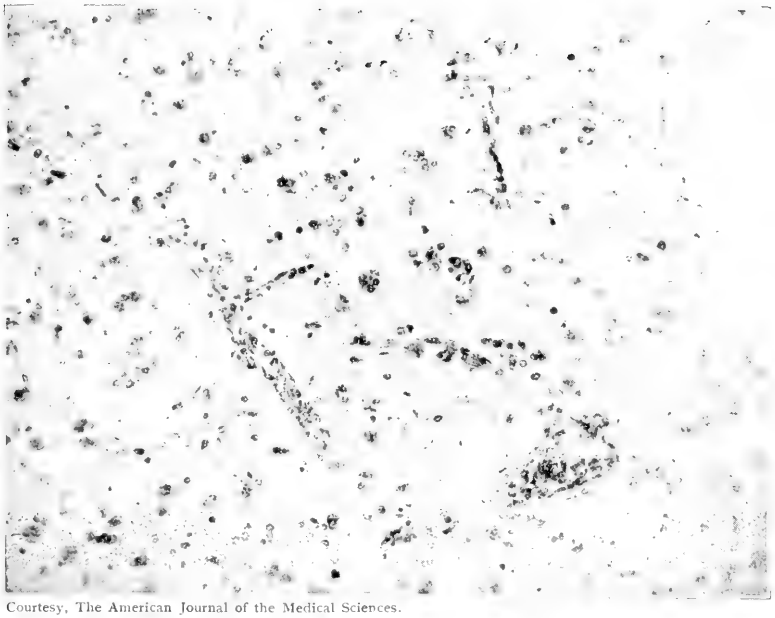

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FIG. 11.—Showing the characteristic picture of paresis, with plasma-cell infiltration.

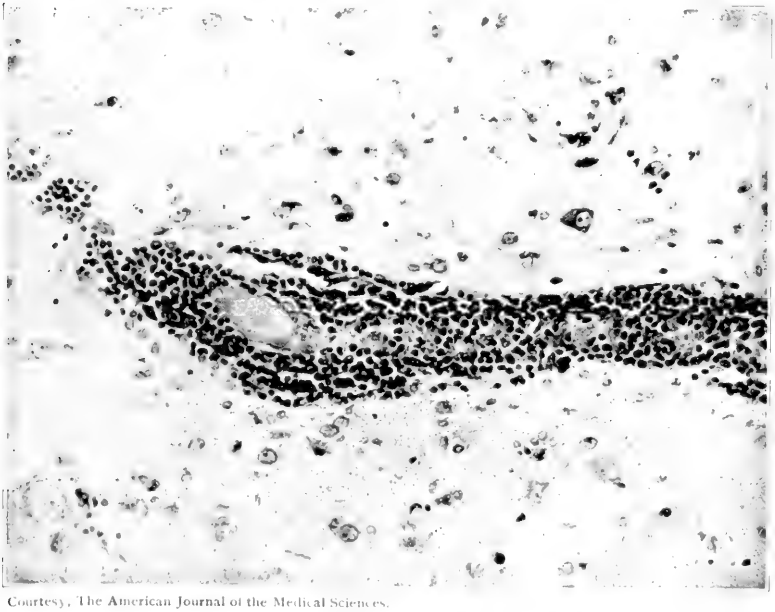

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FIG. 12.—Paresis, showing the grouping of lymphocytes and plasma cells about small vessels in cerebral cortex.

dead spirochætes intravenously, and after five months inoculated them subdurally with a particle of a scrotal syphiloma rich in spirochætes. They all remained well for two months, then became emaciated, and showed a slightly ataxic and spastic gait. The symptoms continued to increase, and after five months the animals were no longer able to jump. Microscopically some of these animals showed identical changes with paresis, but the nerve-cells were intact. In three an exudative meningitis was found and in one a unilateral atrophy of the frontal lobe. The control animals without previous sensitization remained well.

Analogies are frequently drawn between tabes and paresis and sleeping sickness. Spielmeyer, as the result of extensive investigations, came to the conclusion that in incipient cases the diagnosis between paresis and trypanosomiasis is not possible. He was able to produce in dogs with *Trypanosoma brucei* an involvement of the central nervous system which was like human tabes. After nine to ten weeks the process was found in the posterior roots with beginning extension to the posterior columns, sensory trigeminus, and optic nerve. In trypanosomiasis, however, the central nervous system rarely escapes, whereas in syphilis only 1 or 2 per cent. develop tabes and 4 to 5 per cent. paresis.

CONCLUSIONS

Clinical observation and experimental work have shown that no true immunity exists in syphilis, but that an anergy develops during the first incubation period and is complete at the time of the general eruption. This refractory state usually persists as long as the body harbors spirochætes, and when it disappears, with the cure of the disease, the patient is again in a receptive state.

In the transmission of syphilis to the offspring the theory of spermatic infection has been abandoned in favor of placental infection, as the majority of mothers of congenitally syphilitic children have a positive Wassermann or show spirochætes in the intervillous spaces and maternal portion of the placenta.

The histopathology of syphilis is a uniform one. In all stages and in all organs the lesion begins in the perivascular lymph spaces as a lymphocytic and plasma-cell infiltration. The distribution of secondary lesions, according to Ehrmann, is due to a branching of the vascular stems. In the tertiary stage the ulcerative and destructive character of the lesions finds its explanation in a changed tissue reaction, the Umstimmung of Neisser, or allergy, in the sense of an increased susceptibility to the action of the organisms.

Syphilis produces a characteristic type of aortitis and is very common in individuals who have had the infection. It may be the only lesion present in so-called latent lues with a persistent positive Wassermann, and emphasizes the importance of examining all such patients for possible cardiovascular involvement. It is a frequent concomitant of paresis and tabes, especially the rudimentary form, occurring in about 80 per cent. of parasyphilitics.

Infection of the nervous system probably takes place during the secondary stage. A low-grade meningitis may exist for years or the spirochætes may remain quiescent for years until called into activity. In paresis a mantling infiltration of lymphocytes and plasma cells is found in the adventitial lymph spaces of the meninges and encephalon. From its pathological anatomy, which has been studied in all stages, it is much easier to comprehend this condition than the tract degeneration in tabes, as tabetics seldom die during the early period of the affection. The few pathological examinations which have been made reveal, however, changes in the meninges about the posterior roots between the ganglion and the cord. It is not so difficult to understand how an infiltration in this region by pressure could produce degeneration of the afferent fibres extending to the posterior columns and leading to an ascending tract degeneration following the well-known law that a destruction of the neuron is followed by degeneration along its distribution. This explanation is a much more plausible one than a primary degeneration without inflammatory manifestations.

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STRUCTURE AND RELATIONSHIPS OF THE ISLETS OF LANGERHANS*

CRITERIA OF HISTOLOGICAL CONTROL IN EXPERIMENTS ON THE PANCREAS

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THE relationship between the islets of Langerhans and the rest of the pancreatic parenchyma is of great interest to the histologist, not only because of its significance from the stand-point of the localization of the anti-diabetic function of the pancreas, but also because the question of the functional significance of differentiation, as it is understood by histologists, is at stake. If it should prove, as many investigators have claimed, that the appearance of differentiation and specialization, both cytological and histological, presented by the islets of Langerhans when compared with the acinous tissue of the pancreas, is but a mask behind which lurks a substantial functional identity, then differentiation as indicated by structural characters becomes a delusion, and the effort to analyze further the reciprocal and independent functions of the cellular constituents of mixed glands a mere waste of energy. Hence, it seems to me to be worth while to review briefly the salient features of research in this field, and to point out some of the sources of the misconceptions and contradictions which have, from time to time, obstructed its progress.

Although the islets of Langerhans were discovered as early as 1869, the twenty years which elapsed between this discovery and the announcement of v. Mering and Minkowski's experiments on the effect of pancreatic extirpation are notable only for Kühne and Lea's discovery of the peculiar glomerule-like

* Delivered February 27, 1915.

capillary net, now a familiar object to every histologist, and for Lewaschew's experiments, to which reference will be made again, on the effect on the number of islets of excessive activity of the pancreas.

V. Mering and Minkowski's discovery that complete removal of the pancreas was followed by a grave diabetes which soon proved fatal, stimulated renewed interest in the islets and, in the decade which followed the discovery, they were sought and found in the pancreas of representatives of all the vertebrate classes. Their embryonic development was studied in detail, and it was shown conclusively that they were epithelial in nature, derived from the primary pancreatic anlagen, and not, as some had supposed, mesenchymatic or nervous structures. Particularly active in this investigation were Laguesse and Diamare. The former, in a remarkable series of articles extending from 1893 to the present day, not only recorded results of the investigation of the islets in many vertebrate groups, but studied in detail their development in the sheep, and developed, on the basis of his original observations, a theory of their relationship to the other secreting elements of the pancreas, which, while similar to that of Lewaschew's to the extent that it admitted reciprocal transformations of the islet and acinous tissues respectively, claimed for the first time a specific activity of the islet cells in internal secretion. On this account Laguesse suggested that a search of the pancreas in cases of human diabetes would reveal changes involving the islets of Langerhans. Diamare also supported the theory that the islets were engaged in internal secretion.

The grounds for these assumptions by Diamare and Laguesse were purely anatomical. The search for duct connections and for lumina in the cell cords of the islets of mammals had been for the most part unsuccessful. On the contrary, the character of the islet complex and the provisions made for a rich blood supply suggested an important secretory function, the outlet for which could only be provided by the blood stream, since duct connections were apparently not present.

Meanwhile, the production of diabetes by total removal of

the pancreas, now a commonplace of the physiological laboratory, had been confirmed by Hédon, Gley, Lépine, Thiroloix and others, and Hédon, by showing that a small portion of the pancreas detached from the bowel and transplanted under the skin would protect against the onset of glycosuria, but that when this graft was removed a glycosuria equal in degree to that produced by total pancreatectomy immediately followed, had shown that the result was not due to the cutting off of the external secretion and so to the impairment of intestinal digestion, but to some direct influence of the pancreas, exerted through the medium of the blood-stream—in other words to an internal secretion. What could be more natural than to attribute this internal secretion to the very elements in the pancreas, which, anatomically considered, were specialized for that very function?

The islet theory of diabetes, thus founded, acquired great favor among physiologists, pathologists, and anatomists alike, but efforts to demonstrate it were unsuccessful until Ssobolew and Schulze published the results of their investigations on the changes in the pancreas which resulted from ligation of the ducts. These observers found that, if the outflow of the pancreatic juice were thus prevented, the acinous tissue degenerated with great rapidity, leaving, after a certain lapse of time, a gland in which the only secreting elements were islets of Langerhans. The animals showed no glycosuria; therefore, they concluded, the islets were able, by themselves, and without the aid of the acinous tissue, to mediate the antiglycosuric function.

In 1901 Opie published the results of his interesting and important investigations of the pathological conditions in the pancreas after death from various causes, and reported cases of diabetes in which the chief or only lesion involved the islets of Langerhans.

Thus, at this period, it seemed as if the paramount importance of the islets of Langerhans in respect to carbohydrate metabolism of the body was conclusively demonstrated.

Contrary results, however, were not long wanting. Investigations of cases of human diabetes revealed a large number

in which no lesions of the islet could be detected, and studies of non-diabetic pancreases showed some in which the islets were profoundly changed. Wright and Joslin found islet changes in but two out of nine autopsies on diabetic patients, Herzog studied five cases, in one of which the islets were completely absent, in another degenerated, and in the remaining three affected to some extent. Hansemann, on the other hand, found in 34 cases only six in which the islets were degenerated and even in those six a certain number of normal islets were found. Sauerbeck, in a general summary of the pathological findings of various observers published prior to 1904, found that, in 40 out of 157 cases, the islets were reported as normal. Heiberg, while admitting the frequent occurrence of degenerations, considers that the greatest stress should be laid on the quantitative reduction of the islet tissue, a conclusion which he supports by careful counts of islets in normal and diabetic cases. Weichselbaum claims that in the cases where other observers have reported normal islets, there is, in reality, islet exhaustion evidenced by diminution of islet granules, or by early or advanced hydropic degeneration. Cecil, in 90 cases, reported that seven-eighths of the total number showed pancreatic lesions, and that wherever lesions of the pancreas accompanied diabetes the islets were involved. Recently Major has reported thirteen cases, in seven of which the islets of Langerhans were normal in every respect. Thus, the hopes which were raised by Opie's discovery have not been fully realized in subsequent investigations.

The questions raised by Ssobolew and Schultze concerning the fate of the several pancreatic tissues after litigation of the duct have met a similar fate. The results obtained by different investigators are so contradictory that Allen in his book on "Glycosuria and Diabetes" has divided them into four groups, viz., those who report survival of islet tissue alone, those who report survival of acinous tissue alone, those who report survival of both tissues, and those who report survival of neither.

The most serious attacks on the islet hypothesis, however, have been made by certain physiologists who deny to these structures any specificity whatever, and regard them as simply

temporary variants of the acinous tissue, into which they believe they may be readily transformed. Dale was the first to revive the Lewaschew theory that the number of islets increases during activity of the pancreas by transformation of acini into islets, and again diminishes during a period of rest by the converse process of change of islets into acini. He and the others who followed his lead were wholly undismayed by the difficulties involved in the peculiar blood supply of the islets and in the apparent lack of lumina and duct connections. Dale stimulated the pancreas for several hours by means of intravenous secretin injections, and compared the organ, when so exhausted, with the normal resting gland. He claimed that islets in large number were produced by this method and that in addition large areas of the pancreas might be converted into islet tissue without rearrangement of the cells or of the duct and vascular connections. Since he interpreted the islet cell as an acinous cell which had lost its characteristic zymogen, any process which brought about exhaustion should result in increase of islets. In accord with this idea he found that starvation had the same consequences in this regard as stimulation.

Vincent and Thompson confirmed these observations and investigated the effect of starvation, which they reported to be more effective than secretin stimulation in increasing the number of islets.

Laguesse reported similar effects of starvation in pigeons. This observer, however, regarded the transformation of acini into islets not as a result of exhaustion but as a regulatory phenomenon, since he maintained stoutly the internal secretory potential of the islet cells. He thought, however, that the internal and external secretory functions of the pancreas were possessed in like measure by both acinous and islet cells, and that the formation of islets was a sort of reversal of polarity of the cell for the purposes of internal secretion. He rested his argument, however, rather on the presence of intermediate types of cell and on the continuity between the two tissues. He supposed that there was some sort of physiological mechanism of control which determined from moment to moment the rela-

tive amounts of the two tissues which were needed for the external and internal secretory purposes. Laguesse thus occupied a position intermediate between the opponents and advocates of islet specificity, since he accepted all the facts of the former while maintaining the conclusions of the latter.

These views of the non-specificity of the islet tissue found great favor among physiologists, and especially among pathologists who, by reason of the character of their material, found no great difficulty in convincing themselves of the reality of transition forms. There was also doubtless a certain prejudice in favor of results obtained by experimental methods as compared with the results of anatomical study. Anatomists, however, remained for the most part staunch supporters of the theory of islet specificity.

Could any situation be more unsatisfactory? Investigation, instead of illuminating the theme by the discovery of new facts, simply added to the confusion by throwing doubt on the old ones, and the researches of Dale and Vincent and Thompson seemed to question almost the reality of the islets themselves.

Undoubtedly the factors which contributed to this confusion were many. Among them may be mentioned the system of experimental controls which had grown up, particularly in bacteriology, but also in physiology, suitable enough, doubtless, where the question was the nature of the reaction of an animal to a pathogenic organism or to a toxin, or even to nerve stimulation or drug effect, but wholly unsuited to the investigation of variations experimentally produced in the relative amounts of the constituent elements of an organ. Here it is necessary, above all, to know what the normal range of variation is and how it is influenced by age, sex, strain, and conditions of rearing. A second factor, which was, doubtless, to a large extent, responsible for the favor accorded to Dale's views, was the deceptive appearance of accuracy assumed by results obtained by quantitative methods, even though these methods introduced doubtful factors in mathematical computation, and ignored the normal range of variation in the species and in the organ, and the con-

ditions which influenced it, and involved the actual inspection of an almost infinitesimal fraction of the total tissue involved.

The most important factor, however, was unquestionably the vague definition of the islet itself. Since their discovery by Langerhans, the cytological study of islet cells had made little advance. The majority of investigators still commend methods of study which emphasize the negative characters of the islet. Dale's conception of the islet cell was a cell which did not contain the characteristic zymogen granules and basophile substance of the acinous cell, and hence could be distinguished only by location from a duct-cell. Of the host of workers who have concerned themselves with this study only Ssobolew, Tschassonikow, Mankowski, Laguesse, Diamare, in Europe, and a small group of recent investigators in America have concerned themselves with the really significant substances of the islet cells, namely their secretory granules.

Under these circumstances the task of finding transitions between islet cells and acinous cells was an easy matter, for any reduction in the characteristic substances of the latter would, in the opinion of these workers, be a step in the direction of the other type.

These considerations bring us to the question which is of the greatest importance in this controversy, namely, what would constitute a satisfactory definition of the islet cell. The answer to this is obvious, for it is clear that the only fully satisfactory definition would be the recognition of known chemical substances in the cell by microchemical methods. This, however, is not possible, since we know neither what the characteristic chemical substances are nor how to detect them microchemically. The closest approximation to such a microchemical method is, in my opinion, the determination of morphological constituents of the cytoplasm by examination of the surviving tissue in media which are as nearly indifferent as may be, and the comparison of these constituents as regards solubility in reagents, refractive power, etc., with the cells of the ducts and with the acinous cells. Such a study should also furnish us with information on which

a satisfactory staining and fixing technic could be worked up. This would constitute the second phase in the investigation.

The next desideratum in investigations on the pancreas is an accurate quantitative method for the determination of the total islet content, since any method which involves computations from fractional counts introduces so many sources of error, caused by the inherent difficulties of making the counts themselves from sections, the uncertain mathematical factors involved in the computations, and the great variations in local content, on the one hand, and of the relative count, on the other, introduced by the fluctuations in bulk of the intervening tissue resulting from physiological activity, or from general conditions such as reduction of water content from active diuresis or purging in the course of the experiment.

Finally, the definition of the islet cell should be cytologically complete so as to enable us to recognize those more subtle changes which may have deep functional significance without involving a true degeneration of the cell.

The first steps in this direction were taken, as already indicated, by Ssobolew, Tschassonikow, Mankowski and Laguesse who described the cells of the islet as containing many small granules, and by Diamare, Schultze, and Laguesse and Tschassonikow, who recognized that there were two kinds of cells in the islet. The significance of the last observation, however, escaped these observers because, as will appear more clearly later, they interpreted wrongly the second type of cell as an intermediate between islet and acinous.

The investigations of Lane on the cytological characters of the islets marked a distinct step forward. Lane studied the islets after fixation in a larger number of different fluids and found that he could define the islet cells of both types by the way in which they behaved in these fluids—that is, by the solubility of their secretory granules in the solutions, and by their staining properties when fixed. In the same way he could distinguish between the islet cells of either type, on the one hand, and the acinous cells, on the other. He also described morphological differences between the two types of cells which he

designated respectively A cells and B cells, the latter constituting the great bulk of the islet. Lane's division is based on the following characters: the zymogen granules for the most part, and all the granules of the B cells, are dissolved in alcohol, while the granules of the A cell are well preserved and may be indicated in the cell by the use of Bensley's neutral gentian stain; in chrome sublimate solutions the A granules are not seen but the zymogen granules and the granules of the B cells are well preserved, stain in neutral gentian, but take a differential color; in picric acid fixations the zymogen granules are well preserved but both types of islet granules are lost.

Thus Lane's observations not only give strong grounds for maintaining the composite character of the islets and the specific nature of their secretory products, but also supply a much-needed technic for handling the pancreatic tissues, and for distinguishing their several constituents in sections.

With the aid of these technics Cecil and myself independently attacked the questions raised by the claims of Dale, Vincent and Thompson. The results of these investigations will be more fully discussed later, but meanwhile I may state that, although my own results were contrary to the claims advanced by these authors, as indeed were also those of Cecil, I did not feel quite satisfied that the methods gave sufficient information. The work of counting the islets, even though by Lane's method they were sharply outlined, was so laborious, and the results so incomplete, that they did not seem to me quite as convincing as they should be. Consequently, I cast about for methods which would enable me to determine the whole islet content by direct methods without employing computations at all. The search for transitions also made apparent the necessity for combining in one method the advantages of the several methods of Lane for the demonstration of the islet and acinous constituents, and I therefore instituted experiments in fixing and staining the pancreas with this object in view, depending on differential staining, instead of differential fixation, for my results. Furthermore, new methods were needed for investigation of the relation of the islets to the ducts since the injection method and the

silver impregnation method had proven unsatisfactory, and since Laguesse claimed duct connections, which were denied by most observers with objective experience, though admitted by the advocates of variability, because they were a necessary consequence of their claims.

These efforts resulted in the discovery of new methods for the study of the pancreas which, for the first time, in my opinion, give us full experimental control, since they enable us to examine the whole pancreas instead of a part of it, and to determine by direct count without mathematical computations of any sort whatever, and without cutting sections, the real content of the pancreas in islets. I succeeded in securing two methods for staining all of the islets in the surviving pancreas by means of vital stains, thus permitting actual counts of all the islets in a pancreas of moderate size. Two methods were also devised for staining all of the duct system by vital dyes, and of combining in the same pancreas vital staining of the islets by one dye and of the ducts by another. By this means I was able to determine the true relation of the islets to the ducts and to bring to light a new mechanism of much importance in the history of the organ. I was also successful in securing methods of fixing and staining which met the requirements which I have indicated above. These various methods made it possible to repeat, under conditions which would give a definite answer to the questions involved, the experiments of Dale, Vincent and Thompson and Laguesse, as well as the duct ligation experiments of Ssobolew, Schultze and others.

Before proceeding to a statement of the experimental results, however, I shall have something to say about the methods themselves, for it seems probable that they may prove as useful in other experimental work involving histological control of the pancreas as they have been in the experiments mentioned.

The first successful method which I discovered for the vital staining of the islets of Langerhans was by means of janus green. This dye, when injected in the form of a solution of 1 in 15,000 in normal salt solution into the aorta, promptly stains the whole pancreas blue. On inspection with a hand

lens of low power, however, the islets may be seen as deeply stained particles in the midst of the pancreatic lobules. If, now, the pancreas be covered up, and air excluded, the tissue rapidly reduces and breaks down the dye to a safranin, which is red in color, but this change proceeds with so much greater rapidity in the acinous tissue than in the islets, that presently the latter are the only blue stained elements in the organ, the rest of the tissue being an intense carmine red color. If now a solution of ammonium molybdate be injected by way of the duct the preparation may be made permanent. By this means an entire pancreas of the guinea pig may be so prepared that every islet in it is deeply stained blue while the surrounding tissue is stained red. The fixation in molybdate of ammonium leaves the tissue still pliable and sufficiently transparent, so that one can easily see every islet in a fragment of pancreas without cutting a section. The preparation may be made a permanent mount by washing in ice-cold distilled water, dehydrating as rapidly as possible with absolute alcohol, and clearing in toluol. By dividing such a preparation into a large number of small fragments and spreading these on slides, it was possible to count every islet in the pancreas, without counting any twice, and without making a section at any point, and to ascertain by weighing the fragments the ratio between number of islets and weight of tissue.

The beauty, definiteness, and completeness of these preparations cannot be equalled by any other method, especially since it is possible to superpose on the results of vital staining a vascular injection by means of carmine gelatine, or to combine with this method of staining the islets some of the methods to be described for staining the duct system by vital stains.

In the majority of the observations and experiments, however, I employed for the same purpose neutral red, which has the great advantage of being restored by direct oxidation from the air if the reduction should proceed too far. The neutral red solutions are prepared and injected in the same way as the janus green solutions but I have not succeeded in finding any way of making them permanent.

The character of the preparations made by the neutral red method is sufficiently indicated by the drawing I have had prepared from one of my preparations. The ease with which counting can be accomplished in these preparations is, however, better illustrated by the photographs which were exhibited when the lecture was given. These photographs were made from preparations of guinea-pig pancreas stained intravitam in neutral red and simply spread on a slide and covered without either teasing or section cutting. I wish to emphasize here also the fact that my reports of counts in guinea pigs are actually what they purport to be and not the result of computations of the total content made from partial counts. In one or two instances they have been quoted by other authors as estimates. Nevertheless, I have found that if a sufficient amount of tissue from each segment of the organ be counted by this method and the tissue weighed and the total computed from the ratio of number to weight, the results will correspond within one or two thousand of the actual numbers, in a guinea-pig's entire pancreas.

The relations of the duct system were similarly studied by means of new post-vital staining methods which were just as specific for the ducts as janus green and neutral red were for the islets. The best results were obtained by the use of pyronin injected in the form of a 1 in 1000 solution in normal salt solution, but excellent results were also obtained by methylene blue used in the same way as neutral red. Methylene blue, as Ehrlich pointed out, stains the islets, too, but the latter reduce the dye more quickly than the duct cells, and the duct cells take up the dye more readily than the islet cells, so by careful use it can be employed to confirm the results obtained by pyronin staining. I have since found that acridin red is just as effective as pyronin for this purpose. By means of these stains every duct cell in the pancreas may be stained even to the last centro-acinous cell. The pyronin preparations cannot be preserved, but methylene blue preparations may be made permanent by the usual molybdate method. The difficulties of keeping the proper content of oxygen and controlling leaching prevent these

permanent methylene blue preparations from being as perfect as the fresh ones. They have been of much service to me, however, in the study of the relationship of islets to neighboring acini.

The use of the vital staining methods just described has the great advantage that they permit a complete survey of the whole pancreas, which should prove of great advantage in experimental work, since the effect on metabolism of any experimental procedure is determined by the condition of the whole pancreas, and not by that of a part. The antiglycosuric power of the pancreas is the sum of the powers of all the units in it which are physiologically active, and unless one can see and estimate all of these units he must necessarily have but a partial appreciation of their potencies. For example, the islet tissue in the remains of a pancreas of which the duct is ligated may be so dispersed among fat that a section, or even a series of sections, may give the impression that the total quantity of the islet tissue is extremely small, or by unfortunate chance it may be missed altogether in the tissue taken for examination. But in a preparation *in toto* of the whole pancreas, as may be seen, for example, in some of the wall plates which I have prepared, the efficiency and completeness of the regulatory processes in the pancreas may be recognized at a glance.

These methods, however, although they tell us at once whether, or to what extent, islet tissue is present in the pancreas, do not enable us to estimate the nature or extent of changes in the acinous tissue itself, or to answer definitely those interesting questions concerning the presence or absence of true transitions between the islet tissue on the one hand and the acinous tissue on the other. For this purpose it is necessary to resort to section methods. Lane has shown us how to handle individually the different secreting elements and how to preserve and stain the secretion antecedents. To the methods devised by him, however, must be added a method which does all of this in one preparation, relying on differential staining capacity instead of differential solubilities of the cell contents in the process of fixation. This means, of course, that the fixing fluids em-

ployed must fix all of the several secretion antecedents, and that the stains employed must stain them different colors. Furthermore, the fixation must not introduce into the section precipitates which will confuse the interpretation of the cell structures. These conditions I have found to be fulfilled by a fixing solution containing osmic acid, bichromate of potassium and a minimum of acetic acid, the formula of which is given in my article on the pancreas of the guinea pig published in *The American Journal of Anatomy*. This fixing fluid has the disadvantage of slow penetration and, as with all osmic acid solutions, the area of good fixation is very thin, but by care in preparing the pieces and by using sufficient fluid, and, above all, by removing as much as possible of the fat from the tissue, or by increasing the concentration of the osmic acid and the ratio between volume of tissue and volume of solution in proportion to the fat content, these difficulties can be overcome.

For staining these sections a combination of aniline acid fuchsin and methyl green is recommended, by means of which the granules of the A cell are stained red, those of the B cell lilac. The details of these methods may be found in the article quoted above. The results are indicated diagrammatically in the drawing of the islet of the guinea pig shown, and *ad naturam* in the drawing of an islet with the surrounding acinous tissue from the cat, which has been kindly loaned me by Dr. John Homans for this lecture.[1]

It must not be supposed that these technics can be handled successfully by investigators without effort. They require skill in the manipulation of tissue and of stains which, unfortunately, is not often acquired in the routine work of the histological and pathological laboratories, which is concerned almost exclusively with the simple and reliable but, unfortunately, cytologically not very useful hæmatoxylin and eosin methods. They require also an appreciation of the rapidity with which tissues undergo post-mortem change.

In this connection it may be mentioned that, provided proper

[1] These drawings exhibited at the lecture are not reproduced here.

care be taken in the fixation of the material, and if a fixing solution be employed which actually preserves the secretion antecedents, the beautiful staining technics devised by Professor Mallory may be employed with advantage.

The preparations and counts made by the vital staining methods, the quantitative results of which are published elsewhere, at once bring out certain considerations which are of the utmost importance in explaining the contradictory results of observation and experiment. The enormous quantity of the islet tissue becomes at once apparent, and the inaccuracy of estimates made on the examination of sections can be perceived and computed. The number of islets per cubic millimetre of pancreas, in guinea pigs between 300 and 600 grammes weight, averaged 22.28, 19.5 times as great as Dewitt's estimate and 22 times as great as Laguesse's. When the error of observation is of such colossal dimensions, what reliance can be placed on estimates made on the basis of a few sections from the splenic end of the pancreas?

A great number of islets came to light that are ordinarily overlooked, ranging in size from single islet cells, included in the epithelium of an acinus, or of an excretory duct, through groups of a few cells up to islets of the largest dimensions.

The vague outlines, insisted on by Vincent and Thompson, Dale, Laguesse, and other advocates of the variability hypothesis, vanish, and the islets, whether they be one cell or a large mass, are sharply outlined among the acinous tissue. Acinous cells which have lost their zymogen no longer appear like islet cells, because all of the latter are filled with sharply stained secretion granulations.

The islets are found to have a wide range of variation in different guinea pigs, in different parts of the pancreas of the same guinea pig, even in adjacent parts of the same pancreas, thus reducing the so-called quantitative method of estimating the number of islets after experimental procedure by counting the number in similar areas of a few sections from the same portion of the pancreas to the plane of luck and guess work.

Similar observations carried on by Clark by means of the

janus green vital staining technic show that the range of variability in number of islets in the human pancreas is as great as in that of the guinea pig. His observations also show that even the most careful computations made by the section method are far below the real number of islets in the pancreas.

One of the arguments which has been seriously advanced against the islet hypothesis of diabetes, by persons little familiar with the structures themselves, it is true, is that the quantity of the islet tissue in the pancreas is too small to have any such importance. The fallacy of this argument is at once apparent when we find that the total number of these structures in a guinea-pig pancreas may be as high as 56,000 and that in a new-born guinea pig there may be as many of these islets in one milligramme of tissue as 551. The average number per milligramme as well as the average number per animal is far below these numbers, but, nevertheless, considerable.

The vital staining with pyronin and with methylene blue brought to light details concerning the relations of the islets to the duct system and acinous tissue which are interesting because they reveal the normal regulatory mechanism of the pancreas as regards these structures, because they explain fully the contradictory results of various observers as to whether the islets have a duct connection or not, and a capsule or not, and because they explain the predilection of the islets for the centre of the lobule which has been a matter of so much difficulty to the advocates of variability. They have brought to light a system of epithelial tubules of great complexity which form a web of anastomosing ductules around the main duct and its branches whose obvious object is the formation of new islets and acini. The relations of these tubules are brought out in the Figs. 5, 6 and 7 of my article which are displayed for this lecture as wall plates. Connected with these tubules as well as with the main duct are large numbers of islets in all stages of growth, ranging from single islet cells to islets of a diameter of half a millimetre. In fact, with a few exceptions, all the islets of the pancreas are connected at some place to the duct system. In only a few instances, however, does the lumen of the tubule penetrate the

mass of the islet, and even then it is everywhere separated from the islet cells by duct cells. The duct connections become, therefore, merely vestiges showing the origin of the islet, and the position of the islet, and the nature of its relations, or of its investment, is wholly determined by its developmental history. If it has originated from the main duct or one of its branches or from the web of ductules referred to above, its position is primarily interlobular and it will have a more or less well-defined capsule. If, on the other hand, it has originated from several intralobular ductules, it will be in continuity with acini on all surfaces. Furthermore, the fact that the islets originate from the duct system explains fully why they are rarely found on the surface of the lobules, for, as is well known, the ductules ramify in the interior of the lobule and there also we find the islets which have taken origin from them.

The contradictions in the results as regards the relations of islets to ducts become thus a matter of efficient technic and of attention. Those who have concentrated their attention on the large primarily interlobular islets have been perfectly right in concluding that they were discontinuous with the acinous tissue and that they had a sort of capsule, while those who have studied islets which were primarily intralobular in origin have been just as correct in insisting on their continuity with acinous tissue. With both groups the question of the relationship to ducts has been more a matter of rhetoric than observation, except in the case of Laguesse, who not only patiently studied these relations in series, and saw, though not so completely as they are displayed in vitally stained preparations, the short ducts which link up the islets to the ducts, but maintained courageously the reality of these connections when everybody else was asserting the contrary.

The existence of similar duct connections in the human pancreas has been shown by Clark, using the vital stain, and by Weichselbaum and Kyrle, using the section method.

We are now in a position to consider the question of variability of the islets, that is, the possibility of transforming islets into acini or acini into islets directly. The evidence in support

of the theory of variability consists of the statements of Lewaschew, Dale, Vincent and Thompson, and Laguesse, who assert that the number of islets may be greatly increased in a few hours by stimulation of the pancreas or in a few days by starvation of the animal, and that cells transitional in type may always be found in the border of the islets. These claims are also supported by the observations of many pathologists who have seen similar transition figures. On the other side we have the experiments of Opie, Cecil, Dewitt and myself, who agree in stating that neither secretin nor pilocarpine stimulation nor starvation produces any such result. Cecil's results are particularly apposite here because he made free use of the advantages to be gained by the use of Lane's differential staining methods to identify the elements. His results offer no support either to the idea that the islets can be experimentally varied by these methods, or to the existence of transitions. My own results are of the same order, though obtained by the method of total counts of the islets in the whole pancreas. The results obtained in the animals which were stimulated for hours with secretin and in which the pancreas was so exhausted that the majority of the acini contained no zymogen, were all well within the limits of natural variation, and indeed one of the lowest counts in any animal was found in a guinea pig thoroughly exhausted with secretin. Allen also reports a series of experiments on the effect of prolonged starvation in cats, in which the islets of Langerhans, instead of being increased, really seem to be diminished. In many of these he saw appearances which might easily be mistaken for transitions but which, he says, are obviously not such, because the acinous cells, though exhausted and deprived of their usual contents, never show an islet arrangement, while the islets all show the characteristic arrangements of the cells and relations to the blood-vessels. Allen's experiments are doubly interesting, because, in addition to starvation, the animals were subjected to injections of various carbohydrates and fats which should, in accord with the hypothesis of Laguesse, stimulate the new production of islets at the expense of the acini.

On the basis of these experiments, we can now dismiss

definitely from consideration the claim that secretory exhaustion, or exhaustion by inanition, alters in any way the respective relations of acinus and islet, and accept as definitely proven a high degree of functional specificity for the latter. The question of absolute specificity, however, remains for the present undecided and, as I have pointed out elsewhere, must remain so until positive results are obtained by experimental means. No accumulation of negative evidence, however great, would be sufficient to establish the claim of absolute specificity of the islets as compared with the other epithelial elements of the pancreas, for such a claim presupposes the loss of some of the original potencies, which may be considered to be possessed in like degree by all of the epithelial elements of the pancreatic anlagen, or, as my observations by means of vital staining show, by the epithelium of the ducts, and especially by the web of fine tubules which surround the true ducts and have for their sole function the production, by growth and differentiation, of new islets and new acini. Closely bound up with this question is that of the capacity and method of self-regulation in the pancreas, whether it be in the ordinary process of growth or in the processes of repair and restitution which may follow disease or surgical interference.

Meantime, however, I may give a brief account of the grounds on which we have rejected the descriptions of Laguesse, Dale and others of transitions between the acini and the islets. The investigation, as I have already indicated, must rest on the cytological characters of the acinous cells on the one hand, and of the islet cells on the other. Studies carried on by technical methods which were adequate have been published by Lane, Cecil, Homans, Allen and myself. Lane and Cecil used for control of the observations the neutral gentian staining technic which stains particularly well the granules of the B cell and the zymogen granules, so that they were able at once to say whether a given cell contained islet granules or zymogen granules or both. Their results are consistently negative as to a true transition between islet and acinus. Allen used for the most part eosin and methylene blue, but to some extent the neutral gentian technic, with similar results, except that he found large numbers

of cells which might easily be mistaken for transitions but the arrangement of which, in the form of acini, at once revealed as acinous cells. My own observations and those of Homans have been conducted with the Lane methods, and with my new methods of fixation and staining which combine in one preparation differential staining of both kinds of islet cells and of the acinous cells. They agree in their results with those of Lane, Allen, and Cecil. The nature of the results obtained by this method is illustrated by the schematic representation of a guinea-pig islet and acinus, and by the beautiful drawing of an islet and surrounding tissue of the cat, which Dr. John Homans has kindly loaned me for this lecture. The results of the examination of fresh tissues vitally stained are of the same order. Never does one meet, either in the normal or in the exhausted pancreas, cells which cannot on the basis of positive characters be at once diagnosed as acinous cell, islet cell, or duct cell.

We must therefore seek another explanation for the claims that have been advanced by various writers of the existence of such transitionals in the pancreas. We may do this by first inquiring what characters a cell which is intermediate in type between islet and acinous cell ought to present, and then applying this criterion to the transitions which have been described. It is necessary, therefore, to look for a moment at the microscopic characters of the epithelial elements of the pancreas, and to compare them one with another.

The characters of the acinous cell are well represented in the figure made from a preparation fixed in acetic osmic bichromate solution and stained with aniline acid fuchsin and methyl green. The acinous cells in this figure are seen surrounding a narrow lumen into which extend a pair of centro-acinous cells continued from a small duct. Each acinous cell presents two zones, which are always present, except under pathological or extraordinary experimental conditions. The inner zone is occupied by coarse granules, usually called zymogen granules, because it is supposed that they constitute in part at least the mother substance of the enzymes of the pancreatic secretion. The outer zone in the fresh preparations is transparent, but under good

apochromatic objectives shows a faint radial striation. In the specimen this outer zone is seen to be composed of a homogeneous ground substance which is optically undifferentiated, but chemically defined by the fact that it contains a substance which stains with basic stains, the prezymogen of Mouret, prozymogen of Macallum, or, to use a term more general in its scope and less burdened with unsupported interpretations, the chromidial substance of Hertwig. In this homogeneous ground substance are seen, stained strongly with the fuchsin, long filaments, which are responsible for the faint striation of the living cell. These are the mitochondrial filaments, structures common to all protoplasm but characterized in the acinous cell by their unusual size. The nucleus of the acinous cell is characterized by its richness in chromatin and by its large oxyphile nucleolus. By appropriate methods there may be demonstrated in the cytoplasm, at the central pole of the nucleus, a network of fine channels containing a clear fluid, the canalicular apparatus of Golgi and Holmgren. In another preparation, made by the Altmann method, the same details essentially may be noted, and in addition a number of small fat droplets may be seen in the base of the cell, for the most part enveloped within the mitochondrial filaments.

The small centro-acinous cells and the cells of the intralobular duct to which the acinus is attached exhibit an optically homogeneous cytoplasm containing many irregularly scattered mitochondrial rods and granules. They are distinguished from the acinous cells by the fact that they lack both zymogen granules and chromidial substance.

In the islets of Langerhans we see two kinds of cells, the A cells and B cells, each of which is characterized by the possession of secretion antecedents peculiar to itself. I have already given the reasons why we think that these granules are not simply small zymogen granules of the same sort as exist in the acinous cell. The mitochondria of the islet cells resemble more those of the duct cells than those of the acinous cells, since they are minute filaments, scattered throughout the cytoplasm. These cells differ from one another by the solubilities and staining properties of their granules—they differ from acinous cells

by the lack of both zymogen granules and chromidial substance. The nuclei of the islet cells differ from those of the acinous chiefly in not possessing the large oxyphile nucleolus.

Summing up these characters, all four types, acinous cell, duct cell and both kinds of islet cells contain mitochondrial filaments but the character and distribution is characteristic for each. The characters which chiefly distinguish the acinous cell are the possession of chromidial substance and zymogen granules. Those which chiefly characterize the islet cells are the possession of their characteristic granules and the absence of chromidial substance.

Considering these properties of the cell it is obvious that to establish a transition between them we must find cells which partake of the properties of both, or we must find cells which have lost the characters of the type from which it is developing, and other cells representing all gradations connecting it with that type on the one hand and with the type towards which it is developing on the other. We cannot accept the presence of undifferentiated cells *per se* as evidence of transition, but must insist on the intermediate phases which demonstrate progress and its direction.

Examining on the basis of these criteria the assertions of those authors who describe transitions, we find that they fall into three classes: first, those who state that, as the acinous cell loses its zymogen and chromidial substance, it becomes more like an islet cell; second, those who have lightly and without due consideration interpreted the A cells as transitions because they frequently occur on the surface of the acinus; and, third, those who perceived the logical necessities of the case and attempted to prove a real transition by showing the acquisition by the acinous cells of positive islet characters. The first class requires no discussion because the statements rest upon a negative interpretation of the islet cell. The second group has been sufficiently answered by the demonstration by Lane and others that the A cell was not an intermediate but a specialized cell with characters peculiar to itself. The third group, consisting of Mankowski and Laguesse, requires a more extended discussion.

Mankowski, in preparations of the guinea-pig pancreas fixed in Flemming's fluid and stained with safranin, found acinous cells filled with tiny granules, which he thought to be acinous cells changing into islet cells. Apparently the same granules have been seen by Laguesse, who thought that it was an indication of a new form of internal secretory activity of the pancreas.

This appearance is very common in the guinea pig, particularly in animals which have been kept for some time on a diet of hay and oats without green food. It is, however, not a normal occurrence but a degenerative change, all stages of which may be conveniently studied. The granules first make their appearance in the outer portion of the cell, and, as they increase in number, the chromidial substance disappears. At the same time the mitochondrial filaments swell, become spherical, and finally dissolve in the cytoplasm. Soon the granules fill the entire cell and the zymogen also disappears. The change in the mitochondria stamps this change as a pathological one. Moreover, the granules are not islet granules, since they are larger, more highly refractive, and are insoluble in any of the reagents used for dissolving the islet granules. The accompanying illustration,[2] from a preparation of a guinea-pig pancreas fixed in Hermann's fluid and stained in neutral gentian, is sufficient evidence of the fact that these granules are not islet granules. In this preparation the Mankowski granules are well preserved, discrete, and intensely stained. The granules of the A cells are fused into a homogeneous mass, but the protoplasm, as a whole, stains intensely, while the granules of the B cells are dissolved out. These granules therefore have no connection with the formation of islet cells.

Laguesse at first considered the A cells as transitional types, but in the majority of his descriptions relies more upon position and vague differences for the evidence of transformation. In a recent publication, however, he describes a cell intermediate in position between an islet and an acinous which partakes in a measure of the characters of both. I have searched diligently

[2] Exhibited at the lecture but not reproduced.

in my preparations for such appearances but without success, except where it was obviously a case of overlapping of cells, in which case the characters may be optically combined. In these cases, however, the true character is at once resolved by the study of adjacent sections in the series.

Another resort of the advocates of variability is the assertion that the network of wide capillaries described by Kühne and Lea, and confirmed by many observers, is not, in reality, a point of difference between the islets and the acinous tissue, since the capillaries of the islet communicate freely on all sides with those of the general circulation of the pancreatic lobule, but rather an effect of the withdrawal of the mechanical support of the tissue, resulting from a diminution in size of the constituent cells. The implication is that such a glomerular structure might arise anywhere in the lobule for mechanical reasons.

I have indicated in a previous paragraph that it is possible after vital staining with janus green, followed by appropriate reduction of the stain, to inject the pancreatic blood-vessels with carmine gelatine and so obtain a preparation in which every islet large and small is stained, and in which all the blood-vessels are colored. An additional advantage of this method is that, in the process of staining, the arterial walls are stained with the dye, and thus in the preparations the arteries are bluish, the veins carmine red, and the capillaries intermediate in color, thus adding to the advantages of a vital staining of the islets the equivalent of a perfect double injection of the vascular system. After fixation in molybdate of ammonium these preparations may be dehydrated, cleared, and mounted in balsam, or they may be dissected under the binocular microscope to obtain fragments suitable for high power study. Preparations made by this method, in which there is never any doubt as to which is artery and which vein, show that the larger islets have a direct arterial branch running into them, and some of the largest interlobular islets may have three or four such branches as a direct arterial supply. The blood-vessels of the islet are neither sinusoids nor venous retia mirabilia. Even the smallest islets, groups of only

a few cells, have in relation with them a capillary loop which is distinguished from its neighbors by its larger calibre.

The end of all these observations and experiments brings us back to the situation which existed when Opie succeeded in demonstrating the specific changes in the islets in cases of diabetes in man. The object has been to investigate, by methods which were really discriminative, the attacks which have been made upon the theory of individuality and permanence of the islets of Langerhans and to afford thus a firm anatomical foundation upon which experimental inquiry as to the functions performed in the body by these structures may proceed. As a result of these studies, we can now state with assurance that the islets of Langerhans are specialized elements of the pancreas, having secretory powers differing from those of the acinous tissue, developing in embryonic life from the undifferentiated epithelium of the pancreatic anlagen and in post-fetal life from the epithelium of the ducts, having a peculiar blood supply characterized by its direct arterial source, by the larger calibre of its capillary vessels, and by the close association of the latter with the epithelial cells. These experimental morphological studies give us no information as to the nature of the internal secreting function which is obviously indicated by their structure. For enlightenment on this topic we must look to further experimental work.

In this direction, naturally, interest has centred around the phenomena of diabetes, and workers have sought by various experimental methods to show that the work done by the pancreas in connection with the control of carbohydrate metabolism was the function in particular of the islets of Langerhans. That this is a narrow point of view, whether we regard it from the stand-point of the derangements of metabolism in diabetes or from the stand-point of the internal secretory activity of the pancreas, is obvious, for the fact that the islets are themselves composed of two independent sorts of internal secreting cells would be sufficient to suggest that their internal secretory function is two-fold, and many studies indicate that there is more to diabetes than a deficient utilization of carbohydrate material.

The investigation of the relation of the pancreas to carbohydrate metabolism is only a part of the difficult problem of internal secretion in the pancreas. It has, however, the merit of definiteness and so serves as a convenient starting point for investigations designed to determine what the internal secreting functions of the pancreas may be, and to what extent they are mediated by the several epithelial elements which constitute the organ.

The efforts to solve this problem have been directed towards isolating experimentally the two principal tissues of the pancreas and determining the effect of this isolation on the excretion of dextrose in the urine, observations on the pathological changes in the pancreas in human diabetes, and observations on the effect on the pancreas of diabetogenic drugs and procedures.

Most important are the attempts to reduce the pancreas to the level of a pure endocrine gland by ligation of its excretory ducts. The first attempts by Schulze and Ssobolew were most interesting and apparently conclusive, but the results obtained by those who followed them were so contradictory that Allen in his able summary of this work divided the experimenters into groups, the designation of which sufficiently indicates the nature of their results. Allen groups them as follows: (1) authors reporting preservation of islet tissue only; (2) authors reporting preservation of acinar tissue only; (3) authors reporting preservation of neither tissue; (4) authors reporting preservation of both tissues.

In order that experiments of this sort may be definite, it is requisite that the result of the operation be the total exclusion of one of the tissues, and that the identification of the remaining tissue be established beyond a vestige of doubt. Accordingly, in the consideration of this matter, the question of the efficiency of the experimental method and of the completeness of the investigation becomes of the utmost importance. Since exclusion of one of the tissues is a necessary part of the experiment, conclusions based on a partial exclusion, for example, of the acinous tissue may be at once rejected as irrelevant. Concretely stated, what we seek to know is whether, by the method of duct ligation, the acinous tissue may be wholly destroyed, and, if so,

what is the nature of the tissue remaining in the pancreas and what its efficiency from the stand-point of carbohydrate metabolism. We can now accept as something agreed upon by all workers that the profound changes produced in the pancreas by duct ligation do not produce glycosuria, although there may be fluctuations of carbohydrate tolerance during the duration of the experiments.

Although the logical way to treat this matter would be to discuss the changes that take place in the pancreas in the order of the occurrence, the question of prime importance is whether the tissue which is left behind contains islet tissue. We may then proceed afterwards to discuss whether it contains at any period of the experiment anything else of the nature of secretory tissue besides islet tissue.

Here again it is necessary to insist on the identification of the islets by positive cytological characters, and not by arrangement of cells, or by blood supply, or by lack of the characteristics of an acinous cell. If the question is decided in the affirmative, then we can utilize the observations of individuals who did not employ so exact a criterion in considering the general questions involved. In this regard the investigations of Laguesse, Gontier de la Roche, and Kirkbride are significant. Laguesse used for recognition of the islet cells the characteristic secretion granules which he stained by a method similar in its results to my neutral gentian method. Gontier de la Roche worked in conjunction with Laguesse and employed his methods. Kirkbride, working in the laboratory of Professor W. G. MacCallum, used for the identification of the tissues the neutral gentian technic recommended by Lane. Laguesse reported that in a rabbit examined 45 months after ligation of the duct the sole tissue remaining in the pancreas was islet tissue. The animal exhibited no glycosuria. Kirkbride ligated the distal third of the pancreas and examined it 15 months later. She found that it consisted of a cyst-like duct surrounded by masses of fatty tissue in which were imbedded opaque dots or strands, the only remains of the former pancreatic tissue. Studied microscopically by the Lane technic, she could find no

trace of acinous cells, but on the contrary the remains of the pancreatic tissue consisted of small masses of cells which were filled with the fine blue stained granules of the islet cells.

These observations establish beyond doubt the survival, whether by direct continuity or by regeneration, of the islet cells. Since they do not involve a serial study of the whole pancreas they do not establish the complete absence of acinous cells, which is necessary to the argument. Tiberti rejected the demonstration of the islet nature of the tissue by Laguesse on the ground that the latter had not demonstrated the characteristic blood supply of islets. This objection, however, is of small moment, since the physiological efficiency of the islet is directly dependent on the islet cells, and only indirectly on the blood supply.

MacCallum isolated a portion of the pancreas of a dog, allowed the animal to recover from this operation, then seven months later removed the portion of the pancreas not included in the previous ligation, leaving only the atrophic remains of pancreas isolated at the first operation. After this operation the animal showed a large but rapidly diminishing glycosuria for four days, following which it acquired a considerable carbohydrate tolerance. Then, twenty days later a third operation was performed, removing all of the remains of the pancreas. This was immediately followed by grave glycosuria which persisted for five days, when the animal was subjected to a total thyroidectomy, sparing two parathyroids. This operation the animal survived for three days, during which time no further sugar was secreted. This experiment shows that the atrophied remnant was capable alone of securing a high carbohydrate tolerance. As the author states, it is unfortunate that the dog was not kept under observation for a longer period without the thyroidectomy, for some doubt still remains as to the influence of the anæsthetic in producing the glycosuria. This experiment, if repeated and confirmed, is exactly what is required to establish beyond doubt that it is the remains of the pancreas itself which constitute the antiglycosuric factor and not some vicarious adaptation to the new conditions, outside

of the pancreas. The examination of the tissue removed in this case revealed only islet tissue and duct tissue.

Thus, we can say that ligation of the duct is not followed by glycosuria, that true islets are preserved in the pancreas, that the amount of acinous tissue, if any, evades search, and that the removal of the remains of the pancreas produces at once the effect of a total pancreatectomy performed without previous ligation. The one thing that is lacking to a proof of the physiological efficiency of the islets of Langerhans apart from the acini is to prove that a search of the whole pancreatic remnant by adequate means would be equally unsuccessful in revealing acinous tissue, that is to say, that acinous tissue is actually absent. For this task the methods of vital staining which permit of accurate identification of the islet tissue and a search of the whole pancreas under the binocular microscope for other pancreatic elements offer obvious advantages. Investigations by this method have been carried on in my laboratory by Mr. Elbert Clark.

Before proceeding to a general description of the results obtained by Clark, it may be well to emphasize certain aspects of the experiment which doubtless have been responsible for the differences in the results obtained by ligation of the ducts. In the case of the dog especially, Lombroso found very little degeneration of the acinous tissue after ligation of the ducts, while MacCallum obtained results exactly comparable to those obtained by many workers in the guinea pig and rabbit. Pratt even claims to have secured complete degeneration of all pancreatic tissue after an operation which involved complete separation of the pancreas from the duodenum and ligation of all possible ducts. It is obvious that the nature and degree of the reaction will be modified by several circumstances concerning which the experimenter must inform himself when the tissue is finally investigated. In this connection it is to be expected that the degree of the primary reaction will be proportional to the injury inflicted on the pancreas, and that an operation, which requires the dissection of the pancreas away from the duodenum, while sparing the blood supply of the latter, will be

followed by a more intense inflammation and a more thoroughgoing fibrosis of the organ than one which involves the mere finding and ligating of the ducts. It also gives as a result a tissue which is much more difficult to investigate thoroughly. Undoubtedly the chief sources of uncertainty in these experiments and ones which should be rigorously controlled are the presence of supernumerary ducts, and the re-establishment of continuity between duct and bowel. The lack of adequate evidence on these matters throws doubts on the results of the operation. That there may be supernumerary ducts in the dog has been established by the observations of Hesse, and the fact that Lombroso admits that there may be rapid atrophy after ligation of the ducts in some cases, though he insists on the generally adequate preservation of the acinous tissues in others, justifies the suspicion that the differences may be due, in part, to the presence of supernumerary ducts, and also, in part, to the occasional re-establishment of communication with the bowel. To offset this possibility, in every experiment, the proof of adequate and permanent closure of communication with the bowel should be forthcoming. That the re-establishment of communication with the bowel is possible is well illustrated by one of the cases in Clark's series, which he permits me to quote. In this animal, a rabbit, the duct was ligated in two places, and cut between the ligatures. When the animal was killed 15 months later it was found that the duct had effected a new communication with the bowel at a distance of about two centimetres from the stump of the duct, which was also found at autopsy. The patency of the new opening was demonstrated by picking up the duct in the body of the pancreas and injecting a colored mass through into the bowel. In this case the duct was much larger than normal, but the pancreas, though somewhat reduced in size and somewhat richer in fibrous tissue, was, in other respects, normal.

Clark's observations on the rabbit and guinea-pig pancreas after ligation of the duct are not yet complete, but they show certain interesting facts which must be taken into consideration in connection with experiments of this sort. They have been

controlled by means of the vital staining technic, which permits a complete examination of the pancreas instead of a small fragment of it, as is the case when the section method only is employed.

In the guinea pig the primary effect on the acinous tissue is very complete. A majority of the acinous cells degenerate at once; probably, however, not all of them. The examination of the tissue at the end of seven days reveals a large number of cells which, because of their imbrication over the ends of interlobular ducts, we regard as acinous cells which have lost their characteristic substances and are now indistinguishable in structure from duct cells. These cells as well as the remains of the duct system show many mitoses, and it may be considered that, in the regenerative processes which go on in the pancreas from this time forward, cells which were parts of the duct epithelium, and these cells which, with Tiberti, we must regard as dedifferentiated acinous cells, participate in equal measure. In this primary effect apparently all those islets which are of small size and closely related to the acinous tissue participate.

In the rabbit, the general course of the change was the same except that in the primary changes not all of the acinous cells were involved.

Towards the end of the first month regenerative changes become prominent, resulting in the formation of new acini and new islets of Langerhans from the remains of the duct system. At the same time the original islets not involved in the primary effect, that is to say, the larger islets, begin to be involved in the advance of the sclerotic process. The connective tissue invades the tissue of the islets themselves and the cells show to some extent atrophic changes.

Thus, both primary tissues are present in the pancreas for a certain period, and throughout this period acini are being constantly formed, pass through a series of developmental states, and, to some extent, undergo atrophy, or become again dedifferentiated. At the same time the islet tissue is being progressively increased by the addition of new islets developed

from the duct system with which they remain in continuity, and the original islets for the most part atrophy as a result of the invasion of the connective tissue. At five and one-half months practically all of the islet tissue in the pancreas is new islet tissue which has been developed since the ligation of the duct. The oval and spherical islets of the original pancreas are no longer to be found. Instead one sees branching masses of islet tissue which stain quite normally in neutral red and janus green, or, after fixation, in the granule stains. Nevertheless there is present also considerable acinous tissue in the form of bulb-like acini, containing large lumina, and composed of cells which contain abundant zymogen granules. Beyond this period the number of cases which we have investigated is small, but they show unmistakably a progressive increase in the islet tissue, coupled with a tendency for the efforts to reconstitute the acini to diminish. The appearance of the islets at these several stages is represented in the figures which I have had drawn from photographs of the fresh neutral red stained pancreas. These figures show not only the formation of islets from the ducts and the presence of the acini, but also the difference in the morphology of the new islets as compared with those in the normal pancreas. At what period the last vestiges of acinous tissue disappear we have not yet determined, but one case, kept for 533 days after the ligation of the duct, showed a complete absence of acinous tissue. The neutral red preparation is shown in the wall plate. The pancreas tissue consisted almost entirely of islets imbedded in a mass of fat having the shape and situation of the original pancreas. The islets consisted of a few isolated islets of various shapes, racemose masses with bulbous protuberances composed wholly of islet cells, and irregular networks of cords composed of islet cells. In addition there were here and there in the pancreas complicated nets of epithelial cords composed in part of islet cells, in part of undifferentiated cells, and a few masses of dendritic tubules composed wholly of undifferentiated cells. The animal showed no glycosuria. In this case the whole pan-

creas was thoroughly inspected and we can be quite sure of the absence of acinous elements.

In view of Tiberti's objection, another rabbit which had been kept for 21 months after ligation of the duct was injected with janus green and carmine gelatine to show whether the new islets had a characteristic arrangement of the blood-vessels. The conditions found in this animal were identical with those in the preceding, except for the fact that the stump of the duct left in contact with the bowel had regenerated a small mass of pancreatic tissue. The islets were found in this case to have the same characters as in the preceding one, and the usual direct arterial supply and rich capillary net. No acini were found except in the regenerative portion, though the whole pancreas was inspected.

These observations of Clark, which show a capacity on the part of the pancreas to regenerate acini, which persists for several months, lead me to conclude that the experiments along this line, though promising, are as yet not wholly conclusive. The case which I have quoted, where after 533 days had elapsed from the ligation the animal was free from glycosuria and the pancreas free from acini, I think it may be fairly said, is the only case so far reported where the whole pancreas has been investigated, for the presence of acini, by adequate means. With the exception of this case, then, the most we can conclude is that the islet tissue is the predominating tissue in the pancreas if the animal is kept a sufficient length of time after ligation of the duct, and that the length of time necessary for this purpose has been for the most part under-estimated.

It should be remarked, here, however, that the failure of one investigator to get complete results in no respect invalidates the results of another who did obtain complete results from ligation, that is to say Lombroso's results in the dog must simply be placed in the category of unsatisfactory experiments which help in no way to decide the question whether the islets alone mediate the carbohydrate function, and in no respect invalidate the results of MacCallum who did obtain complete results in the dog. MacCallum and Pratt's results, on the other hand, as

well as Kirkbride's, Laguesse's and Gontier de la Roche's can be challenged, in my opinion, only on the basis of the fact that inevitably by the section method only a small portion of the pancreatic tissue can be inspected, and so the proof of absence of acinous tissue or of islet tissue, as the case may be, cannot be made complete.

The difficulties of this line of experiment, as well as the expense of it are very great. The accidents which happen in the operation, such as missing an accessory duct, or during recovery, such as re-establishment of communication with the intestine, or from causes outside of the pancreas which may cause the death of the animals before the experiment has lasted long enough to be conclusive, all add to the difficulty of obtaining definite results. It is clear for reasons which Allen has pointed out that these results would better be obtained in the dog, and that two things are necessary which have not been met in any investigation of this sort hitherto, viz., a complete investigation of the remains of the pancreas, without missing any part of it, by means of the vital staining method, followed by confirmatory examination of all portions which remain doubtful after the vital staining, by adequate discriminative section methods, and a complete history of the carbohydrate tolerance throughout the duration of the experiment.

Pratt's results are subject to the same criticism as the results obtained by other workers with the ligation method, that is, the proof of the absence of islet tissue is not adequate. These experiments also should be repeated and the results tested by injection of the animal with neutral red and janus green.

Experiments and observations which show a destruction of islet tissue leaving the acinous tissue unimpaired include the observations in human diabetes, and the experiments of Homans and Allen on the changes produced in the pancreas of dogs and cats which have become diabetic as the result of the reduction of pancreatic tissue. The pathological observations in human diabetes are interesting and suggestive but inconclusive, since many authors report cases of grave diabetes in which the islets are apparently unaffected.

The experiments of Homans and Allen, done independently by these two observers with consistent results, constitute just what is needed to establish the islet hypothesis when the results of the ligation experiments furnish unequivocal results from the other side. These observations show in animals which have lost a sufficient amount of their pancreas to produce a mild glycosuria, in Homans's observations, an exhaustion of the islets, indicated by a disappearance of their secretory granules, and in two animals in which a grave permanent glycosuria was produced a degeneration of the islet cells. Allen found that removal of nine-tenths (more or less) of the dog's pancreas, leaving the remnant in communication with a duct, led to a regular and definite result, viz., a diabetes which, in the earlier stages, was relatively mild, but increased with time, till, apparently, there was complete inability to utilize dextrose. "The microscopic, apparently, agree with the clinical observations. At the outset the diabetes is in one sense functional, viz., in the sense that the islets show no visible alteration." Homans's experiments controlled by my fuchsin methyl green method show at this stage an exhaustion of the islet granules. "At later stages, under the typical conditions, a typical condition of the islets appears to be present in every instance, viz., a visible degenerative change involving all the islets and showing regularly the same picture, viz., loss of cells, deficiency of protoplasm in remaining cells, degenerating (generally pyknotic) nuclei, occasional naked nuclei." Homans's results show by the anilin fuchsin methyl green technic hydropic degeneration of the B cells. In both these series of experiments the acini were well preserved and apparently normal.

These experiments constitute a most important contribution to the proof of the islet theory and I think we may confidently predict that the proof on the other side, namely, the competence of the islets *per se* in the absence of acinous tissue to care for the carbohydrate function, will before long be satisfactorily established by means of the methods which I have outlined. The experiments of Homans and Allen establish in my opinion the incapacity of the acini alone to mediate this function. I have

had the pleasure of examining Dr. Homans's preparations and can vouch for the accuracy of his descriptions. He has also loaned to me for the purpose of this lecture the beautiful colored drawings, one a preparation showing a normal islet from the cat, and one from a diabetic cat in an advanced stage of hydropic degeneration. An interesting consequence of his observations also is the fact that the B cells of the islet are mainly concerned in this function.

As for the pathological observations on cases of human diabetes, it is my opinion that this method will only lead to satisfactory results in the hands of those who are competent to handle the tissue technically by methods which tell us what the secretory potential of the islet tissue is. Homans's results show that the first evidence of pancreatic deficiency is accompanied, not by a gross pathological change, but by exhaustion of the secretory granules of the islet. To the ordinary pathological technic such islets would appear wholly normal. Moreover, the application of adequate histophysiological technic requires a promptness in the performance of autopsies which cannot usually be obtained in human cases. Accordingly, all negative results reported in human cases should be rejected, unless the time at which the autopsy is performed, and the ante-mortem history, and the methods of investigation justify the presumption that adequate precautions have been taken to detect by microscopical means changes which would indicate a depression of the functional potential and rate in the islet tissue.

One of the most interesting aspects of the work of Allen and Homans is the question they raise as to the capacity of the pancreas for self-regulation, and the conditions which influence this capacity. In neither series of experiments does there seem to be much evidence of a response on the part of the pancreas in the way of compensatory hyperplasia, notwithstanding the fact that in many of the experiments the reduction of the pancreas was carried to a point where the organ was incompetent to carry on its function of internal secretion adequately. The reaction in these cases was an exhaustion and finally a degeneration of the islet tissue.

On the contrary, the observations of Tiberti, Gontier de la Roche and Clark indicate a high capacity for restoration of the pancreas after the duct has been ligated. Clark has shown that for a period lasting for at least five and a half months after ligation of the duct in guinea pigs and rabbits, the remains of the duct system are actively mitotic, and new acini and new islets are being constantly produced. There is indirect evidence also that if the duct continuity be established at a sufficiently early period the pancreas may be entirely restored. The case of the rabbit which I have described, where, after ligation of the duct in two places and cutting between the ligatures, a new opening was effected into the intestine, illustrates this. The dilated duct and a certain degree of fibrosis of the pancreas indicated that the occlusion of the duct had persisted for some time before the new opening was re-established, and, when we consider the rapidity with which degenerative changes go on in the pancreas of the rabbit after duct ligation, it seems probable that in this case the acinous tissue had first been almost completely destroyed and subsequently completely restored. A number of similar results were obtained in guinea pigs. Whether our interpretation of the process in these cases is correct or not, the fact remains that the pancreas after ligation of the duct shows a high capacity for regeneration of its parts, while reduction below the point of physiologic competence is followed by very slight indications of this capacity. It is obvious, therefore, that the magnitude of the physiological demand made on the organ, whatever that may mean, is not the only thing to be considered, but that there is a biological equilibrium in the cells of the organ themselves which influences profoundly the reaction. One of the things which, it may be supposed, influence the reaction is the physiological activity of the cells themselves. In general it may be said that mitotic activity in secreting cells means a suspension of secretory activity, that the two processes are to some extent mutually exclusive. That being the case any condition which would favor mitosis would by so doing inhibit secretion, and conversely any condition which would inhibit secretion would favor mitosis. In other words

regeneration of pancreatic tissue would be favored by experimental procedures which would check secretion, and stimulate cell division. The progress and course of regeneration so initiated would depend upon the degree of participation of the several tissues involved, and on the chance of survival, under the conditions, of the several tissues so regenerated. Under the conditions which obtain after ligation of the duct both these needs are realized. Mitosis is favored by the inflammatory reaction which follows the operation and which involves the whole pancreas, and the external secretion of the pancreas is abruptly brought to an end. Thus regenerative processes are initiated which result in a continuous increase of islet tissue because it is the only tissue which can survive. The new formed acini, as soon as they reach the point where they are able to secrete, become cystic and undergo retrograde changes.

The differences between the pancreas as a whole and fragments of pancreas in this respect may be simply due to an extension of the process which destroys the acinus in the former case to the islet in the latter. The pancreas as a whole contains a great excess of islet tissue, so the work of the original islets is not increased markedly by the operation of tying the ducts. The degeneration of the original islets, though inevitable according to Clark's experiments by reason of the invasion of the islets themselves by the process of fibrosis, does not proceed rapidly enough, so that when one considers the sum of all islet tissue in the pancreas old and new there is at any point a marked decrease in the excess of islet tissue present, and so the new islets survive and do not fall a prey to the degenerative changes which Homans and Allen have demonstrated to be a result of overwork. The explanation of Allen's results which show that a smaller fragment of pancreas will protect against glycosuria when the duct is tied may be simply on the one hand that the operation favors mitosis in the pancreatic cells, and on the other that the exclusion of the external secretion from the intestine with its reduction of the efficiency of carbohydrate digestion diminishes the load on the islet cells which are left behind,

so permitting them to survive, the increased formation of new islet tissue accounting for the gradual raise in carbohydrate tolerance as time goes on.

Though the results are suggestive, the matter obviously requires further investigation. The indications of pancreatic hypertrophy after reduction of the pancreas should be tested not only by the increase in size over that observed at operation, but also by careful measurements of the fragment left, and by search for mitoses in the fragment at different intervals. The effect of pancreatic feeding on the rate and amount of regeneration in animals in which the duct has been tied should also be determined, both in cases where the whole pancreas has been left and in those where a large portion of pancreatic tissue has been removed. In the latter case of course it would be necessary to proceed with caution because of the fact that pancreatic feeding increases glycosuria, and according to Kirk's recent experiments, also, acetonuria in totally diabetic dogs.

It has been my purpose in this paper to emphasize the necessity in experimental work on the pancreas of full histological control, and to illustrate by reference to the results as well as by means of colored illustrations drawn from actual preparation how this histological control may be accomplished. I have already indicated some of the directions in which further experimentation is desirable, and doubtless many others will occur to those who hear this address or who afterwards read it.

One of the most inviting fields, apart from the investigation of diabetes, is undoubtedly the investigation of the interrelation of the various internal secreting organs. There has already been a great deal of stimulating speculation in this field, but as yet very little in the way of objective proof. The influence of experiments on the other endocrine glands on the structure of the pancreas is susceptible of more accurate study perhaps than any other. In the guinea pig, my counts of the islets in 99 guinea pigs, of all ages and of both sexes, would serve as a convenient starting point for such an investigation. The necessity of examination of the whole pancreas indicates for these investigations the use of small mammals in which the vital

staining methods work well. The white rat offers many advantages, also, because the statistical studies of Donaldson, Hatai, and Jackson provide information of the normal range of variation which is available to the same extent in no other animal, and also much interesting information concerning the correlations between the various organs, and their plasticity under varying experimental conditions.

CARBOHYDRATE UTILIZATION IN DIABETES BASED UPON STUDIES OF THE RESPIRATION, URINE, AND BLOOD *

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IN the classical work of Naunyn [1] upon diabetes mellitus occurs the following sentence: "In general, even in severe diabetes, at least in man, the carbohydrates ingested are not completely excreted in the urine again as sugar. A portion of the starch, as well as of the dextrose, will be burned in the organism." This view was also shared by Kulz. Naunyn, however, refers to a case in which von Mering records an excretion of all the sugar ingested, and attention is called in the report of the cases of Kulz to four instances in which apparently a similar condition existed.

Von Noorden [2] defines diabetes as "a disease in which the capability of the organism to adequately burn grape sugar is pathologically lowered," and in another place he says: [3] "One cannot help thinking that, in man, even when death has resulted from coma, the diabetes has not always been quite 'complete'—that is to say, the pathological processes which produce diabetes have not developed so far, and the factors which favor the storing up of glycogen have not been so completely destroyed as is the case in a dog whose pancreas has been entirely ablated."

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* Delivered March 13, 1915.

From the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Mass.

I wish to acknowledge my grateful appreciation of the help received from Mr. Emmes, Miss Babcock, Miss Tompkins, Miss Corson and Miss Sandiford, of the Nutrition Laboratory, as also my indebtedness to Mr. Higgins, who controlled several of the experiments with the Tissot apparatus, and to my secretary, Miss Helen Leonard, for cheerful work upon long computations and puzzling charts.

Notwithstanding all the work upon diabetes, this question of the utilization of carbohydrates in human diabetes has not been settled. In diabetic dogs evidence has accumulated pointing to the complete loss of this power to utilize carbohydrate, and the work of Murlin and Cramer [4] has given definite results upon this point, although so recent a writer as Landsberg,[5] working from a different point of view with other animals, comes to the opposite conclusion. The present paper is concerned with diabetes in man. At this time I wish to call to your attention certain observations bearing upon this problem which are related to the body weight, the urine, the storage of carbohydrate in the body, the respiratory metabolism of diabetics both fasting and following the administration of food, and the remarkable disappearance of acidosis in diabetics with prolonged fasting, which is associated with a rise in their respiratory quotient.

I. THE INFLUENCE OF WEIGHT UPON THE DETERMINATION OF THE UTILIZATION OF CARBOHYDRATES IN DIABETES

The changes in weight which occur in a normal individual, following a slight increase of the carbohydrate in the diet, are so striking that one might hastily conclude that a study of the weights of a diabetic patient would give some idea as to his utilization of starch and sugar. A closer scrutiny of the problem, however, reveals many difficulties. In the first place, the diet employed in most cases of diabetes and all severe cases is low in carbohydrates, and seldom reaches 10 per cent. of that of normal individuals. In other words, it amounts to less than 50 grammes carbohydrate—200 calories—per day. The effect of 200 calories upon the weight is possible of determination theoretically, but practically such an experiment is difficult because the protein, fat, and carbohydrate must be kept at uniform levels for a long period. But in a severe case of diabetes some of even this small amount is lost in the urine, which renders the available carbohydrate for increasing the weight still less. There are other complications. A severe case of diabetes with 50 grammes of carbohydrate in the diet usually excretes more than 50 grammes of sugar in the urine, and it is

difficult to assign in proper proportion this excess of urinary sugar between the carbohydrate ingested and the carbohydrate already stored in the body on the one hand, and the protein simultaneously ingested and the body protein on the other.

Remarkable changes in the weight of normal as well as of diabetic patients will also occur, although the caloric value of the diet remains constant, if the proportion of fat to carbohydrate is altered. A diet rich in carbohydrate brings about an increase in weight, whereas a diet of exactly the same number of calories, although chiefly made up of fat, lowers the weight. These changes undoubtedly are due simply to the retention of water by the tissues upon a carbohydrate diet, and loss of water upon a fat diet. Such changes appear reasonable because the storage of one gramme of carbohydrate in the body demands the retention of three grammes of water, one gramme of protein requires the storage of 0.75 gramme of water, and one gramme of fat requires only 0.1 gramme of water. These changes are well illustrated by the following table:

TABLE 1

CARBOHYDRATE DIET

Date, 1904	Food and drink			Body Weight	Gain(+) or loss (−)
	Solid Matter	Water	Total		
	Gm.	*Gm.*	*Gm.*	*Kilos.*	*Gm.*
April 16	...	....	....	75.086	.....
April 16–17	970	3577	4547	75.443	+357
April 17–18	966	3553	4519	75.414	− 29
April 18–19	966	3491	4457	75.269	−145
	FAT DIET				
April 19–20	750	3108	3859	74.319	−950
April 20–21	745	4150	4896	73.480	−839
April 21–22	747	4152	4899	72.528	−952

Average gain per day, carbohydrate diet	+ 61
Average loss per day, fat diet	−914
Water stored per day, carbohydrate period	+165
Water lost per day, fat period	−906

It is important for the clinician to bear this in mind, because it explains the rapid change in weight which often follows the initial diminution of the carbohydrate in the diet of diabetic patients and its replacement with fat.

An increase in weight following a marked increase of carbohydrate in the diet is strikingly illustrated in severe diabetic patients under the oatmeal treatment. Under these conditions the weight may rise 4.5 kilogrammes in one or two days. Undoubtedly you all have seen œdema during the course of an oatmeal cure. It is significant that some of these cases show little or no carbohydrate in the urine. I cannot give proof that patients showing this increase in weight fail to give evidence of burning more than a trifling amount of carbohydrate, but from other similar cases I suspect this often to be the case. This point deserves further study. However, I think there will be general agreement that the gain in weight following the sudden introduction of large quantities of carbohydrate is accounted for by the storage—temporarily, perhaps—of carbohydrate in the body. That this storage or delay of excretion is accentuated in the presence of diseased kidneys is common knowledge. Barrenschen [8] showed that excretion of milk sugar was delayed upon the day following an oatmeal cure.

The administration of sodium bicarbonate is frequently followed by a gain in weight. Thus, in Case No. 220,[9] the changes in weight during the administration of sodium bicarbonate were as follows:

TABLE 2

Date	Sodium bicarbonate	Body weight	Date	Sodium bicarbonate	Body weight
	Gm.	*Kilos.*		*Gm.*	*Kilos.*
Nov. 2.....	0	48.1	Nov. 7.....	20	50.7
Nov. 3.....	0	48.6	Nov. 8.....	20	51.5
Nov. 4.....	0	49.0	Nov. 9.....	20	52.4
Nov. 5.....	0	48.6	Nov. 10.....	20	52.3
Nov. 6.....	20	49.3	Nov. 11.....	20	53.3

In order to show that this gain in weight was not directly due to the alkali, but rather to retention of salt, the weights

of another diabetic patient, Case No. 135,[10] were taken while upon a salt-free diet.

TABLE 3.—Case 135

Date, 1908	Intake						Urine									
	$NaHCO_3$	Carb.	Protein	Fat	Alcohol	Liquids	Vol.	N	NH_3	Acetone and diacetic acid	β-oxy. acid	P_2O_5	Cl.	Sugar	Weight
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*c.c.*	*c.c.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*lbs.*
Jan.															
26	0	135	110	185	..	3500	3720	21.8	4.2	7.9	29	4.4	8.2	160	88¼
27	0	135	110	185	..	3500	3940	19.6	4.3	7.8	29	4.5	6.3	165	89¼
28	0	135	110	185	..	3500	3210	20.5	4.4	7.3	24	4.6	5.9	160	86¾
29	0	135	90	155	..	3500	3210	19.2	4.1	7.3	26	4.2	4.8	163	85¾
30	25	135	70	185	..	3500	3190	16.3	3.5	8.7	33	4.1	1.6	146	85
31	25	120	60	95	23	5370	4600	19.1	4.3	12.6	51	5.1	2.3	146	83¼
Feb.															
1	37	130	100	130	45	5250	4050	18.7	3.3	10.7	39	4.3	2.0	137	82¼
2	52	70	60	95	45	5370	3510	16.0	3.5	10.2	37	3.9	2.1	121	81¾
3	..	15	15	30	45	800	360	15.0	...	...	..	...	...	86	....

It will be seen that while upon the salt-free diet the weight steadily fell, and despite the administration of sodium bicarbonate later, no increase in weight occurred. This observation has been elsewhere confirmed. I might here make the clinical observation that a salt-free diet in diabetes is inadvisable. It is also interesting that I have never seen the death of a diabetic patient from diabetic coma who had dropsy, nor have I encountered such in the literature.

The simple enumeration of these various facts affecting the weight shows how complicated is the determination of the utilization of carbohydrate from it alone. Changes in weight, however, do afford, when combined with other methods of clinical investigation, new fields for work.

The changes in weight which a healthy fasting man undergoes at the beginning of a fast are known. The fasting man at the Nutrition Laboratory lost 2850 grammes in 3 days, and consumed during these 3 days body substance equivalent to 161 grammes of protein, 149 grammes of carbohydrate and 407

grammes of fat. It is possible that from a series of observations upon diabetic patients similarly fasted conclusions of value as to the storage of carbohydrate in the body might be secured. Ten of my patients who were available for this purpose showed upon an initial fast a loss of weight considerably less, and occasionally a gain in weight was recorded. Following the termination of the fast, although very little food was given, an increase in weight out of proportion to the amount of food given was almost invariably observed.

In one case no mineral waters or alkalies were taken, and yet gain in weight occurred during fasting. It is not unexpected that the gain in weight was often coincident with a fall in the excretion of urine. A gain in weight during fasting raises the question as to whether new carbohydrate has not been formed in the body, and as a result of its formation water retained. This line of investigation deserves attention. It will be referred to later in the discussion of severe cases of diabetes treated by prolonged fasting, the method which Dr. F. M. Allen [11] has had the courage to introduce and has so accurately defined that it is safe for any practitioner to employ.

II. THE UTILIZATION OF CARBOHYDRATES BASED UPON INTAKE IN DIET AND OUTGO IN URINE

The comparison between the carbohydrate ingested and the sugar excreted in the urine is the common method of determining the utilization of carbohydrates. It would appear to be a simple procedure, but, as a matter of fact, the problem is far more difficult than has heretofore been considered. Your attention is first directed to the possibilities of error in reckoning the carbohydrate in the diet. Most severe diabetics under careful observation live upon diets low in carbohydrate, seldom in excess of 50 grammes. Therefore errors of 5 grammes in the estimation of carbohydrates, though actually small, are proportionately large. It is seldom that the actual quantity of carbohydrate in the diet has been analyzed. In many of the cases food has not even been carefully weighed, and approximate portions of food have been supposed to contain definite quantities

of carbohydrate. Take, for example, cream. The quantity of carbohydrate contained in half a pint may vary 5 grammes, making an error of 10 per cent., if the total carbohydrate for the day amounted to 50 grammes, or 20 per cent. if limited to 25 grammes.

Vegetables constitute a considerable proportion of the diet of these severe diabetics. Often in the literature—and I plead guilty to the charge—the quantity of carbohydrate in the mixture of vegetables chosen from those containing less than 10 per cent. carbohydrate for the day has been roughly estimated. Recently I have taken more careful account of the amount of vegetables eaten, and it has come out that the quantity of vegetables prescribed and eaten frequently varies from 300 to 1000 grammes. Any accurate computation, therefore, of a carbohydrate balance must be based not alone upon the total quantity of vegetables eaten in the day, but upon the actual quantity of each vegetable, even in these low carbohydrate groups. Furthermore, varieties of the same vegetable vary in per cent. of carbohydrate. It makes a difference of 5 grammes in a day whether 500 grammes vegetables contain 1 per cent. more or less of carbohydrate. But this is not all. Analyses of carbohydrate in vegetables include the cellulose contained in them as well as the starch and sugar. How much shall we subtract from our total carbohydrate intake on account of this undigested cellulose which is lost in the fæces?

The other foods commonly used in the study of the metabolism of diabetic patients are potato, oatmeal, bread, fruit. The potato, oatmeal, and bread are usually carefully weighed, and the analyses of these foods are fairly constant, but the per cent. of carbohydrate is so large that I should not dare to be positive about the quantity of carbohydrate which my patient received unless standard varieties of these foods were employed. With fruit frequent errors exist, because usually an orange or grapefruit is allowed and seldom the actual weights of the portions eaten are determined. A further error occurs in that the intake of carbohydrate is reckoned indifferently as starch or sugar. As a matter of fact, 100 grammes of starch when con-

verted to sugar amount to 105 grammes. Errors of 5 and 10 grammes a day in computing the carbohydrate intake may easily occur and in a period of a week form notable amounts—35 to 70 grammes. Physiologists and physicians must not take too seriously clinical statements about the carbohydrate in the diet, and greater accuracy must be employed in the future. We need, first, a standard test diabetic diet, and, second, we need to employ it for at least five days. Unfortunately even at the end of this time the results may be unsatisfactory, because the condition of the patient's tolerance may have changed in this period either for better or worse.

The estimation of sugar in the urine is far more accurate than that of the carbohydrate in the diet, provided the analysis is made in one of our best laboratories, but I would hesitate to accept as final in accurate computations many routine analyses made in private practice or in hospitals. Too often the method employed in the estimation of the sugar is not mentioned and I suspect many results are obtained with the polariscope which may involve an error of 20 grammes or more, due to the presence of lævorotatory bodies. However, urinary analyses are usually far and away ahead in accuracy of that observed in the collection and measurement of the urines of diabetic patients. The admirable methods adopted in the ward of the Russell Sage Foundation at Bellevue Hospital and at the Rockefeller Hospital have been seldom followed by experimenters in the past. I pass over errors of forgetfulness or design on the part of the patients, both as regards diet and collection of urine. Dogs may not be any more honest, but we do not expose them to temptation or trust their memory. How often a patient states that a trifling amount of urine has been lost at stool! I realize this is trite, but a good share of the arguments based upon the utilization of carbohydrate rests on data which are not above reproach.

The variability of excretion of urine and urinary constituents from day to day is another source of error. If the diet is not constant the variation may be great. In one of our tests designed to determine the utilization of levulose, during seven

days prior to the administration of levulose the average volume of urine was 1079 c.c. Upon the day the levulose was given the volume of urine was 966 c.c., the next day 390 c.c., and on the following day amounted to 1175 c.c.; it then returned to near the average quantity. Yet the habits of this patient's daily life were nearly constant, and except for the one levulose day changes in the diet were not extreme. Such marked variations in the volume of the urine on successive days must be reckoned with, because with such great changes in volumes of urine, the quantities of the constituents of the urine change too, though to a much less extent. In this same case, the average daily excretion of nitrogen for the 55 days which included this period was 7.3 grammes, but on the day when 81 grammes levulose were given with very little other food, it fell to 6.53 grammes, and upon the next day to 4.34 grammes. This low point was never reached by this same patient upon a fasting day, and the quantity of levulose is considerably less than would be supposed to exert so strong a positive action, particularly when delayed or diminished oxidation is taken into consideration. Consult Table 4 and also Table 6 on pages 299 and 306.

Experiments designed to test the utilization of carbohydrate should be conducted upon patients who are in equilibrium both as regards weight and urinary excretion.

III. THE IMPORTANCE AS WELL AS THE INFLUENCE OF CARBOHYDRATE STORED IN THE BODY UPON THE UTILIZATION OF CARBOHYDRATE INGESTED

It is well known that following a period of fasting large quantities of carbohydrate can be administered without subsequently appearing in the urine. The best illustration of this is von Noorden's oatmeal treatment. Thus, Case No. R of the Benedict and Joslin [12] series showed a positive carbohydrate balance of 520 grammes during an oatmeal cure, although he never after this cure became sugar-free save for occasional days, despite rigorous dieting. A more spectacular demonstration is the severe diabetic of Klemperer,[13] who took 100 grammes of glucose in divided portions during 24 hours without more than

a few grammes appearing in the urine. Almost as striking is that of Case No. 785, a boy of seventeen, who came to me in the twentieth month of the disease. By consulting Table 4 it

TABLE 4.—Case No. 785. Male, Age, 17; Weight, 42 Kilos

EFFECT OF LEVULOSE

Output					Intake				
Vol.	Di-ac. acid	Sugar	Nitrogen	Ammonia	Carbohydrate	Protein	Fat	Alcohol	Calories
c.c.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
1079[1]	+	11.1 but none on 6 days	7.83		17	53	127	9	1506
966	+	7	6.53	0.69	90[2]	21	30±	3	735
390	++	5	4.34	0.35	20	63	110	9	1385
1175	+	3	8.35	0.74	20±	63	110±	9	1385±

[1] Average for previous 7 days.

[2] Levulose, 81 gm., carb. in diet 9±gm. in the form of vegetables.

will be seen that only 7 grammes of sugar appeared in the urine following an intake at one time of 81 grammes of levulose, although by observations before and after the tolerance was known to be low. A summary of his metabolism is given in Table 5.

TABLE 5.—Case No. 785. Male, Age at Onset, 15. Duration Since Onset, 20 Months. Severe Diabetes. Weight, 42 Kilos

Period	Nitrogen balance		Carbohydrate balance	
	Urine and fæces[1]	Diet	Urine	Diet
55 days	440.	384.	190.	919.
Daily average	8.0	7.0	3.5	16.7
		Sugar present in Urine 20 Days		
Daily average	8.9	7.8	8.8	15.4
		Sugar absent from Urine 32 Days		
Daily Average	7.5	6.4	0.0	15.1

[1] Nitrogen in fæces estimated at 10 per cent. of nitrogen in diet.

During the 55 days he was under my observation the average daily nitrogen in the diet was estimated at 7.0 grammes, and in the urine and fæces 8.0 grammes. The carbohydrate in the diet was 16.7 grammes and in the urine 3.5 grammes. During 32 of the 55 days, sugar was absent from the urine and upon 20 days it was present, although the average daily carbohydrate in the diet was the same. A study of Table 5 would suggest this being due to the slightly lower nitrogen intake on the sugar-free days. This is not quite justifiable, because another factor enters in,—namely, starvation,—for on several of the 32 days the patient received no food at all. These starvation days evidently played an important rôle. How very important is shown by the test already recorded in Table 4, where 81 grammes of levulose were administered and only 7 grammes of carbohydrate appeared in the urine.

Is it possible for the body to store so large a quantity of carbohydrate as 520 or even more grammes? Furthermore in what form may it be retained in diabetic patients?

Nearly all experiments upon the utilization of carbohydrates in the past have been based upon the difference between the carbohydrate intake and the carbohydrate excreted. Unless the amount of the carbohydrate stored in the body is known, it is unjustifiable to say that the carbohydrate excreted represents a part of that ingested during the same 24 hours. All data in reference to the D : N ratio are confused by the possibility of stored carbohydrate. The importance of the storage of carbohydrate thus becomes evident.

The influence of carbohydrate so stored in the body is also great. Whatever virtue the oatmeal cure possesses, all agree that it depends in major part upon preceding starvation, which has tended to exhaust the carbohydrate depots of the body.

Glycogen.—Carbohydrate is stored in the body in various ways, but most of it is supposed to be in the form of glycogen, and this is about equally divided between the liver and the muscles. An old estimate of Bunge that the body had 400 grammes is roughly approximated by experiments upon fasting men and professional athletes doing severe work without food.

This figure may be taken as a fair average, but there are enormous variations. This statement is based upon glycogen which has been shown to be burned; it does not exclude the possibility of some glycogen still remaining in the body, and in fact Benedict [14] says: "It would appear that the estimate of 400 grammes of glycogen for the content of the body is if anything too small rather than too large." Experiments on fasting men show that they may burn from 93 to 232 grammes in the first three days. In diabetic patients the quantity of glycogen is universally considered to be far below this amount, but Frerichs [15] found, upon puncturing the liver of two diabetics, a small amount of glycogen in one and a considerable amount in the other, and Kulz[16] found 10–12 grammes of glycogen in the liver of a diabetic who had been for a long time on a diabetic diet. Examinations of the tissue removed from the livers of living diabetic patients show appreciable quantities of glycogen, and it is the experience of pathologists that the organs of diabetic patients contain more than traces of glycogen. It is most unfortunate that no data exist which enable us to determine what this minimum is. It is quite conceivable that, although it might be extremely small at any one moment, a small quantity might be frequently formed and destroyed, and the sum of these small quantities reach a substantial amount in 24 hours.

The recent work of Helly[17] throws new light upon the problem. He points out the striking contrast between the constant presence of glycogen in the liver of human diabetes and the very small quantity which is found in the severe diabetes of depancreatized dogs, yet even in the latter the power of the liver to form or deposit glycogen is shown when levulose is administered. If a milder form of diabetes is produced in the dog, more glycogen remains in the body and there is a closer resemblance to human diabetes, whereas with total removal of the pancreas there is only 0.065 per cent. of glycogen in the liver; on the other hand, with partial removal, even though there be 8 to 10 per cent. of sugar in the urine there is 0.3 per cent. of glycogen. By microscopic examination so considerable a quantity as this appears small.

Blood Sugar.—Sugar is also stored in the body in the form of blood sugar. The normal quantity of sugar in the blood of healthy individuals varies between 0.07 and 0.11 per cent. and for convenience in calculations may be considered 0.1 per cent. This rises quickly after a meal rich in carbohydrates, but soon falls to its former level. In 55 observations upon 15 of our diabetic patients the per cent. of blood sugar varied from 0.12 to 0.36. But the blood of these diabetic patients does not behave like that of normal individuals following the ingestion of food. It is true that the per cent. of sugar rapidly increases following a carbohydrate meal, but it does not as rapidly fall, and in my own experience most diabetic patients, even after prolonged fasting, show values for blood sugar which are far above normal. Certain types of diabetic patients, namely, those with disease of the kidneys, are especially prone to maintain high percentages of sugar in the blood for many days after their urines have become sugar-free. It is impracticable to consider that the per cent. of blood sugar is maintained independently of the other tissues in the body, first, because the per cent. is so unstable; second, there is no constant relation between the sugar in the blood-serum and the sugar in the total blood; and, third, because the capacity of the blood for storage of sugar is so slight. If we assume an individual of 70 kilos body weight and consider that 7 per cent. of the weight is made up of blood, we have 4.9 kilos of blood with a normal sugar content of 0.1 per cent. This would amount to 4.9 grammes, even taking the highest for the normal individual, and should we take the highest figures we have encountered even after the administration of food with our diabetic patients, namely 0.36 per cent., the total quantity of sugar stored in the blood would not be far from 18 grammes—a trifle more than a half ounce.

Falta [18] has called attention to the slow return of the blood of diabetic patients to its former sugar level, and emphasizes this point as of fundamental importance in diabetes. He points out that the disturbance of blood-sugar utilization is not the same as the disturbance of glycogen formation, for the blood-

sugar regulation may be interfered with when the glycogen formation is not.

Kleiner and Meltzer [19] have also beautifully shown this same difference in depancreatized dogs. Whereas the sugar in the blood of normal dogs increases fourfold, namely, from 0.20 per cent. to 0.79 per cent., following the injection of 4 grammes of dextrose per kilo body weight, and of depancreatized dogs threefold, that is, from 0.38 per cent. before to 1.19 per cent. after the injection, the blood sugar of the former returned nearly to normal at the end of an hour and a half, while the diabetic dogs even then showed 0.86. It is significant that in these experiments the quantities of sugar excreted in the urine were practically the same. Interesting as these figures are from this point of view, they are still more interesting from another point of view. It is impossible to account for all the sugar ingested by adding together the sugar found in the blood and that in the urine. Where did the sugar go? You may say it was burned, and this possibility, though not probability, must be admitted in the normal animal, but no one would contend this to be wholly the case in the depancreatized animal.

At the Nutrition Laboratory we have been able to carry these experiments to their logical conclusion, for we have had the opportunity to determine the respiratory quotient following the administration of levulose to severe diabetics. In Table 21 will be found a report of the effect of levulose when administered to severe diabetic patients in amounts to 2.51, 2.42, and 1.95 grammes per kilogramme body weight. In the first and third cases there was no increase in the respiratory quotient. A considerable portion of the levulose was probably excreted in the first case, but in the third little or none. The explanation of this difference in behavior in the storage of levulose is probably that the first case had not fasted beforehand, and that the third had been on a low carbohydrate diet for a long time; this is confirmed by the second case, which also excreted little of the levulose when administered following a period of strict dieting. An increase in the respiratory quotient occurred in this case, but it was so slight as to preclude any considerable quantity of

the sugar having been burned. It should also be recorded that in all the cases the levulose was given at one time and not spread out through the 24 hours, as in Klemperer's test. This gives added emphasis to the possibility of the presence of an empty storehouse for carbohydrate in the body. I also have evidence that the gradual administration of carbohydrates is of little value, provided the body is not prepared to retain it. Following etherization, a patient, Case No. 808, while fasting for the first 24 hours, was sugar-free, but on the next day, although only 2 grammes of carbohydrate per hour were administered, he excreted practically all of it, although formerly his tolerance amounted to 50 grammes of carbohydrate.

The small amount of glycogen and the still smaller quantity of blood sugar represent an amount of carbohydrate far too low to account for the phenomena above described in diabetes. Other sources for storage of sugar in the body must be sought, as has been emphasized by Ivar Bang. If we should assume that the per cent. of sugar in the blood was the same for all the fluid in the body, certain amounts of sugar might be stored in this manner. While such an assumption is not wholly justifiable, it has some basis, for we know that sugar exists in the spinal fluid of diabetics, as well as in other fluids. In normals Dr. Jacobson tells me that he has not found it so closely to follow the blood, but the opposite was true in his cases of diabetes mellitus. It gets into the spinal fluid and cannot seem to get out. Notable percentages of sugar, not very different from those in the blood, have been found in pleuritic and ascitic fluids, and Husband found even 0.7 per cent. in the amniotic fluid. There is some doubt about its presence in sweat, but we do have a record of sweet tears. Yet, granted that the assumption is correct, we cannot increase our storage capacity very much that way. For example, assuming the total quantity of fluid in the body as 60 per cent. of the body weight of 70 kilogrammes, we have 42 kilogrammes of body fluid, from which we must deduct 4.9 kilogrammes already reckoned as blood. This leaves us a remainder of 37.1 kilogrammes of fluid in the body, and using the highest figure, 0.36 per cent., for blood sugar which we have

encountered, the quantity of sugar in this mass of fluid would be only 133 grammes. This is not enough relatively to explain Kleiner's and Meltzer's experiment.

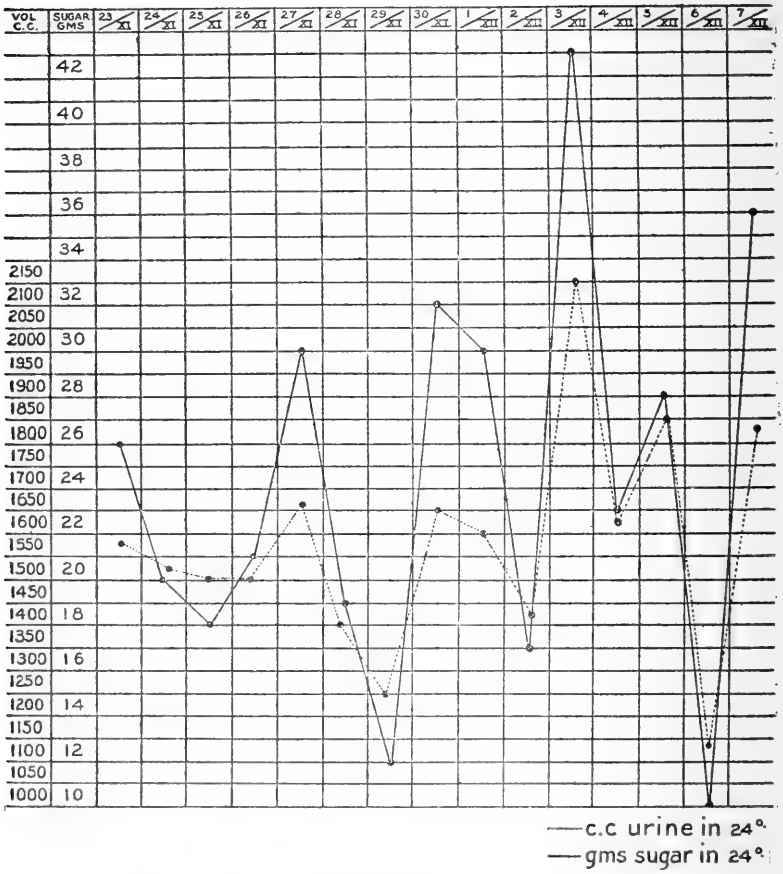
Another source for the formation, although perhaps not for the storage, of carbohydrate in the body has long been recognized in protein. The close connection which is maintained between protein and carbohydrate in diabetes would make a clinician with modest chemical knowledge seek for some combination of carbohydrate in the protein molecule—some arrangement by which a portion of the sugar molecule could be stored in protein or given up as occasion arises, just as water is squeezed out of a sponge. Good chemists, and I have asked many, assure me that even with glucoproteids sugar can only be extracted from the protein molecule when the molecule itself is disintegrated. The large quantity of movable protein and fat in the body suggests a large carbohydrate reservoir, too. Few realize how large this quantity of movable protein is. It has been shown by Albert Müller [20] that by overfeeding, 210 grammes of nitrogen, the equivalent of 1260 grammes of body protein, in turn the equivalent of 6.3 kilos of muscle tissue, can be retained by the body, and, conversely, it has been shown by Benedict [21] that even more—277 grammes—can be removed. This movable protein amounts to about one-third of the total body protein. The readiness with which fat can be increased and decreased in the body is universally recognized.

Although we are not allowed to say that carbohydrate can be extracted from the protein molecule, leaving it intact, we do know that in severe diabetes sugar can be formed out of protein. Professor Lusk [22] has demonstrated that in completely depancreatized dogs and in his now famous diabetic patient, 3.65 grammes of dextrose appeared in the urine for each gramme of nitrogen therein contained. This represents approximately 60 grammes of dextrose for each 100 grammes of protein. If we should assume that in diabetic patients there were 1200 grammes of movable protein, this would furnish a possible source of 720 grammes more of carbohydrate.

Unfortunately, one cannot be sure that in the disintegration

of the protein molecule the nitrogen and carbohydrate leave the body hand in hand. As a rule, the nitrogen loiters behind, greatly to our annoyance in estimating the source of the sugar

TABLE 6.—Illustration of Variations in Excretions of Urine and Sugar of a Severe Diabetic Upon a Constant Diet. (From Naunyn.)



Diet constant, meat, 1000 gm., fluid, 1750 c.c.

in the urine. Mendel and Lewis [23] have recently shown that this delay was increased if either indigestible substances or cotton seed oil form a prominent part of the diet—just the sort

of foods which our diabetic patients eat. Consequently if an attempt to determine the quantity of carbohydrate from protein (dextrose-nitrogen ratio D : N) is made, this irregularity in the excretion of nitrogen must be considered. When one adds to this difficulty that of determining what share the quantity of residual carbohydrate in the body bears to the total sugar excreted, and when one considers that even under an absolutely uniform diet of 1000 grammes of meat and 1750 c.c. of fluid intake for 15 days Naunyn [24] found variation of sugar excretion from 12 to 43 grammes, and frequently of 100 per cent., I feel very modest about asserting that my patients are producing any given quantity of sugar for each gramme of nitrogen excreted.

Naunyn says that these spontaneous variations may reach even 70 grammes. Kulz has emphasized this same point. If under ideal conditions for 15 days such variations exist, it behooves one to accept with caution reported D : N ratios for a period of a few days as being of value, or to base arguments, as is sometimes done, upon the D : N ratio of single isolated days selected from a series. In the tables of Rumpf, Allard, Hesse, and some of Lüthje's, D : N ratios are recorded which Professor Lusk and I would feel indicated a far larger per cent. of carbohydrate coming from protein than is actually the case. It is arbitrary selection to pick from these tables all ratios above 3.65 : 1 and say they are wrong and to class the remainder as correct. It is furthermore remarkable that with fasting all D : N ratios cease to exist. It is also hard to understand how a patient one day fails to burn the protein of an ox, but the next day burns his own body protein with ease. Fasting diabetics will afford unusual opportunities to study this point. As a rule, the high D : N ratios are found when the nitrogen excretion is high, and it may be that to produce these high ratios large quantities of protein may be required.

The small part which the blood plays in the storage of carbohydrate has been pointed out. This is peculiarly unfortunate, because one would hope from the per cent. of sugar in the blood to derive some knowledge of the course of metabolism in diabetes. As if to emphasize the independence of the metabolism

to the content of sugar in the body, I submit at this point Table 7, which gives the respiratory quotients obtained upon individuals whose blood sugar was determined at the time of the test, reserving discussion of the same to a later portion of the paper.

TABLE 7.—The Blood Sugar and Respiratory Quotient in Severe Diabetes

Case No.	Condition	Per cent. of sugar in blood	R. Q.
806	Fasting	0.12	0.71
806	Fasting	0.14	0.68
786	Fasting	0.17	0.69
806	After potato (60 Gm. carb.)	0.18	0.69
765	Fasting	0.18	0.74
810	Fasting	0.19	0.72
806	After 1 egg and 20 Gm. vegetables	0.19	0.72
765	Fasting	0.23	0.76
765	Fasting	0.24	0.73
714	Fasting	0.25	0.78
786	After oatmeal (60 Gm. carb.)	0.25	0.74
765	After oatmeal (10 Gm. carb.) and potato (48 Gm. carb.)	0.26	0.74
773	Fasting	0.27	0.70
773	After oatmeal (80 Gm. carb.)	0.30	0.70
746	Fasting	0.30	0.70
746	After oatmeal (40–60 Gm. carb.)	0.36	0.74
786	After oatmeal (120 ± Gm. carb.)	0.36	0.83

IV. THE RESPIRATORY METABOLISM AND ITS RELATION TO THE UTILIZATION OF CARBOHYDRATES

An examination of the composition of the carbohydrate molecule will show that it contains sufficient oxygen to unite with all the hydrogen present. Consequently, for each volume of oxygen used in the oxidation of carbohydrate a volume of carbon dioxide will be produced. The relation which the volume of carbon dioxide produced bears to the oxygen required for the oxidation of a food constitutes its respiratory quotient. It is obvious, therefore, that the respiratory quotient of such a carbohydrate as glucose ($C_6H_{12}O_6$) is 1. It matters not whether the oxidation takes place rapidly outside of the body in a flame,

or less obtrusively in the body during 24 hours. Protein, on the other hand, does not contain sufficient oxygen for the hydrogen atoms contained in its molecule. As a result, in the burning of protein, oxygen must be used not only for the carbon in the molecule, but for the hydrogen as well. The denominator of the fraction is thus increased, and the final quotient of protein must be less than 1, and is 0.81. The protein molecule is made up of many component parts, and while the respiratory quotients of these parts greatly vary, yet for protein as a whole the above quotient, 0.81, holds. With fat a similar condition exists to that in protein, only there is still more hydrogen present to require oxygen, so that the amount of oxygen necessary for the combustion of fat is still greater, and as a result the respiratory quotient falls to 0.70. The respiratory quotient of alcohol is still lower, namely, 0.67. Beta-oxybutyric acid, which can be taken as the chief one of the group of acid bodies formed in diabetes, has a respiratory quotient of 0.89, diacetic acid has a respiratory quotient of 1.00, and acetone of 0.75, so that one will not go far astray to take 0.89 as a common respiratory quotient for these three acid bodies.

The respiratory quotient of an individual can be determined by measurement of the quantity of carbon dioxide exhaled and the oxygen absorbed. When this is done, information is obtained concerning the character and total amount of the combustion taking place in the body. Since the urinary nitrogen gives us a definite idea of the quantity of protein metabolized, if we calculate what this represents and subtract it from the total material burned, we have left the combustion derived simply from fat and carbohydrate. Knowing the respiratory quotients of fat and carbohydrate, as well as that of the individual, it is possible, by computation, to determine the share which these two variables have taken in the total metabolism.

The Technic of the Determination of the Exchange of Carbon Dioxide and Oxygen in Man.—Two types of apparatus are employed to learn the exchange of carbon dioxide and oxygen in man; the calorimeter and respiration apparatus. In the closed chamber of the calorimeter the oxygen admitted and the carbon

dioxide withdrawn can be accurately determined in periods usually of one hour's duration, but it is better to take the average of the results obtained in three successive periods. Occasionally each period may be shortened to three-quarters of an hour, exceptionally to half an hour, but the large size of the calorimeter increases the chances for error. The calorimeter is cumbersome, expensive to construct and to maintain, and the length of the experiment is not only disagreeable to the patient, but disadvantageous in studying the results of rapid changes in the metabolism, which are desirable in a study of the utilization of foods. On the other hand, the respiratory apparatus is advantageous because the exchange of gases can be determined

TABLE 8.—The Respiratory Quotient (R. Q.) of a Food is Obtained by Dividing the Volume of Carbon Dioxide Produced During Its Oxidation by the Volume of Oxygen Absorbed

	R. Q.
Carbohydrate: $C_6H_{12}O_6+6O_2=6CO_2+6H_2O$ Oxygen is required for oxidation of carbon alone $\frac{6CO_2 \text{ produced}}{6O_2 \text{ absorbed}} =$	1.00
Fat: $C_{51}H_{101}O_6$ Oxygen required for carbon and a large quantity of hydrogen	0.71
Protein: Occupies an intermediate position	0.81
Alcohol: C_2H_6O	0.67
Beta-Oxybutyric acid: $C_4H_8O_3$	0.89
Di-acetic acid: $C_4H_6O_3$	1.00
Acetone: C_3H_6O	0.75

during short periods of 15 minutes. It is disadvantageous, however, because the periods being so short, errors at the beginning and end of the same are magnified, and because the individual must breathe through a nose- or mouth-piece, and this introduces an abnormal state. Fortunately, in each form of apparatus the error of a leak falls chiefly on the oxygen, because the patient and the apparatus constitute a closed circuit, and any diminution in gas in this circuit must be offset by the addition of oxygen. A more troublesome source of error and one difficult to avoid arises from the possibility of the patient exhaling carbon dioxide, which has previously accumulated in the body, at a

more rapid rate than corresponds with the oxygen inhaled. The patient is said to "pump out" carbon dioxide. There is also another error due to carbon dioxide which is lost by cutaneous respiration, and it has been calculated that this would lower the quotient 0.01 to 0.15.

Many pitfalls, therefore, arise in the determination of the respiratory exchange of an individual. The carbon dioxide is the more easily estimated of the two gases, and in early experiments upon metabolism investigators attempted this alone. The determination of oxygen is far more difficult. Hence, in dealing with the respiratory quotient which depends upon the relation of these two determinations to each other, one treads on very dangerous ground, and all statements regarding the respiratory quotient of individuals must be accepted with caution. The general picture of the respiratory quotient in an individual based upon several experiments is far more valuable as a guide to his true metabolism than the result of a single experiment. Similarly, it is probably safer to average the results of a series of cases than to attach too much importance to figures obtained in one.

The Respiratory Quotient of the Normal Individual.—The respiratory quotient of the normal individual is best determined at least 12 hours after a meal. It has been shown that if this rule is not followed the composition of the meal will have a marked influence upon the result. Under these circumstances the respiratory quotient of normal individuals does not greatly vary. Benedict, Emmes, Roth and Smith,[25] working at the Nutrition Laboratory of the Carnegie Institution, have studied the basal gaseous metabolism for 89 men and 68 women and their average results are shown in Table 9.

TABLE 9.—Respiratory Quotient and Total Metabolism of Normal Individuals at Rest at Least 12 Hours After the Last Meal

Individuals	R. Q.	Calories per kilo per 24 hours
89 men	Average =0.83	25.5
68 women	Average =0.81	24.9

I would call attention to the slight difference existing between the respiratory quotient of men and women—0.83 and 0.81. I have also incorporated the heat production, calculated from the oxygen intake, which was approximately 25 calories per kilogramme per 24 hours. This latter figure is lower than we are apt to consider, but it should be remembered that it is based upon fasting periods when the patient is purposely endeavoring to be quiet. It would be absolutely wrong, from such determinations covering periods of 15 minutes, or even a few hours, to draw conclusions upon the total heat production for the day. In illustration of the method and at the same time of the difficulties of determining the respiratory quotient of normal individuals I give my own chart.

TABLE 10.—Normal Individual (E. P. J.) Fasting Experiment. *December 23, 1914.* Weight, 64.9 Kilos; Height, 177.8 Cm.

Duration		CO_2 per min. c.c.	O per min. c.c.	R. Q.	Calories per kilo per 24 hours
Min.	Sec.				
15	6	152	192	0.80	20.45
14	59	150	194	0.77	20.53
15	0	153	196	0.78	20.78

Average =0.78

Naturally I took the greatest possible pains to be quiet and breathe in a normal manner, but it will be seen that, whereas the values for the carbon dioxide of themselves, and of oxygen for themselves, vary to an extremely small degree from period to period, yet they differ sufficiently to make a considerable variation in the respiratory quotient. This experiment emphasizes the possibilities for error in the determination of the respiratory quotient even under most favorable circumstances.

The respiratory quotient of individuals fasting for long periods is well exemplified by the studies made by Benedict [20]

upon a man, who went 31 days without food. These are illustrated in the following tables:

TABLE 11.—The Respiratory Quotient of a Man During a Prolonged Fast

Period	Time	R. Q.	Calories per kilogramme body weight
Preliminary period....	4th day before fast.....	0.81	33
	3rd day before fast.....	0.89	32
	2nd day before fast.....	0.89	29
	1st day before fast.....	0.82	27
Period of fast.....	Days 1– 5 of fast.....	0.77 (Average)	26
	Days 6–31 of fast.....	0.72 (Average)	23
	Days 6–31, early a. m.....	0.73 (Average)	23
After period.....	2nd day after breaking fast [1]	0.78	25
	3rd day after breaking fast [1]	0.94	27

[1] Twelve hours after food.

TABLE 12.—Quantities of Protein, Carbohydrate and Fat Oxidized by Fasting Man at Nutrition Laboratory

Determined from the Daily Metabolism, the Urinary Nitrogen and the Calculated Non-protein R. Q.

Period of fast	R. Q.		Quantities oxidized			Calories per kilo per 24 hours
	Actual	non-protein	Protein Gm.	Carb. Gm.	Fat Gm.	
1st day.....	0.78	0.76	43	69	135	30
2nd day.....	0.77	0.74	50	42	142	30
3rd day.....	0.74	0.74	68	39	130	29
4th day.....	0.75	0.71	71	4	136	28
5th day.....	0.76	0.72	63	15	133	28
6th to 31st day average.....	0.72	0.70	53	0[1]	114	26

[1] Actually a total of 32 Gm. carbohydrate were burned during the 6th to 13th day inclusive, and later none.

It will be seen that, prior to the experiment, the respiratory quotient differed little from that of the group of normal individuals above mentioned. With the withdrawal of all food the respiratory quotient fell, and after the fifth day reached a point which Magnus-Levy [27] has said theoretically represents the quotient which is obtained when the metabolism consists of 85 per cent. of fat and 15 per cent. of protein, namely, 0.72. In other words, five days of starvation removed the last discernible influence of carbohydrate remaining stored in the body, and the individual lived wholly upon body fat and body protein. It is possible to discover how much fat and how much protein take part in the metabolism.

Knowing the nitrogen in the urine, one can calculate the amount of oxygen employed by the body for the oxidation of the protein ‡ which it represents, and, correspondingly, the amount of carbon dioxide given off can be determined. If we subtract these computed figures from the total carbon dioxide and oxygen obtained by direct experiment, we have left the carbon dioxide produced by the non-protein metabolism in the body, and the relation of the two gives the non-protein respiratory quotient. In a useful table constructed by Lusk,[28] the per cent. of carbohydrate and of fat for any given non-protein respiratory quotient between 70 and 100 can be calculated. On this basis it was shown that Benedict's fasting man burned either none or only a trace of carbohydrate after the sixth day. When the respiratory quotient of this man was 0.73 on the seventh day, it represented a non-protein respiratory quotient of 0.70 and no carbohydrate was burned. A respiratory quotient of 0.74 gave a non-protein respiratory quotient of 0.71, which represents the oxidation of 3.8 grammes of carbohydrate; a respiratory quotient of 0.76 gave a non-protein respiratory quotient of 0.72, which is evidence that 15 grammes carbohydrate were burned.

Respiratory Quotient in Normal Individuals after Food.— The respiratory quotient following the ingestion of food is shown well by the fasting man at the Nutrition Laboratory

‡ In estimating the quantity of body protein burned from nitrogen in the urine the equivalent 6.00 is employed instead of 6.25.

for the periods before fasting commenced. It will be seen that 12 hours after food it varied from 0.81 to 0.89 in the four days. Similarly, following the termination of the fast, the respiratory quotient rose, indicating the combustion of large quantities of carbohydrate.

An experiment was performed upon myself which was comparable to those later carried on with the diabetic patients when tests were made of the influence of food upon their metabolism. The changes in my own respiratory quotient following the ingestion of 60 grammes of carbohydrate in the form of oatmeal are given in Table 13.

TABLE 13.—Metabolism of a Normal Individual After Food. Weight, 64.9 Kilos; Height, 177.8 Cm.

Date, 1914	Condition	CO_2 per min. c.c.	O_2 per min. c.c.	R. Q.	Calories per kilo per 24 hours
Sept. 9...	1–2 hours after breakfast...	205	241	0.85	26
Sept. 10...	1–2 hours after breakfast...	192	237	0.81	25
Sept. 30...	9 A. M., fasting.....	159	194	0.82	21
	10.30 A. M., after 60 Gm. carbohydrate as oatmeal	189	212	0.90	23
Dec. 23...	8 A. M., fasting.....	152	194	0.78	21
	1 P. M., fasting.....	151	196	0.77	21

It will be seen that the respiratory quotient within an hour rose some 8 points after eating 60 grammes of carbohydrate in the form of oatmeal. It has been calculated that if 48 grammes carbohydrate are burned in 24 hours at the rate of 2 grammes of carbohydrate each hour continuously for the 24 hours, the respiratory quotient would rise 3 points; in other words, would be about 0.75 instead of 0.72, which is a fat-protein quotient. I wish to emphasize the change in respiratory quotient of only 3 points when approximately 48 grammes of carbohydrate are burned at the rate of 2 grammes of carbohydrate per hour per day, and the rise of 8 points following directly upon the ingestion of 60 grammes carbohydrate. The continuous combustion of small portions of carbohydrate amounts to the combustion of a considerable quantity of carbohydrate during the whole

day, and yet it will raise the respiratory quotient very little. The combustion of 24 grammes of carbohydrate at the rate of 1 gramme per hour could scarcely be detected with our present methods, and yet a tolerance for 24 grammes of carbohydrate is relatively a high tolerance when one is dealing with serious cases of diabetes.

The Respiratory Quotient in Diabetes.—In mild cases of diabetes, when the urine is free from sugar and the carbohydrate in the diet large, the respiratory quotient differs little from that of normal individuals. The respiratory quotient of these same mild cases of diabetes will be lowered by fasting or by the withdrawal of carbohydrate, as just shown in the case of the normal fasting man. Undoubtedly the limited quantity of carbohydrate in the diet in cases of severe diabetes is responsible to a large degree for the low respiratory quotient which such patients show. Magnus-Levy called attention to this, and so have other observers. It is well exemplified by the change in the respiratory quotient of Case No. 714. This patient, with only moderate acidosis, became sugar-free upon April 16, 1914, following 14 days of treatment. On December 3, 1914, she re-entered the hospital with 4.4 per cent. of sugar, but under fasting treatment became sugar-free after the omission of four meals. The respiratory quotient on successive days is shown in Table 14.

TABLE 14.—Illustration of Fall in Respiratory Quotient of Mild Diabetic. Case No. 714. Female

Date	R. Q.	Urine sugar	Diet [1]			
			Carbohydrate	Protein	Fat	Alcohol
			Gm.	*Gm.*	*Gm.*	*Gm.*
Dec. 3.....	....	4.4 per cent.	++	++	++	0
Dec. 4– 5....	....	20 Gm.[2]	+	+	+	10
Dec. 5– 6....	0.78	0	0	0	0	25
Dec. 6– 7....	0.75	0	15	40	45	10
Dec. 7– 8....	0.75	0	15	45	60	7
Dec. 10–11....	0.73	0	15	55	100	9

[1] Approximate. [2] In 14 hours.

Tests were made fasting at 8 A. M., which was one hour after the collection of the 24-hour urine.

It will be seen that, whereas the respiratory quotient was 0.78 on entrance, due undoubtedly to the oxidation of some of the carbohydrate ingested, though much at the same time was being lost in the urine, this rapidly decreased to 0.73 under starvation followed by a low carbohydrate diet. Yet this woman could not be considered a severe case of diabetes. The quotient was low simply because she was living almost exclusively upon a fat protein diet.

The problem of drawing inferences from the respiratory quotient in diabetes is complicated by the fact that much of even the little carbohydrate which is given to a diabetic patient is lost in the urine. The patient really approaches the condition of the fasting man in that he is living exclusively on fat and protein, although in this case not that of his own body. If all the carbohydrate ingested is lost in the urine, his respiratory quotient would be 0.72. But there are other complications. Occasionally cases of diabetes are seen where the sugar in the urine exceeds that of the diet, and speculation at once arises as to the source of this excess of sugar. Magnus-Levy [29] has pointed out that if the sugar in the urine amounted to 60 grammes and the protein in the diet to 100 grammes, the additional quantity of oxygen which would be demanded to form this amount of sugar out of protein would lower the respiratory quotient to 0.70. The situation is still further complicated by the presence of unoxidized acid bodies in the urine, amounting frequently to 20 to 40 grammes and occasionally to 60 grammes calculated as beta-oxybutyric acid. The amount of oxygen consumed in the formation of these bodies—for beta-oxybutyric acid is far more rich in oxygen than are protein and fat—would again lower the quotient, and it has been calculated by Magnus-Levy that the respiratory quotient of a case of diabetes presenting 60 grammes of sugar in the urine for 100 grammes of protein in the diet, and excreting 20 grammes of beta-oxybutyric acid, would fall as low as 0.69. For convenience, these figures are summarized. The respiratory quotient of the fasting man at the Nutrition Laboratory was 0.72. The calculated respiratory quotient of a

normal individual who is burning 15 per cent. protein and 85 per cent. fat is 0.72. The theoretical respiratory quotient of a diabetic individual excreting all the carbohydrate in the diet, and 60 grammes of glucose for each 100 grammes of protein in the diet, is 0.70. The theoretical respiratory quotient of the diabetic individual excreting 60 grammes of glucose for 100 grammes of protein and 20 grammes of beta-oxybutyric acid as well, is 0.69. These calculations presuppose that the sugar and beta-oxybutyric acid excreted were formed during the same 24 hours, but who knows whether this is the case? The theoretical non-protein respiratory quotient of a case of diabetes living upon fat and the non-carbohydrate part of the protein molecule, as calculated by Lusk, is also 0.69.

TABLE 15.—Theoretical Respiratory Quotients
(From Magnus-Levy)

Diet	Calories	R. Q.
Protein, 100 Gm. (100×4.1 =410) Carbohydrate 567 Gm. (567×4.1 =2325)	2735	0.97
Protein, 100 Gm. (100×4.1 =410) Fat, 250 Gm. (250×9.3 =2325)	2735	0.72
Loss in Urine Sugar, 60 Gm. (60×4.1 =246)	2489	0.70
Loss in Urine Sugar, 60 Gm. .....(60×4.1 =246) β-Oxy. acid, 20 Gm. (20×4.7 = 94) Total loss =340	2395	0.69

Table 15 shows the theoretical respiratory quotient, which should be reached under varying conditions of diet for a normal individual, and the changes which theoretically are present under special conditions in diabetes. Figures of this type have dominated the discussions of the metabolism in diabetes from the start, and whenever experiments have not produced figures comparable with these, they have often been considered erroneous. We are taught to believe that diabetic patients are not severe unless the respiratory quotient is 0.69. It is questionable, however, whether the experimental data at our disposal enable us to say that our theories are backed up by the results which

we obtain. If one looks over the lists of respiratory quotients obtained in successive periods with any variety of respiratory apparatus or calorimeter, he will be shocked at the discrepancy and is forced to the belief that any argument based on a change in the respiratory quotient of one point is unjustifiable, and any argument which is based on a change in the respiratory quotient of two points really rests on a very slender thread. A change of three points is, however, deserving of consideration, but modesty should rule in conclusions which are to be drawn from any given set of experiments.

It is appropriate to discuss here what constitutes a severe diabetes. At the outset it can be said for our own encouragement that Naunyn did not pretend to be able to distinguish accurately between the various types. Usually by severe diabetes is understood those cases in which, to quote von Noorden, "notwithstanding a prolonged, extreme carbohydrate-free diet, the urine contains sugar." By an extreme carbohydrate diet von Noorden undoubtedly meant one containing protein, fat, and a few green vegetables, in other words, a diet with 10 grammes of carbohydrate, more or less—not a strictly fat-protein diet. The definition is also open to objection, because one frequently meets with cases of diabetes of long duration who excrete in the urine but a small per cent. of the carbohydrate intake, yet this persists for many days upon an extreme carbohydrate-free diet, but the patient could not be classed as severe.

Another method of classification is adopted by Lusk, who considers cases severe which, when put upon a protein-fat diet have a dextrose-nitrogen ratio of 3.65 : 1. By this he means that 3.65 grammes of dextrose appear in the urine for 1 gramme of nitrogen, or the 6 grammes of protein which it represents. In other words, 60 per cent. (actually $3.65 \div 6.25 = 58.4$) of the protein burned by the body appears in the urine in the form of sugar. Lusk considers that this is the greatest possible amount of sugar which can appear in the urine on a carbohydrate-free diet, and he assumes that it comes wholly from protein. This conclusion has been reached with many observations upon dogs,

following injections of phloridzin, and by one case of diabetes coming under his personal observation, and he refers to other cases selected from the literature.

Unfortunately, or perhaps fortunately, neither of these methods of classification at the present time is wholly satisfactory, because, thanks to Dr. Allen, our patients now become sugar-free very readily. It is possible that fasting will not remove the sugar from the urine of all diabetic patients, but this has been my experience with every case when I have followed Dr. Allen's directions, and my experience coincides with that of many others. It may be that recent cases of diabetes have been of a different type from those hitherto encountered, but this is hardly possible. Consequently we cannot adopt the definition of von Noorden, and it is embarrassing to adopt the precise definition of Lusk. The dextrose-nitrogen ratio vanishes with fasting, and the clinician does not wish to expose his patient before beginning fasting to the dangers of a protein-fat diet simply to determine his severity. I am hoping that, with the added knowledge of diabetes which the introduction of fasting has brought about, Professor Lusk will pursue his studies further and give us definite rules for testing the severity of the disease. Perhaps definite quantities of protein alone or some special form of protein or derivative of protein could be administered to these patients, and the amount of sugar in the urine determined. Should this method not furnish satisfactory results, another series could be carried out in which varying quantities of fat as well as protein could be added, and, if a third factor were necessary, the calories per kilo could be standardized. But we can trust Professor Lusk to give us help. Of course dextrose-nitrogen ratios are of little significance without simultaneous reports of the blood sugar.

In the data which will follow, consideration will be taken of both von Noorden's and Lusk's classifications, but also the severity of the cases will be indicated by a statement of the time intervening between the period of observation and death in coma. It would seem as if the severity of the type of diabetes which resulted in death in coma should challenge criticism.

As the periods of observation before death in coma are of importance, the intervals between the determination of the respiratory quotient of the patient and death are given. See Table 17, which will later be discussed more in detail. This appears far more rational than to give the duration of the course of the disease, for many patients present a mild type of diabetes for many years, changing over to a severe type at a comparatively short period before death.

The following table summarizes the respiratory quotients of cases of diabetes considered severe by their observers:

TABLE 16.—Respiratory Quotient in Severe Diabetes

Year	Cases	Observers	R.Q.
1894.....	1	Weintraud and Laves: Ztsch. f. Physiol. Chemie., 1894, vol. xix, p. 603.....	0.70
1897.....	2	Nehring-Schmoll: Ztsch. f. klin. Med., 1897, vol. xiii, p. 59.....	0.72
1905.....	2	Magnus-Levy: Ztsch. f. klin. Med., 1905, vol. lvi, p. 86	0.71
1907.....	1	Mohr: Ztsch. f. Exp. Path. u. Therap., 1907, vol. iv, p. 910.....	0.72
1908–1911.	19	Benedict and Joslin: Carnegie Inst. of Washington, Publications 136 and 176, 1910, 1912.....	0.73
1912.....	8	Rolly: Deut. Archiv. f. klin. Med., 1912, vol. cv, p. 494	0.74
1912.....	3	Grafe and Wolf: Deut. Archiv. f. klin. Med., 1912, vol. cvii, p. 201.....	0.74
1912–1914.	7	Benedict and Joslin: 1914–15.....	0.73
Total...43		Average.....	0.73

It will be seen that there is surprising unanimity of agreement among the different groups. It should be stated that Leimdorfer [30] has obtained much lower quotients, varying between 0.64 and 0.68, with five cases which he considered severe. His figures, however, have not been generally accepted. One of the cases which he considered mild at no time showed a respiratory quotient above 0.70. According to the computations given above from Magnus-Levy, it was shown that, theoretically, in a diabetic patient with 60 grammes of sugar in the urine for each 100 grammes of protein in the diet—in other words, ap-

proximately the Lusk dextrose-nitrogen ratio—and with 20 grammes of beta-oxybutyric acid, the respiratory quotient would not go below 0.69, and he further points out that, in order for the ratio to sink to 0.653, 150 grammes of sugar must be formed from 150 grammes of protein and 40 grammes of beta-oxybutyric acid must appear in the urine when the patient is upon a diet of 150 grammes protein and 250 grammes fat. A respiratory quotient of 0.653 is a figure so low that it should be entertained with scepticism. The average respiratory quotient of 0.73 for 43 cases of clinically considered severe diabetes is a far safer figure to follow than to pick out one, two or three from the 43 cases and say that these represent severe cases of diabetes and the others do not. The errors of the determinations of the quotients are so great that the average figures are safer than the individual ones. These respiratory quotients, as Grafe and Wolf [31] pointed out, show that at least some carbohydrates were being oxidized by severe diabetic patients. They also pointed out that with the improvement of patients the respiratory quotients increased from 0.743 to 0.817 in a fasting condition.

These figures suggest at the first glance that very little carbohydrate was burned in this group of severe cases of diabetes. The respiratory quotients are identical with the quotients obtained under similar conditions with the fasting man at the Nutrition Laboratory, though his average for the whole day for the fasting period was 0.72. But we must remember that two corrections are to be made in these figures: first, sugar has been lost in the urine which has been formed from protein, and second, there have been varying amounts of beta-oxybutyric acid, diacetic acid and acetone excreted. Both of these processes represent processes of oxidation, and by demanding additional oxygen for which no carbon dioxide is produced tend to lower the respiratory quotient. Therefore, if we grant that the series represents cases of severe diabetes, we must reach the conclusion that these diabetic patients utilized some carbohydrate, and that their respiratory quotients would have been several points above

0.73 had they not been lowered to 0.73 by the production of sugar from protein and the formation of acid bodies.

Are the cases reported in the above table severe? At least no cases of greater severity have been hitherto published. By von Noorden's criterion they might be considered severe, for they did not become sugar-free with restricted diet, yet it is true that this restricted diet was not so rigid as is often employed on account of the marked acidosis. If we accept Lusk's criterion (and I am not ready to do so until a second human case is studied under modern conditions §) they were not severe. Not one of Benedict's and my cases showed a persistent D:N ratio of 3.65:1. Yet the clinical facts point to severity. Of the first group of 19 cases of diabetes reported in 1908–1912 by Benedict and myself, 18 are dead, and of these 15 died in coma. This fact can be taken as a measure of their severity. I do not believe, however, that this alone justifies us in saying that a diabetic patient is of the severest type. I conceive it possible, for a moderately severe case of diabetes, by sudden changes of diet, to be driven into coma accidentally. This was done years ago, when diabetic patients, who were living on a free diet, upon coming to the hospital were suddenly deprived of carbohydrate and the fat and protein were increased. It appears to me quite probable that most cases of coma in diabetes have occurred long before the disease had reached its greatest severity, and I wish to point out that therein lies great hope for the future.

However, it will be of interest to note the respiratory quotient of a group of six cases of diabetes all ending in coma,

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§ By modern conditions I mean (1) exclusive fat-protein diet; (2) under surroundings which make errors in diet impossible; (3) a duration of at least 7 days to exclude the washing out of stored carbohydrate; (4) a constant (not falling) D:N ratio of 3.65:1 for the last 3 of the 7 days; and (5) several daily blood sugar determinations to furnish some proof, inadequate though it be, that the sugar in the urine has not come from that left over in the blood. At present I cannot advocate such a test because of the danger of acidosis, and believe it better to leave the question, in this form, undecided.

who died within a period of 44 to 14 days from the time of observation, and to compare these with a group of patients whose respiratory quotient was observed at a greater interval from death in coma. This is shown by the following table:

TABLE 17.—Respiratory Quotient in Fatal and Living Cases of Severe Diabetes Compared

Fatal cases			Living cases		
No. of Cases	Days before coma	R. Q.	Case Number	Days before [1] March, 1915	R. Q.
6	44–14	0.71	552	801	0.72
8	442–70	0.74	765	125	0.73
			786	111	0.71
			806	72	0.70
			4 cases	801–72	0.715

[1] All of these cases were in good condition May 1, 1915, which would add 61 days to the duration since the observations were made.

A consideration of this table suggests that with approaching death the respiratory quotient falls. It will be seen that the cases dying within a period of 44 to 14 days from the time of observation gave a quotient of 0.71, as contrasted with a quotient of 0.74 in cases dying in coma at an interval from death of 442 to 70 days. If we had these figures alone, the inference might be justified, but caution is necessary before drawing such a conclusion. Four living cases of diabetes show a respiratory quotient almost as low—0.715. Instead of progression toward death in coma, their general condition has improved. In other words, a falling respiratory quotient does not necessarily mean approaching death in coma. It does mean that these patients have lived for prolonged periods upon an almost exclusively fat-protein diet, and suggests that they are forming carbohydrate out of protein and producing acid bodies.

It should be said that all of these living cases have been treated either by much restricted diets or by fasting as advocated by Dr. Allen. When they were first seen they appeared

to be quite as severe cases of diabetes as those earlier studied which died in coma. What shall we say of them at present? None of these cases can be considered well, but all lead a comfortable life at home.

The group of cases dying within a period of 44 to 14 days deserves further comment. The average quotient of these cases was 0.71. From four of these the non-protein respiratory quotient has been reckoned, and it amounted to 0.695. This respiratory quotient implies that much material must have been formed in the course of the metabolism which used a portion of the oxygen. This was especially true of Case 246,[32] who had a respiratory quotient of 0.69, which was based upon an average of 29 periods, most of which were fasting. Stimulated by inquiries from Professor Lusk, I am fortunately able to show the cause of the particularly low quotient in this patient. His diet and urinary analyses will be found in Tables 18 and 19.

TABLE 18.—Metabolism of a Severe Diabetic with a Respiratory Quotient of 0.69. Case C. No. 246. Male. Acute Onset at 28. Death in Coma in 15 Months. Month of Disease, 13

Day	Urine					Diet				Sodium bicarbonate
	Vol.	N.	NH_3	β-oxy.	Dextrose	Carbohydrate	Protein	Fat	Alcohol	
I.....	2935	16.3	4.8	29	72	15	13	55	15	0
II.....	3710	13.3	5.0	34	106	98	22	225	30	0
III.....	4370	19.6	5.5	61	134	65	100	200	30	60
IV.....	4035	19.4	5.4	61	107	65	100	200	30	60
V.....	3330	14.7	5.6	46	100	125	45	100	30	25
VI.....	3765	16.3	5.0	48	93	65	100	200	22	25
August.....	3691	16.6	5.2	46.6	102	71	65	165	26	28

Carbohydrate in diet..... 72 Gm.
Dextrose in urine..... 102 Gm.
Carbohydrate balance..... 30 Gm.

D : N Ratio 1.9 : 1.0

Daily protein metabolism estimated at 100 Gm. Total acetone bodies estimated at 60 Gm.

TABLE 19.—To Supplement Table 18. Case C. No. 246

Diet	Calories	O_2	CO_2	R.Q.
	Grammes	*Litres*	*Litres*	
Protein	100×4.1 = 410	96.6	78.2	
Fat	165×9.3 =1535	333.1	235.5	
Carbohydrate	71×4.1 = 291	58.9	58.9	
Alcohol	26× 7 = 182	37.9	25.3	
	2418	526.5	397.9	0.756
Lost in urine				
Dextrose	102×3.7 = 337	76.1	76.1	
Acetone bodies as β-oxyb	60×4.5 = 243	58.1	51.6	
	620	134.2	127.7	
	1798	392.3	270.2	0.692

The respiratory quotient found, based on an average of 29 periods, chiefly fasting, was *0.69*.

The average daily urinary nitrogen for the six days of observation was 16.6 grammes, and it was considered that this represented approximately the metabolism of 100 grammes of protein. The beta-oxybutyric acid was 46.6 grammes daily, and allowing for acetone and diacetic acid the total excretion of acid bodies was assumed to be 60 grammes. The fat in the diet as originally recorded was probably inaccurate, and I believe 165 grammes daily near to the exact quantity. From these tables it will be seen that the daily carbohydrate in the diet was 71 grammes, and the dextrose excreted was 102 grammes, giving a minus balance of 31 grammes. This, with the 16.6 grammes of nitrogen in the urine, gives a D : N ratio of only 1.9 to 1. In Table 19 are placed the data from which the respiratory quotient can be calculated from the diet and urine, and they show that after deductions for dextrose and acetone bodies, the theoretical quotient would be 0.692, which it will be remembered was identical with the respiratory quotient found by experiment. These tables are submitted as proof that a quotient of 0.69 does not necessarily mean that the capacity for burning carbohydrate has been totally abolished.

Computations of a similar character by Grafe and Wolf [33]

lead to the same conclusion. According to these writers, "the conception which, on the whole, appears to have the greatest probability is that even the severest diabetic has at his disposal 20 to 30 grammes of glycogen for combustion or synthesis, 13 to 20 hours after a meal containing a minimal amount of carbohydrate. Perhaps the complete loss of the power of combustion of sugar is, broadly speaking, no longer consistent with life."

Effect of Food upon Utilization of Carbohydrates in Severe Diabetes.—A moderate number of experiments upon the effect of food on severe diabetics has been recorded, but the actual number of experiments to determine the effect of carbohydrate upon the respiratory metabolism is very limited. Such experiments have been published by Leo,[34] who considered that the respiratory quotient did increase in two cases of severe diabetes, although this was not uniformly the rule, and he concludes that even in severe diabetes a part of the sugar ingested or formed in the body is utilized.

Nehring and Schmoll [35] tested the effect of carbohydrates also in two severe cases of diabetes, but were unable in either to show an increase in the respiratory quotient. Frequently a fall instead of a rise in the quotient took place. Benedict and Joslin,[36] in a series of experiments chiefly with bread and dextrose, state that "the ingestion of carbohydrate produced no very material alteration in the metabolism as a whole," and later, "no evidence can be found of a combustion of carbohydrate. . . ." Two years later a series of experiments with oatmeal and levulose was reported, but without comment.

Schilling, in an Inaugural Dissertation, Leipzig, 1911, tested the effects of various meals upon the respiratory quotient of 1 severe, 1 mild, and 2 moderately severe cases of diabetes, and demonstrated no specificity for oatmeal. With the severe case the results were inconstant, but usually tended to show a slight increase.

Rolly,[37] in a series of experiments, tested the comparative effects of oats, rye, wheat, lentils and green corn-meal upon diabetic patients. Unfortunately, few of the experiments were preceded by control periods. Two of his cases he considers severe. In Case I, at 3, 5 and 6 hours after 70 grammes of oatmeal were administered, the respiratory quotient was 0.73.

After 70 grammes of wheat meal it was 0.76. The respiratory quotient of his Case V after 80 grammes of oatmeal was 0.71, after 80 grammes of rye meal was 0.73, and after 80 grammes of wheat meal was 0.71. Two of his other cases were only moderately severe, and the other only a light case, and all showed an increase in the respiratory quotient after their meals reaching up to 0.83, 0.85 and 0.84 respectively. It will be noted, furthermore, that of the two severe cases, in the first the quotient following administration of wheat meal which was given after oatmeal reached 0.76.

Roth [38] records slight increase of the respiratory quotient following the administration of carbohydrate. The experiments, however, lose much of their value because of the absence of fasting controls upon the day the carbohydrates were given.

Falta [39] has mentioned several experiments designed to show the effect of the oatmeal cure upon the respiratory quotient. The data of the experiments are not given, but he states that with one moderately severe diabetic the respiratory quotient rose only on the third day of an oatmeal cure, in which 400 grammes had been given daily. Despite this enormous quantity of oatmeal, no glycosuria was observed. It is unfortunate that I have not been able to find a later publication, which was promised. He furthermore makes the interesting statement, which is so remarkable as to invite confirmation, that a similar result was encountered with a normal individual, whose carbohydrate depots had been robbed by living upon a diet poor in carbohydrates for a long time. It would appear that only after these depleted carbohydrate stores were replenished, the normal individual, like the diabetic, began to burn carbohydrate. His results are in striking contrast to the changes in respiratory quotient which were shown by the fasting man at the Nutrition Laboratory. At the end of his fast of 31 days he ate food almost exclusively in the form of carbohydrate and the quotient promptly rose to 0.79 and 0.96 on the second and third days respectively. Falta emphasized the fact that in a mixed diet carbohydrates are burned much earlier. He further states that on a meat diet or on a diet with a moderate amount of carbohydrate the diabetic patient seldom shows a quotient above 0.74, and

he also noted the fact, to which attention has been called by Nehring and Schmoll, which is also borne out by our own series of cases, that, following the administration of carbohydrate, a considerable quantity of carbohydrate not only remains in the body, but the respiratory quotient remains low. Intravenous injections of sugar (30 grammes) given by Falta to severe diabetics, who had eaten 300 grammes of oatmeal for three days without glycosuria, brought about an evident glycosuria, but the respiratory quotient rose proportionately little. In the case of a severe diabetic there was no increase, but a still further lowering of the already low respiratory quotient.

The present series of experiments with foods which I have to report represent a part of the experiments upon diabetics whose metabolism following the administration of food was studied at the Nutrition Laboratory since 1910.

TABLE 20.—Effect of Levulose Upon a Severe Diabetic. Case No. 332. Female, Age 37; Weight, 40 Kilos

Date, 1911	Condition	CO_2 per min.	O_2 per min.	R. Q.	Calories per kilogramme per 24 hours
March 31	Fasting	c.c.	c.c.		
10 : 50	Fasting	151	205	0.74	35
11 : 23	Fasting	145	211	0.69	36
	100 Gm. levulose				
12 : 16		172	271	0.63	46
12 : 44		184	261	0.70	44
1 : 30		180	246	0.73	42
2 : 05		172	235	0.73	40
2 : 28		171	250	0.68	42
2 : 53		166	240	0.69	40
April 2					
8 : 17	Fasting	148	199	0.75	34
8 : 45	Fasting	151	203	0.74	35
9 : 14	Fasting	154	213	0.72	36
	Oatmeal =70 Gm. carb., 38 Gm. butter				
10 : 13		163	234	0.70	39
10 : 39		167	228	0.73	39
11 : 08		177	238	0.75	40
12 : 12		170	230	0.74	39
1 : 28		154	206	0.75	35
2 : 37		163	209	0.78	36

TABLE 21.—Effect of Levulose Upon Respiratory Quotient of Diabetic Patients

Case	Duration months	Month observed	Carbohydrate preceding day	Levulose	Sugar in urine 24 hours	R. Q.	
	Dead		*Gm.*	*Gm.*	*Gm.*	*Before*	*After*
332	28	23	100±	100	120	0.72	0.69
	Alive						
552	32	18	30	100	3	0.72	0.76
785	23	20	20	81[1]	7	No increase	

[1] 81 Gm. levulose and later,
9 Gm. carb. as vegetables.

90 Gm. total.

Three experiments have been conducted with levulose. Case No. 332 was given 100 grammes of levulose when fasting. This patient was a severe diabetic, weight 40 kilogrammes in the twenty-fourth month of her illness, and died five months later. The respiratory quotient before the levulose was administered was 0.72, and following the levulose the quotient was determined in six different periods during the following three hours and showed an average of 0.69. Despite the fall in the respiratory quotient, the total metabolism increased markedly, although apparently most of the levulose was excreted in the urine. Unfortunately, it is impossible to state how much of the 120 grammes of sugar in the urine for this 24 hours came from the levulose and how much from carbohydrates of the preceding day. Our records indicate that the patient was on a diet containing approximately 100 grammes of carbohydrate. This fact is of interest in comparison with the next two cases, to whom levulose was also given.

Case 552, age thirty-seven, weight 40 kilogrammes, in the twenty-third month of her illness, received also 100 grammes of levulose, but this was given after a prolonged period of low carbohydrate feeding. Upon the day previous to the experiment the carbohydrates in the diet amounted to 30 grammes. The quantity of sugar in the urine in this case during the 24 hours of the experiment was 3 grammes. The respiratory quotient rose four points, namely, from 0.72 to 0.76 after the levulose.

The third case, No. 785, was that of a boy of 16 years of age with severe diabetes of 20 months' duration, weight 42 kilogrammes. He had been made sugar-free by prolonged fasting and had been kept upon a diet low in carbohydrate and protein, as well as fat. During the 24 hours of the test, the urine contained but 7 grammes of sugar. Notwithstanding this fact, the respiratory quotient showed no increase, but a fall of two points. The actual figures are not published now, but the comparative values may be considered trustworthy. The evidence in these three cases, therefore, points to no utilization of the levulose in two of the cases. In one of these most of the levulose was probably excreted, but in the other only a negligible quantity. In the third case there was an increase of three points in the respiratory quotient, indicating a slight utilization of the levulose and there was no excretion of levulose of account.

It was possible to determine the effect of the administration of potato in two cases. In the first case the experiment was complicated in that the patient was given a small quantity of oatmeal at the start, but on account of her dislike of the same it was stopped and potato substituted. In this case, No. 765, no

TABLE 22.—Effect of Potato Upon Respiratory Quotient of Severe Diabetics

Case No.	Duration months	Month observed	Carbohydrate intake		Sugar in urine 24 hours	R. Q.	
			Preceding day	Test day			
			Gm.	*Gm.*	*Gm.*	*Before*	*After*
765	7	3	15	63[1] 22 85	29	0.74	0.73
806	6	3	10	60[2] 6 66	3	0.68	0.70

[1] 48 Gm. carb. as potato, 10 Gm. carb. as oatmeal, 5 Gm. carb. as cream, —total 63 Gm. Later in day, 22 Gm. carb. as potato and vegetables. Also 1 egg and 30 Gm. of butter.

[2] 60 Gm. carb. as potato. Later in day, 1 egg, butter, 6 Gm. carb. as vegetables.

TABLE 23.—Effect of Potato Upon the Respiratory Quotient of a Severe Diabetic. Case No. 806. Male, Weight 62 Kilos

Date, 1914	Condition	CO_2 per min.	O_2 per min.	R. Q.		Cals. per kilo per 24 hours		Blood sugar
December 22		*c.c.*	*c.c.*					*Per cent.*
9 : 25	.....	156	223	0.70		24		
9 : 54	Fasting...	150	224	0.67	0.68	24	24	
10 : 22	.....	155	228	0.68		25		
10 : 45	.....	.....	.....	.....		.....		0.14
	Potato =60 Gm.carb.							
10 : 55	.....	.....	.....	.....		.....		0.18
11 : 59	.....	181	257	0.71		28		
12 : 22	.....	168	252	0.67	0.69	27	27	
12 : 55	.....	172	250	0.69		27		
3 : 00	.....	170	233	0.73		26		
3 : 26	.....	157	227	0.70	0.72	25	25	
3 : 54	.....	166	231	0.72		25		
4 : 45	.....	.....	.....	.....		.....		0.19

change in the respiratory quotient took place, but in the second, Case No. 806, a slight increase was noted, and apparently rather more than would be accounted for by the limits of error.

TABLE 24.—Effect of Oatmeal Upon the Respiratory Quotient of a Severe Diabetic. Case No. 773. Female, Weight 40 Kilos

Date, 1914	Condition	CO per min.	O per min.	R. Q.	Cals. per kilo per 24 hours	Blood sugar
		c.c.	*c.c.*			*Per cent.*
X 8 : 00.	Fasting.....	146	212	0.69	36	
	Oatmeal =42 Gm. carb.					
11 : 00.	.....	178	249	0.72	43	
XIII 8 : 00.	Fasting.....	138	189	0.73	33	0.32
11 : 00.	.....	.....	.....	.....	.....	0.27
XIX 9 : 00.	Fasting.....	135	195	0.70	34	0.27
	Oatmeal =80 Gm. carb.					
12 : 00.	.....	167	237	0.70	40	0.30
XX.....	After breakfast.....	.....	.....	.....	.....	0.34

Diet contained 15 Gm. carb. Oct. 9 and Oct. 18.

TABLE 25.—Effect of Oatmeal Upon the Respiratory Quotient of Severe Diabetics

Case No.	Duration		Date	Carbohydrates ignited		R. Q.		Sugar in urine	Carb. intake	Carb. balance
	Onset to coma months	Mo. of test		Day preceding	Before test	Fasting	After oatmeal			
				Gm.	*Gm.*			*Gm.*	*Gm.*	*Gm.*
194	34	31	Sept. 22	15	.....	0.74	.....	42	15	−27
			23	15	100+	0.71	0.71	50	165	+115
			24	165	.....	0.72[1]	.....	19	15	−4
246	15	11	Aug. 9	50	40	0.71	0.67	124	?	?
		13	Oct. 29	65	60	0.68	0.70	100	125	+25
			30	125	.....	0.71	.....	93	65	−28
			25–31	71	.....	0.69	.....	102	72	−30
281	19	17	Dec. 1	15	.....	0.74	.....	69	135	+66
			2	135	29	.....	0.76	58	45	−13
			3	45	0	0.76	.....	38	30	−8
332	28	13	May 19	100	25±	.....	0.73	15 in 3 hrs.		
			26	95	.....	0.73	.....	3 in 3 hrs.		
		24	Apr. 2	?	52	0.74	0.74	97		
		26	June 2	?	48	0.71	0.69	36		
336	132	127	May 18	20	.....	0.73	.....	26	45	+19
			21	45	25	.....	0.75	31	45	+14
441	11	9	Sept. 29	15	75	0.70	0.71	65	165	+100
		10	Oct. 9	15	73	.....	0.69	?	79	
561	33	23	Feb. 7	60	.....	0.75	.....	31.1	60	+30
			8	60	116	0.71	0.74	128.4	185	+57
			9	185	200	0.72	0.72	209.3	205	−4
			10	200	.....	0.76	.....	101.86	60	−42
591	50	44	Apr. 10	?	.....	0.74	.....	63	30	−33
			11	30	.....	0.73	.....	37	15	−22
			12	15	80	0.70	0.70	85	165	+80
			13	165	80	0.73	0.69	77	165	+88
			15	40	.....	0.69	.....	29	?	
773	20	18	Oct. 8	115	70	.....	0.70	175.6	165	−10
			10	15	47	0.69	0.72	95.4	130	+35
			13	50	.....	0.73	.....	83.97	50	−34
			19	15	80	0.70	0.70	96.50	115	+18
746	22 prior to March, 1915	18	Oct. 7	65	26	.....	0.73	93.11	65	−28
			9	15	50	0.73	0.71	86.69	163	+76
			10	165	.....	0.72	.....	34.88	25	−10
			15	165	80		0.74	96.28	165	+69
786	17 prior to March, 1915	14	Nov. 12	15	60	0.69	0.74	0	62	+62

[1] One line under a figure indicates that the R. Q. was taken following an oatmeal day, and two lines that it was subsequent to two oatmeal days.

Eleven experiments have been carried out upon severe diabetics with oatmeal. These were arranged in some cases to determine the immediate effect of the administration of oatmeal, and in other cases to determine the effect of the prolonged administration of oatmeal. It will be seen from a study of the charts that as a rule the respiratory quotient remained stationary or fell, in one case it rose four points, and in two other cases it rose one point. It will be noted further that the respiratory quotient, when taken fasting upon the morning following an oatmeal day, amounted in three cases to 0.73, 0.72 and 0.73 respectively, and that upon the morning following a second oatmeal day was 0.69 and 0.76. The respiratory quotient also determined in three experiments after the administration of carbohydrate on the second oatmeal day was 0.72. If one looks at the table as a whole, it will be seen that little change in the respiratory quotient took place; in fact, none of any account except upon the morning following the second oatmeal day.

The sum total of the results following the feeding of levulose, potato and oatmeal to severe diabetics affords little evidence from the respiratory quotient that the carbohydrate was burned, save in the case of one of the experiments with levulose, one with potato, and one with oatmeal. These results correspond closely with what has been recorded in the literature. Personally, I believe that before a final decision upon this point can be reached from this particular line of study, further experiments must be performed.

Unfortunately, in the experiments recorded, no stated agreement was noted between changes in respiratory quotient and variations in the quantity of blood sugar. From Table 7 it is evident that there is a general tendency for the respiratory quotient to rise with an increase in blood sugar, but this may be accidental. Studies now in progress will soon throw light upon this phase of the question.

V. ACIDOSIS AS A MEASURE OF THE UTILIZATION OF CARBOHYDRATES

It has been generally accepted that acidosis will appear when carbohydrate food is either withdrawn from the diet or

excreted in the urine. It has been unquestionably the universal clinical experience that the patient who excretes quantities of sugar in the urine equal to or in excess of that in the diet exhibits acidosis, and that patients do not show acidosis who are able to utilize approximately 70 grammes of carbohydrate, or large quantities of protein from which carbohydrate may be formed. This statement cannot be so unqualifiedly made, because I have under observation a woman who, in her sixth month of pregnancy, showed over 6 per cent. of sugar, and later under a careful diet became sugar-free, acquired a tolerance for approximately 100 grammes of carbohydrate, and yet a slight acidosis persisted. Nevertheless, the general mass of evidence points to the disappearance of acidosis when carbohydrates are burned, and upon this general concept arguments have been based for and against the utilization of carbohydrate in severe diabetes.

During von Noorden's oatmeal treatment a considerable quantity of carbohydrate ingested is usually retained or burned in the body, and the decrease of acidosis at the same time is usually considered evidence of the latter supposition being correct, but occasionally the acidosis persists although the carbohydrates are not excreted. I doubt if we are in a position to accurately explain the disappearance or non-disappearance of acidosis under these conditions. Oatmeal and other carbohydrates are better retained in the body following starvation, and it is quite possible that with the retention of carbohydrate goes hand in hand a mechanical retention of acid bodies. Magnus-Levy pointed out long ago that these were seldom excreted in concentration of more than 1.5 per cent., and that the fall in acidosis during an oatmeal cure may be simply apparent, because the volume of urine excreted has diminished. The influence of preceding fasting is also important, because this undoubtedly regulates to some extent the storage of carbohydrate. Despite these possibilities, which lessen any argument for combustion of carbohydrate based on the decrease of acidosis following the ingestion of the same, the slight amount of acidosis which is usually found when diabetic patients are on a full

carbohydrate diet points strongly to the fact that some carbohydrate is burned. The increase in respiratory quotient on the last days of an oatmeal cure, which Falta observed and we also have noted, is confirmatory of this position.

Various writers have observed that the acidosis in diabetics decreases upon a vegetable day or fasting day, but it remained for Allen to conclusively demonstrate the remarkable fact that acidosis vanished in practically all severe cases of diabetes under these conditions, and that in the remainder, if carbohydrates to a moderate extent are allowed temporarily, the acidosis wholly clears up. If a normal individual fasts, it has been the universal experience of observers that acidosis appears. In other words, the normal fasting individual corresponds with the concept that when carbohydrates are withdrawn from the diet (and this implies carbohydrates which might be formed from protein) acidosis appears. Thus, in the fasting man at the Nutrition Laboratory, acidosis appeared upon the second day and continued until the fast was terminated. How can we reconcile these two opposing facts: the one that fasting dissipates acidosis in diabetes, but produces it in normal individuals. Must the prevalent conception be given up that carbohydrate oxidation and acidosis are unrelated and must we acknowledge that here is an instance where the absence of the burning of carbohydrates does not lead to acidosis? Such a conclusion appeared unavoidable until observations at the Nutrition Laboratory upon severe diabetics during prolonged fasting began to accumulate, showing that, whereas at the beginning of the fast the respiratory quotient was the ordinary respiratory quotient of severe diabetes, 0.72, with a continuance of the fast this had a tendency to rise several points, occasionally even to the neighborhood of 0.80. Later experiments, as yet unpublished, at the Russell Sage Laboratory made under the direction of Dr. DuBois and Professor Lusk upon one of Dr. Allen's patients suggested a similar condition. In other words, whereas the normal individual showing acidosis exhibits a respiratory quotient based upon the combustion of protein and fat alone, the severe diabetic during fasting shows a respiratory quotient which could only

be accounted for by the combustion of notable quantities of material other than fat and protein. That this material was not protein was evident, because the amounts of nitrogen in the urine excreted during these periods were not abnormal. The explanation why the severe diabetic shows no acidosis when fasted, in contradistinction to the normal individual, is found in this increase in the respiratory quotient.

Several explanations for this increase in the respiratory quotient of fasting diabetics are available. During fasting the diabetic may be able to draw upon sources of carbohydrate in the body which the normal individual cannot. Furthermore, the diabetic has in the body undoubtedly more carbohydrate stored than we have hitherto supposed, and the supposition must be entertained that the diabetic really may actually have more carbohydrate in some form in the body than exists in the normal individual. A third supposition for the increase in the respiratory quotient is that considerable quantities of acid bodies have accumulated and that with the improvement of the condition of the patient during fasting these are burned. It will be remembered that beta-oxybutyric acid, diacetic acid and acetone all have relatively high respiratory quotients, namely, 0.89, 1.00 and 0.75 respectively, and therefore the oxidation of a small quantity of these substances would markedly raise the respiratory quotient. Which of these suppositions is correct will be eventually known because of the improved methods of estimating carbohydrate and acid bodies in the blood, fluids and tissues of the body, and also by the help which is afforded from the estimation of the carbon dioxide tension of the blood.[40]

I should like to point out this further possibility—during prolonged fasting, acidosis tends to disappear, in part because the sources of the acid bodies, save for body fat and protein, have been eliminated. So soon as acidosis begins to decrease, there is, as we and others have found, a lessening of the total metabolism, and with this lessening of total metabolism an improvement in the combustion of carbohydrate takes place. This in turn favors the combustion of acid bodies. It might

well be that the first step to take in the treatment of a case of diabetes is to completely abolish acidosis.

All may be ready to concede that all cases of diabetes under fasting conditions are burning carbohydrates, but some may say that the character of the disease has changed, and instead of being a severe type of diabetes the case has become one of moderate severity. Such a criticism is hard to answer. It, however, presupposes that an individual can readily change in the space of a few hours from a state in which death is imminent to one of safety, and that so fundamental a function as the loss of power to utilize carbohydrates can be quickly regained. This would be a remarkable phenomenon. Against this explanation also is the fact that many who have employed fasting treatment with severe cases of diabetes have regretfully acknowledged that either very slight or no increase of tolerance for carbohydrates has been produced in these patients. This would make it still more unlikely that the diabetic patient by fasting altered his nature. It would rather point to the view that the diabetic condition remained unchanged, but that during fasting the diabetic was able to secure and burn material which under other conditions he could not reach, and that the normal individual could not secure.

In conclusion it is gratifying to be able to record that the recent experimental evidence confirms the old clinical view that the severe diabetic still retains a power to utilize a portion of the carbohydrate of his diet, small though it may be.

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