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THE CHEMICAL BASIS OF IMMUNITY.

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PREFACE.

This thesis is composed of a series of papers, three of which have been published by the author in conjunction with others, or the author alone, one article prepared for publication and a preliminary report of the studies which are now being made.

Much of the work necessary for the final proof of the ideas advanced in this thesis can not be completed before this thesis must be submitted. Many new methods for the study of minute changes of foreign substances in the body must be devised and applied to this problem before the hypotheses presented here can be rounded into complete, precise theories. The author therefore makes no claims to a finished piece of work, but wishes only to present certain new and interesting facts and to deduct from them tentative theories concerning immunity and its chemical basis.

The articles and experiments are assembled as nearly as possible in chronological order. They present therefore the trend of thought present through out the work as well as the necessary modifications of ideas as new facts presented themselves. However each experiment is complete in itself and the whole makes more or less a larger unit of thought.

Chemical Basis of Immunity.

Some Important Concepts of Disease and Immunity.

By

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Introduction.

Since immunological terminology is often so shaded by the conceptions of various writers, we shall briefly discuss the ideas which are foremost in our minds concerning the nature of immunity. However before we can understand the nature of immunity we must understand the nature of disease.

The Nature of Disease:

The conception of the nature of disease has varied as immunologists have emphasized its various phases. With the exceptions of diseases due to physical and chemical agents, nutritional deficiencies, metabolic disturbances such as diabetes, gout, asthma, obesity, hypo- and hyper-glandular derangements, certain mental diseases, neoplasms, and diseases of unknown origin; the greater bulk of the ailments of mankind have been shown to be due to the invasion of the body by living organisms such as bacteria, fungi, proto- and metazoan

parasites, and viruses. What role if any is played by bacteria in these exceptions is not definitely known, but many authorities feel that while these diseases may not be due to specific organisms that the presence of an infection often predisposes to their occurrence.

The conception of the mechanism by which the bacteria are able to harm the body has also changed. Various theories have arisen from time to time as new discoveries have been made. Simple mechanical conceptions such as occlusion of the vascular or lymphatic systems, changes in the colloidal state of the body fluids, etc. brought about by the mere presence of the bacteria have been of interest. But it soon became apparent that bacteria were not inert but were chemical substances which had the property of growth and reproduction. The following quotations from the writings of Ludvig Hektoen (1) shows the conception of this mechanism in 1907; "At the present time the pathogenic effects of infectious microbes are regarded as chemical rather than mechanical in nature. In a few diseases in which there is a great multiplication of the infectious agents in the blood, mechanical effects may play some role; and in a pyaemia infectious embolism is of great importance; but only a few of the results of infection can be explained on mechanical grounds. Since the discovery

by Roux and Yersin of the specific poison of diphtheria bacillus in 1888, followed soon by Kitasato's discovery of tetanus toxin, the intoxication by bacterial products has been accepted generally as the real explanation of the changes that occur in the infected body. To a certain extent the period of incubation may be regarded as indicating the velocity of chemical reactions that follow infections. Specific poisons have not been discovered for many of our infectious organisms as they grow in artificial media, and this is explained by many as due at least in part to the profound differences in the conditions about the bacteria when growing artificially and in the animal body."

Vaughan (2) has emphasized the fact that, altho bacteria are morphologically simple, they are not chemically simple, but are perhaps more complex than the body cells which they attack. An exact knowledge of their chemical composition and rate of multiplication is of greatest importance in the study of the various diseases. Accurate quantitative and qualitative chemical knowledge of bacterial metabolism is also essential to a complete picture of disease.

The chemical composition of bacteria varies greatly (3), (4), (5). This is especially true of the quantitative analyses which vary not only between the different bacteria but also the same strain of organism grown upon different

media. Actively growing bacteria contain from 80 to 90% of water. This varies considerably with the media. The ash content varies with the salt content of the media, in the case of cholera vibrios (3) from 13 to 25%. Phosphoric acid is a prominent constituent of the ash, in some organisms as high as 50% of the total. Sulphur, potassium, chlorine, calcium, magnesium, iron, silica, etc. may also be present. The amount of protein also varies with the media. In protein or peptone rich media the protein may be as high as 70%. These proteins may be simple protein such as albumins and globulins or they may be compound proteins such as glyco-proteins, nucleo-protein, etc. The character of the proteins appears to be relatively independent of the media and more or less characteristic of each strain. The importance of the proteins to disease will be discussed more fully when we discuss the nature of immunity. While the complete amino-acid content of all bacteria has not been worked out, it is quite certain from the work of Vaughan and his pupils, and others that certain bacteria may be deficient in certain of the common amino-acids and rich in others, e.g., B. mesentericus (3) is deficient in di-amino acids, tyrosine, glycocoll, and contains 16.6% of glutaminic acid. Chromatin altho not usually present as a nucleus is probably the chief constituent of bacteria. There is still some question as to whether the

carbohydrate portion of the chromatin is a pentose or a hexose. The amount of ether soluble material also varies with the media. The character of the lipins appear to be independent of the media. Simple carbohydrates appear to be rather scanty in most bacteria. Substances which give a reaction with iodin have been described in some forms (6). Various workers have described what they thought was cellulose, but Vaughan (5) is of the opinion that what they thought was cellulose or hemicellulose was really a nitrogenous carbohydrate, (a chitin like body). Vaughan believes that carbohydrates are present as glyco-proteins and combined with phosphorus from which it is not easily detached, but it may be that he washed away some of the carbohydrates, in purification of this material for analyses. Levene (7) describes a glycogen-like carbohydrate in the tubercle bacillus which was grown upon various media. This substance could be extracted with salt solutions, and gave all the chemical tests for glycogen. As yet the vitamin content of the various bacteria has not been worked out but it is quite probable from the work of Hosoya (8) and others that certain forms will be found to be rich in these substances and other forms deficient in them. From this it is evident that unless the bacteria are grown under conditions nearly identical to those of the body the quantitative analyses will give us little

more than a general notion of the nature of disease. However the value of this qualitative notion must not be under estimated.

In this brief discussion of bacterial metabolism we can do no more than mention a few of its most important features. A study of the nutritional requirements of bacteria is of interest for it is evident that if the bacteria require conditions for growth and reproduction different than those found in the body they will not be pathogenic. If the bacteria require and consume or destroy substances essential to the well-being of the host, such as vitamins, essential salts, rare amino-acids, internal secretion, etc. the body may suffer in this manner from bacterial invasion. The importance of this latter mode of action of the bacteria is discussed in detail in sections 4 and 5. While not the same, this conception is similar to the exhaustion theory of Pasteur (10). A study of the digestive enzymes and the ferments of bacteria and the conditions under which they are able to bring about disintegration of the body fluids and tissue shows that the bacteria are dependent upon these for many of their metabolic activities. These enzymes may play an important role in disease but as yet our knowledge of the action of enzymes is too meager to properly evaluate their importance in disease.

The bacterial products of metabolism may be harmless, very poisonous, or their toxicity may be relative to the amount formed or to alteration of the host by previous invasion. These bacterial products may be (a) manufactured by the bacteria, (b) or formed incidently by the disintegration of the substratum i.e. the body fluids, (c) or the decomposition of the dead bacteria.

The synthetic products of bacteria of special importance are the true soluble toxins which are formed independently of the media and those specific toxins not formed in the body but dependent upon the media. The bacteria capable of forming true toxins (i.e. toxins which circulate in the body immediately causing disease symptoms, and against which the body is able to form antitoxins) are very few, e.g. B. diphtheriae, B. tetani, and perhaps the hemolytic streptococci of scarlet fever (9). Those forming the specific toxins dependent upon the media are the B. botulinus and B. pyocyaneus.

The bacterial products of a non-specific toxic nature formed incidental to bacterial metabolism by the disintegration of the proteins of the media are the amines and the cholins, e.g. such as:

Amines	Formula	Choline	Formula
ethyl amine	CH_3NH_2	Choline	$CH_2OH-CH_2-N(CH_3)_3-OH$
i-methyl amine	$CH_3NH.CH_3$	Neurin	$CH_2=CH-N(CH_3)_3-OH$
ri-methyl amine	$(CH_3)_3N$	Muscarin	$CH(OH)_2-CH_2-N(CH_3)_3-OH$
so-amylamine	$(CH_3)_2=CH_2-CH_2-CH_2-NH_2$	Betain	$COOH-CH_2-N(CH_3)_3-OH$
utrescin	$NH_2-CH_2-CH_2-CH_2-CH_2-NH_2$		
adaverin	$NH_2-CH_2-CH_2-CH_2-CH_2-CH_2-NH_2$		
to.			

Since so few bacteria form true soluble toxins it is evident that the nature of disease is not primarily one of specific toxins. The following quotation taken from Kolmer's book (12a) on immunity shows that some immunologists are still seeking these true soluble specific toxins as a solution of this problem. "In addition to these typical toxin producers, it is highly probable that the majority of bacteria, including the pyogenic cocci (staphylococci, streptococci, pneumococci, gonococci, and meningococci), the pathogenic bacilli not already mentioned (typhoid, colon, cholera, influenza, etc.), and even many saprophytic bacteria are capable of producing some of these exotoxins which exert influence on the pathogenesis of disease. It appears that these toxins possess certain properties in common, although quantitatively they vary within extreme limits, and hence in relative importance

in the causation of disease."

The amines, cholins, etc., i.e. the non-specific toxic products of bacterial metabolism, cannot be the principal cause of the disease since (a) they are incapable of producing all the effects of bacterial invasion, (b) are far less poisonous than the original bacteria, (c) some of the most highly poisonous ptomains are formed by non-pathogenic bacteria, (d) these ptomains (13) do not excite the formation of immune bodies, (e) furthermore, in certain diseases the symptoms are least severe where the greatest absorption of the autolytic products is taking place. These substances, however, probably contribute in an important manner in the production of oedema (11), fever and other important phenomena of disease (12b). However much work of a quantitative nature must yet be done before their importance in this respect is definitely established. It is, therefore, extremely doubtful if ptomains are produced in important quantities by pathogenic bacteria infecting living tissues (17).

Before leaving the discussion of the toxic substances, which probably arise from the substratum as a result of bacterial action, we must mention certain facts which will be discussed more fully later. If the blood of an animal is incubated under definite conditions with bacteria, bacterial cell substance, kaolin, agar, Vaughan's poison, etc. (16) it becomes very toxic for that animal. Since the serum is the

one constituent that is constant in all these reactions it is logical to conclude that the toxic substance arises from it. It is theoretically possible that the protein equilibrium may be so upset by the presence of bacteria or their metabolic products that the proteins of the host act as foreign proteins and bring about severe changes.

The fact that dead bacteria are poisonous when injected in sufficient quantities, combined with the failure to demonstrate the production of these true soluble specific toxins by all pathogenic bacteria, led immunologists to seek a solution of the nature of disease in the so-called endo-toxins. These endo-toxins were looked upon as specific poisons preformed and firmly fixed in the bacterial bodies which were set free only when autolysis or disintegration of the bacteria by the digestive enzymes of the host occurred. They differ from the true toxins in that they do not give rise to anti-toxins (14), but generate in the serum of the animal only the usual types of anti-bodies for the bacteria from which the endo-toxin was prepared such as: agglutinins, bacteriolysins, etc., when animals are immunized (14) against these endo-toxins. The conception of the nature of these anti-bodies is plainly set forth in the following quotation (15). "On the other hand certain bacteria which develop toxic qualities do not set these free as they are formed but store them in or build them into the structure of their

bodies whence they are freed only after death or disintegration of the bacterial cell through autolysis or some external agency. Such toxic substances stored in the texture of the bacteria are called endo-toxins and they do not appear directly to stimulate the organism to the formation of antibodies, although the bodies of the bacteria have specific antigenic powers due to the characteristic proteins."

Vaughan (2) has shown that not only bacteria but practically all proteins of animal, plant, or bacterial origin possess a toxic group which can be liberated by digestion with a 2% alkali in absolute alcohol. He believes that this poison is set free when the proteins are disrupted by the enzymes of the body tissues or fluids. If Vaughan's theory is correct it somewhat doubtful. It is probably safe to look upon these endotoxins makes the existence of these endo-toxins as products of aberrant foreign protein cleavage, since they produce according to most observers (18), (5), the development of symptoms and lesions which resemble closely those of acute or delayed anaphylaxis such as dyspnea, pruritus, coughing, sneezing, partial emphysema, focal hemorrhages in lungs, stomach, intestines, appendix, etc., with the occasional gradual wasting, fever, and death.

Although the clinical picture of disease is generally that of a local or general toxemia, investigators

have as yet failed to demonstrate the production of either soluble toxins or non-specific poisons (ptomains, chloins, endo-toxins etc.) of bacterial metabolism, in artificial media or in the infected body, in sufficient quantities and of a proper character to explain the nature of disease; except in three or four diseases. This has led certain immunologists and biochemists to new conceptions of the nature of disease which would explain not only the apparent specificity of disease and immunity but also the general features of the various diseases. They have sought this explanation in the protein part of the bacteria which should act as any other foreign protein if introduced parenterally.

Foremost among these are the theories advanced by Friedberger (19), (20), Thiele and Embleton (19), (21), abroad, and by Vaughan (5) and Mathews (22), in this country. Although these theories differ in certain essential points they have a somewhat common basis, in that they all consider disease symptoms as being due mainly to foreign proteins. The fact that the anaphylatoxin of Friedberger, the endo-toxin of Thiele and Embleton, and the poisonous protein of Vaughan may be of endogenous origin rather than exogenous (i.e. from the serum proteins of the host) does not distract from the attractiveness of these theories but rather adds support to

the theory advanced by Professor A. P. Mathews. Of these theories perhaps the most promising and attractive is the one advanced by Professor A. P. Mathews (22), since it gives us for the first time a logical explanation for many of the perplexing phenomena of disease and immunity, as well as a new and stimulating hypothesis for further work.

The Nature of Disease according to Mathews.

In the following quotation (22) Professor Mathews gives a few of his reasons for abandoning the specific conception of disease, "first, because most pathogenic bacteria do not form these soluble toxins, diphtheria and tetanus being exceptional; and, second, because of the discovery of anaphylaxis, or sensitization, by Richet, and because of Vaughan's discovery of a toxic principle in all proteins".

Professor Mathews advances the theory that not only asthma, hay fever, and eczema are due to an anaphylactic sensitization of the body, or special parts of it, to certain specific proteins, but that all infectious diseases, as well as many which are really or apparently non-infectious in nature, are also due to specific anaphylactic sensitization of the body to strange or partly digested proteins, coming either from bacteria, parasites, foods, etc. There is therefore one mechanism for all or nearly all disease, and only a few

like diphtheria and tetanus have added to this mechanism an additional poisoning by a true soluble toxin. The symptoms of disease differ, depending upon the principal tissues or organs sensitized. According to this theory the incubation period of acute infectious diseases may not only be a period during which the bacteria are developing but a time during which the body cells are becoming sensitized to the proteins of the invading organism. The period of greatest activity of the disease is the shock. The crisis is the desensitization. The natural cure of the disease is desensitization.

Clinically, most diseases do not appear to be as simple as this, due to the fact that many various factors complicate the picture, such as difference in: structure of the invading organism, ability of the organism to grow in the body, rate of growth and reproduction, portion of the body first invaded, etc.; but we believe that hypersensitivity is the principle and fundamental mechanism of most diseases.

Nature of Immunity:

We may now, with this new conception of the nature of disease clear in our minds, discuss the nature of the powers possessed by the living organism to resist or to overcome a particular infection, i.e. the nature of immunity. So great is the number of factors, which go to make up this polyphasic resistance, that it is with great difficulty that

any one factor may be selected as the basic one. Certain of the earlier hypotheses should be mentioned, not only for their historical interest, for in these are found the inceptions of our more recent theories. In the exhaustion hypothesis of Pasteur (10) is found a possible explanation of the anaphylatoxin of Freidberger. This will be considered under the discussion of the mechanism of anaphylaxis. Chauveau's theory (23) of the retention of inimical substances is in many respects similar to the theory of bacteriolysin, hemolysins etc. Buchner's theory (23) of increase of local resistance of the organs by means of an inflammatory reaction, which protects the tissues against reinfection by the same germ, is somewhat similar to some of our more recent conceptions. For many years the cellular theory of Metchnikoff and the humoral theory of Ehrlich have claimed most of the attention of the immunologists, bacteriologists and biochemists. The cellular theory ascribes cure and protection to the activity of certain white corpuscles of the blood such as the polymorphonuclear leukocytes, large mononuclear leukocytes, (includes the endothelial cell) and the small and large lymphocytes. The humoral theory ascribes these properties to the body fluids, i.e. to the presence of specific anti-bodies such as: antitoxins, bacteriolysins, opsonins, agglutinins, precipitins, etc.

More recently, to these two concepts has been added the conception of local resistance or immunity, e.g. the

fixed tissue immunity of Gay, the cuti-immunity of Besredka, the intestinal immunization of Calmette, etc. This theory of fixed tissue immunity is discussed in the excellent review of this subject by Gay. That Gay believes in the necessity of this additional conception is plainly set forth in the following quotation (46). "But even after admitting a complete cooperation between these two factors (of cellular and humoral theories) on the evidence submitted by the proponents of both cellular and humoral theories of immunity there remain unexplained instances of protection against bacterial agents in which no direct participation of either Metchnikoff's phagocytes, in the narrower sense of polymorphonuclear leucocytes, or of the humoral properties of the blood or both together, can be invoked. There remains a 'tertium quid' to explain,---. It is precisely in these debatable and unexplained instances of protection (often of an extreme grade) that we believe that fixed tissue or local immunity is operative and it is our purpose here to sift the evidence for its existence, extent, nature, and practical utilization."

Melalnikov (24) (25) believes that the basis of every immunity is the immunity of the cell, and that the presence of anti-bodies in the fluids of the body is a secondary phenomenon which appears mostly in higher animals. According to him, coexistence of a cellular immunity and anti-bodies is the perfect form of immunity which occurs only in

certain bacterial infections, and that in most diseases there is no evidence that the anti-bodies play any part in the immunity.

Vaughan, apparently, does not believe that the anti-bodies, such as agglutinins and precipitins, are of prime importance in immunity for he says, (26), "The active constituents of the culture, the agglutinable substance, is not, in my opinion, an essential constituent of the bacterial cells, but consists of one or more proteins closely associated with the bacterial cells. My reasons may be stated as follows: (a) Agglutination does not destroy the viability or the virulence of bacteria; therefore, the reaction does not disrupt the living bacterial cell. (b) Thoroughly washed typhoid bacilli are not agglutinable. (c) When typhoid bacilli are thoroughly shaken in salt solution so as to remove their flagellae and the bacilli are deposited in a centrifuge, the emulsion of flagellae is agglutinable. (d) Neufeld has shown that when cholera bacilli are thoroughly cleansed by being shaken with one per cent alkali, which does not destroy them and only washes away adherent matter, they are not inagglutinable but produce no agglutinin when injected into animals."

Although fixed tissue immunity, phagocytosis, etc. are of importance in the immunological consideration; we believe that there is a phenomenon, of prime importance in immunity, more fundamental than those set forth in any of

these theories. This phenomenon, paradoxical as it may at first appear, is, according to Professor A. P. Mathews (22), M. J. Rosenau (27), Ch. Richet (28), A. T. Henderson (29) and many others, anaphylaxis, i.e. the resistance to infection is probably due to hypersensitivity of the tissues to the proteins of the invading bacteria.

That the process of anaphylaxis is closely allied to immunity, appears to have been clear in the mind of Richet, who first gave the name to this phenomenon; for he says (30), "We have called anaphylaxis (the opposite of immunity) that property possessed by a venom of diminishing rather than increasing immunity, when it is injected in non-fatal doses. It is probable that many poisons (or toxins) have this property, but since we have been attracted by their vaccinating or prophylactic properties we have not as yet carefully studied them from this point of view." A few months later (32) he calls attention to the necessity of a lapse of several days, in order that the resistance be decreased rather than increased and he suggests that anaphylaxis resembles a sort of vaccination. Still later, he (31) expresses this idea very clearly when he says, "The anaphylactic reaction is a function of defense, which maintains intact and homologous the chemical constitution of each species of animals by preventing the entrance into the protoplasm of the cell of the foreign proteins, which would modify the specific chemical structure of

these cells."

Rosenau (27) suggests that hypersusceptibility (anaphylaxis) might explain the incubation period of certain communicable diseases. He also calls attention to certain important relations between anaphylaxis and immunity for instance he says, "We foresaw last year that the problem of hypersusceptibility has important bearing upon the question of immunity and expressed the opinion (J.A.M.A. xlii, No. 13, Sept. 29, 1906, p. 1007-1010) that resistance to disease may be largely gained through a process of hypersusceptibility. --- The phenomenon of hypersusceptibility has been produced in guinea pigs by extracts obtained from the colon bacillus, yeast, hay bacillus, anthrax, tubercular bacillus, and typhoid bacillus. The hypersusceptibility produced by the colon and the typhoid bacilli was followed by a definite immunity to the corresponding infections." However he fails to realize the vast importance and general applicability of his suggestion as shown by what follows, "In the case of anthrax, however, immunity does not follow hypersusceptibility to the anthrax proteid. We are, therefore, not dealing with a general law applicable to all infections, but with certain limitations as in the case of antitoxic immunity".

It is probable that this and many other exceptions are more apparent than real and that most of them may be

explained, either by special structures of the invading organism, such as the capsules which either make them acid fast or Gram positive (Dyer (33) believes that the attenuation of B. anthracis, according to Pasteur's method of growing between 42-93° C., prevents the formation of the normal fatty substances by the bacteria, and the bacteria as a result, is easily digested by the body producing immunity) or by special modes of action of the invading bacteria, e.g. a clogging of the capillaries, etc. (34).

Professor A. P. Mathews (22), in his paper entitled, "The Nature of Disease and its Natural Therapy", advances the theory, that immunity is due to sensitization, in such a clear and definite manner that there can be no doubt as to the importance that he places upon this phenomenon. He says, "Immunity is a result of this sensitization, rather than of the production of specific antibodies. It may be due to the following process: The sensitization to the proteins of the bacteria causes an intense and quick reaction of the mechanism of resistance when the bacteria are reintroduced, and the invader is overwhelmed, eaten and digested before it can multiply. The production of antibodies is only one phase of this reaction."

In these discussions of the nature of immunity and disease, we have attempted to consider them only in their

broadest sense. We leave for future consideration the many other factors which tend to obscure this general biological truth. We have already pointed out how Rosenau failed to grasp this general conception, because of the apparent exception of anthrax. (The secrets of nature are hidden by being revealed all at the same time.) We have not in this paper divided immunity for purposes of discussion, according to the classical divisions, e.g. into natural, and acquired; for we can not help but believe that this division is apt to misload rather than to clarify our minds. The difference being quantitative rather than qualitative.

There is little doubt that the phagocytes play a part ~~in this~~ in natural as well as acquired immunity. The importance of the white corpuscles in natural immunity was first suggested by Sternberg, G. M. (35), 1881 and later given experimental support by Metchnikoff, Elie (1884). The fixed tissue immunity of Gay and others are also of importance in the natural immunity. We give these two concepts first consideration for we believe that immunity is primarily a cellular phenomenon and only secondarily a humoral (body fluids) one. Our main reason for this belief is the necessity for a conception of immunity that will explain 'bacteriaemia' as well as the many other phenomena such as: the persistance of the organisms in the blood stream after the crisis, the lasting quality of an active immunity as compared with that

They differ from those of Vaughan on several points: 1st. We believe that the proteins which are loosely bound to the bacteria which Vaughan removed when he washed the bacteria with sterile salt solutions or coagulated by alcohol are essential to the action of the bacteria. These albumin-like proteins probably play an important part in determining the specificity of the infection and of the body's defense. 2nd. The pathogenicity of the bacteria depends upon the sensitization and poisoning power of their proteins, as well as their ability to live in the fluids of the body. This point finds its strongest support in the work of Vaughan who describes the existance of an organism (as shown by positive blood cultures over a long period of time) in the blood for forty to fifty days, and yet could find no evidences of pathogenicity of the organism (42), i.e. a true bacteriaemia. 3rd. The symptoms of disease are due to the reaction of the hypersensitive tissues to the proteins of the bacteria rather than to the parential digestion of the invading proteins. It may be possible that this sensitivity depends upon the rate of parential digestion. 4th. Natural immunity may be due to many factors, but we feel sure that inherited hypersensitivity (transmitted from parents to offspring either intra-uterine or extra-uterine by the colostrum etc.) must play an important part. 5th. Acquired immunity is due to hypersensitivity of the tissues rather than the development of

specific ferments. This holds alike for that immunity conferred by vaccination, by an attack of a disease etc., and possibly for all forms of immunity except possibly the antitoxic immunity and the lytic immunity of d'Herelle. 6th. We differ further in that we are inclined to place the phenomenon of immunity as primarily cellular rather than humoral as Vaughan places it.

We have considered the cure of disease as anti-anaphylaxis or desensitization. The name desensitization was first given by Weil and later by Besredka to the refractory phase of anaphylaxis, during which an anaphylactic animal would tolerate without severe symptoms many times the lethal dose of a given protein to which it had been previously sensitized. The name desensitization is to be preferred to the one first given by Besredka, i.e. anti-anaphylaxis, since it more aptly describes the phenomenon. We shall not here enter into a discussion of the nature of this desensitization. This subject has been recently reviewed by Longcope, W. T. (44). If space permitted, it would be interesting to compare the methods used successfully in desensitization with those employed in non-specific protein therapy for they are very similar. The subject of non-specific protein therapy has been extensively reviewed by Petersen, Wm. F. (45). The theory of Professor Mathews suggests that, in as much as nearly all diseases are

due to a single mechanism (anaphylaxis), the natural cure of disease is due also to a single non-specific process (desensitization). He says, "It consists in desensitizing the tissues of the body by means of a partially digested protein derived from the body's own tissues by a process of autolysis. These albumoses, peptides, or other substances probably desensitize by replacing in the sensitized cells the sensitizing substance. The cells are accordingly no longer irritated, the bacterial protein products entering the body cease to be toxic, and as a result all symptoms of disease subside. The brain loses its restlessness and goes to sleep; oxidation is reduced and the temperature falls to normal or below; the capillary wall no longer irritated, no longer pours out plasma; the bronchiole musculature relaxes and respiration becomes easier; the heart picks up its load; the kidneys, no longer poisoned, renew their activity and help in getting rid of the sensitizing substance".

Summary:

We have reviewed many of the conceptions regarding the nature of disease, immunity, and natural therapy. We do not contend that we have thoroughly covered all of the various phases of this vast subject. It has been our purpose in this review to suggest a new conception and to set forth a few of the many reasons for doing so.

Briefly then, we have based our studies on the following concepts: 1st. The symptoms of most diseases are due to hypersensitivity of the tissues of one or more organs of the body. 2nd. Immunity is the result of this sensitization. 3rd. The natural cure of disease is brought about by desensitization of the tissues to the invading protein.

Conclusions:

We may then conclude that the chemical basis of immunity is the chemical basis anaphylaxis. This consists in a study of the substances which bring about sensitization, desensitization, or the modification of either of these processes.

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The Chemical Basis of Immunity

I.—The Influence of Vitamin B Upon Anaphylaxis

BY PAUL E. WEDGEWOOD AND AGNES H. GRANT.

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A RELATION of vitamins to infectious disease and resistance to disease has now been observed by many investigators, but the exact nature of this relationship is still very obscure. Thus it is certain that a deficiency of vitamin A, the active principle of cod liver oil, predisposes to tuberculosis and xerophthalmia; and that the vital resistance of the body to these infections appears to depend in some way upon this vitamin. The healing effect of cod liver oil on both these troubles is well known. Similar observations have been made as regards other infections. Thus hens fed on diets deficient in A and B (5) were more susceptible to roup than fowls equally exposed but on a complete diet.

Similarly as regards the anti-scorbutic vitamin. It is well known that the symptoms of this disease are certainly complicated even if they are not caused by bacterial invaders which attack the gums, joints and other parts of the body where there appears to be a lowered resistance or diminished vitality.

It has been suggested also that certain of the symptoms of disease may be due to the bacterial invaders consuming directly, or indirectly causing the consumption of the stored vitamins of the body and thus causing a real vitamin deficiency. A person or animal suffering from scurvy, for example, has the feelings of lassitude, pain, fever and so on which are common to many diseases. Is there in various diseases a real vitamin deficiency in the body which thus predisposes to the spread of the disease?

These are questions to which answers are very badly needed.

We have attacked the problem by studying first the relation of vitamins to a single factor of disease, namely, anaphylaxis, or hypersensitivity. The general basis of the work is that given by Professor A. P. Mathews in his paper entitled, "The Nature of Disease and of Its Natural Therapy." In that paper Professor Mathews developed the theory that the symptoms of infectious disease of all kinds were due to a hypersensitivity of certain tissues of the body and in particular the nervous tissues, produced by their sensitization through the partially digested proteins coming from the bacterial bodies. This, the process of anaphylaxis, he considered as the real basis of immunity. The natural cure of the disease was due to the substitution of similar products from its own proteins, for these partially digested bacterial protein fragments, thus leading to a temporary desensitization during which the bacteria could be destroyed or walled off.

Whether this hypothesis be true or not, it puts anaphylaxis as the cardinal process in infectious disease, where it has also been placed by numerous other writers, and for this reason we began our study on the relation of vitamins to this process.

Abderhalden and Wertheimer (1), who have recently attacked this problem, have obtained positive evidence that vitamin B is related to anaphylaxis. They found that pigeons which were on a diet deficient in B had a markedly increased anaphylactic shock as compared with sensitized pigeons on a normal diet. Abderhalden (1 and 6) from effect of vitamin B deficiency upon respiration in the whole animal and Hess (3 and 7) from the effects upon respiration of tissue itself, ascribe the symptoms produced by lack of B not to the accumulation of toxic products in the intestines, but to a general lowering of oxidative processes.

G. A. Hartwell (2) made a curious observation, which may have some relation to anaphylaxis. He observed that nursing young rats had convulsions

and spasms if the mothers were eating a heavy protein diet without vitamin B, but not if this vitamin was given with the heavy protein diet.

These are all the observations we have found in the literature bearing on this problem.

We chose young white rats for experimentation. It is impossible to produce either active or passive anaphylaxis in an adult white rat when the latter is on a complete diet, as was shown by W. T. Longcope (4). Such rats show no effect whatever on the second or third parenteral injection of a foreign protein.

We have found, however, that young white rats may be sensitized and will die upon the second or third parenteral injection of a foreign protein (anaphylaxis) if they are kept on a diet deficient in vitamin B. This proves that a deficiency of vitamin B either predisposes to sensitization or increases the effects of sensitization in these animals. We found, just as had Longcope, that adult rats on a complete diet, or even young rats on such a diet, showed no effects from second or third injections of protein. We have not tried adult rats on a diet deficient in B vitamin, since it is known that young white rats are more sensitive to vitamin deficiency, and we began with them.

To show that a variation of salt in the diet produced no change in susceptibility to anaphylaxis, we first tried an experiment on twelve young albino white rats about twenty-eight days old, and weighing at the first injection about eighty grams each. These rats were divided into two groups each on a complete diet of corn meal, oat meal, linseed meal, whole wheat flour with a little casein, butter and yeast and a pinch of Na Cl. After fifty days on this diet all were sensitized by intraperitoneal injection of 1 c.c. of a 2% solution of egg albumin. They were then divided into two groups, Nos. 1-8 being continued on the above diet, while 9-12 were given the same diet minus the sodium chloride, and had distilled water in place of tapwater. This difference of salt in the diet made no difference in anaphylaxis. For at the end of fourteen days each of the rats received a shock dose of 3 c.c. of a 10% solution of egg albumin intraperitoneally without any appreciable effects. The rats weighed about ninety-five grams at the time of the shock dose.

Having found that such a salt variation produced no change in anaphylactic response, we then proceeded to test the effect of a diet deficient in vitamin B.

Five normal white albino rats, Group A (rats 1-5), that were five to six weeks old, were injected intraperitoneally with a sensitizing dose of 1 c.c. of a 2% solution of egg albumin, June 12, 1923. They were placed on a diet which was deficient in vitamin B, as follows: Polished rice 52%, which had been soaked and washed under running water for several hours, crisco, an artificial lard made from vegetable oils which have been partially hydrogenated 15%, casein 15%, and McCollum's salt mixture 4%. Small amounts of butter were given daily for vitamin A. This diet has been found to be free from vitamin B. After two weeks on this diet, on June 26, 1923, they were given by intraperitoneal injection a shock dose of 3 c.c. of a 10% solution of egg albumin. Rat A 3 died during the night following this injection. The rest were kept on this deficient diet for two weeks more and again injected with 3 c.c. of a 10% solution of egg albumin, on July 10, 1923. Within three and one-half hours rat A 2 and A 4 were dead. The two that were still alive were continued on this diet for two weeks longer, and on July 24, 1923, were again injected with 3 c.c. of a 10% solution of egg albumin. Rat A 5 died within four and one-half hours. Rat A 1 died during the night following the injection. The weights and sex of these rats are given in Table I.

TABLE I.

Weight Charts of Rats. In Grams.

Date	Rat A 1 Male	Rat A 2 Male	Rat A 3 Female	Rat A 4 Male	Rat A 5 Male
6-26-23	34.5	41.5	73.5	39.4	40.
7-10-23	45.	49.5	....	38.2	47.4
7-24-23	55.2	....	....	....	67.5

Thus all five rats on a diet deficient in vitamin B died after one to three injections of egg albumin, while healthy ones on a full diet did not die nor did they show any symptoms of anaphylaxis.

In this and subsequent experiments, the first shock dose was followed by other shock injections of egg white for two reasons. First, it was thought possible that rats, like rabbits and dogs, may be harder to sensitize than guinea pigs, and that they might require several injections for sensitization to a foreign protein. Second, it was also thought possible that the degree of sensitization of a rat might depend upon the degree of the vitamin deficiency,

hence a longer period of exposure to the diet was tried.

We thus found a deficiency of B vitamin in a diet otherwise complete caused rats to become susceptible to egg albumin, so that they were killed by subsequent injections of this protein. An experiment tried at the same time on rats when on diet deficient in both A and B vitamin, showed that B alone was important.

Five normal white albino rats (Group B, rats 1-5), age five to six weeks, were given by intraperitoneal injection a sensitizing dose of 1 c.c. of 2% solution of egg albumin, June 12, 1923. They were placed on the same diet as Group A, except that no butter was given, so that the diet was deficient in both vitamins A and B. After two weeks on this deficient diet the rats were injected (June 20, 1923) with 3 c.c. of 10% solution of egg albumin. They showed no effects following the injection. They were kept on this diet for two weeks longer. On July 10, 1923, they were again injected with 3 c.c. of 10% solution of egg albumin. Rats B 2 and B 5 died within three and one-half hours, and rat B 4 died within nine hours after this injection. Rats B 1 and B 3 were kept for two weeks longer on the same diet, and on July 24, 1923, were again injected with 3 c.c. of 10% egg albumin solution. Rat B 1 died within three and one-half hours. Rat B 3 appeared to be very sick and had a hemorrhage from the nose, but recovered. The weight and sex of these rats are given in Table II.

TABLE II.

Weight Chart of Rats. In Grams.

Date	Rat B 1 Male	Rat B 2 Male	Rat B 3 Female	Rat B 4 Male	Rat B 5 Female
6-26-23	50.5	44.	72.5	28.	43.5
7-10-23	54.5	40.	77.3	32.	45.3
7-24-23	52.	....	73.3	....	....

This experiment thus confirmed the foregoing, showing that a diet deficient in B vitamin predisposes to sensitization. The absence of A vitamin does not seem to have made any difference in the result. We conclude that rats can be sensitized to egg albumin if their diet is deficient in vitamin B, from which it is concluded that vitamin B either prevents sensitization, desensitizes, or protects in some other manner against the effects of sensitization.

There appears to be no immunity established when there is no response to the first shock dose, or if there is any immunity, it does not last for a period of two weeks.

Having thus shown that vitamin A appeared to exert no influence on sensitization, but that only vitamin B was effective, we also tried an experiment on the effects of a deficiency in C in rats. It is well known that rats appear not to need vitamin C. They thrive on a cereal diet, which produces scurvy in guinea pigs. Whether this shows that rats are capable of making C vitamin themselves, or whether they do not need it, is still uncertain. We found, as the following experiment shows, that on a diet with plenty of B vitamin, but deficient in C, rats showed no effects whatever from several doses of egg white.

Five normal white, albino rats (Group C, rats 1-5), age five to six weeks, and of the same litters as Groups A and B, were sensitized with injection of 1 c.c. of 2% solution of egg albumin on June 12, 1923. They were kept for two weeks on a cereal diet, and injected with 3 c.c. of a 10% solution of egg albumin (shock dose) on June 26, 1923. They showed no ill effects of the injection.

The diet given during this experiment consisted of whole wheat flour 1½ parts, yellow corn meal 1 part, oat meal 1 part, linseed meal, bone meal, and McCollum's salt mixture ½ part. This has plenty of vitamin B, but not vitamin C, and rats grow normally on it. They were continued on the same diet for two weeks longer and again injected with 3 c.c. of a 10% solution of egg albumin on July 10, 1923. No ill effects from this injection following, they were continued on the diet for two more weeks, and again injected with 3 c.c. of a 10% solution of egg albumin on July 24, 1923. Following this injection, rat C 5 died during the night. The others remained well. The weight and sex of the rats are given in Table III.

TABLE III.

Weight Chart of Rats. In Grams.

Date	Rat C 1 Male	Rat C 2 Male	Rat C 3 Female	Rat C 4 Male	Rat C 5 Male
6-26-23	100.	58.5	43.	35.	39.
7-10-23	132.7	87.5	61.2	57.	45.
7-24-23	152.5	103.	70.	67.	68.

All the rats that died during these experiments were autopsied. No pathological conditions were found in any except in rat C 5. The upper lobe of the right lung of this rat was congested. It

not present in all cases. There was irritation of the mucous membranes in a few, as evidenced by sneezing, scratching, etc., but this was not the case in all, and these symptoms, moreover, were shown by some that did not die. The one rat (D 2) whose death the authors were able to observe, as most died at night, first lost its equilibrium, then became depressed, turned over on its back, kicked a couple of times, gasped for breath, and then stiffened out. No attempt was made to measure the temperature changes.

CONCLUSION.

While normal rats can not be sensitized, a deficiency of B vitamin in the food of young rats makes it possible to sensitize them to foreign protein so that they will die on a second or third dose of the protein. Deficiency in A vitamin or C vitamin appears to be without influence in this regard.

It was also found possible to produce protein (anaphylactic) death in young rats when on a complete diet, if these rats had an infectious disease of the lungs. Such rats acquire a sensitivity to foreign protein similar to that produced by deficiency of B vitamin, and it is suggested that this disease has exhausted the B vitamin reserves. This possibility is being investigated.

It is concluded that vitamin B either desensitizes, prevents sensitization, or protects against anaphylactic shock.

It is a pleasure to express our indebtedness to Professor Mathews, who suggested this investigation, and to Professor Tashiro, for their advice and aid.

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The Chemical Basis of Immunity

II.—The Influence of Vitamin C on Anaphylaxis

BY PAUL E. WEDGEWOOD.

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HAVING found (1) that a diet deficient in vitamin B, but otherwise complete, made young white rats susceptible to protein disease (anaphylaxis) so that they were killed by repeated injections of egg white, we desired to test the effects of a diet deficient in vitamin C also. The white rat is not a favorable animal for such experiments, because that animal does not require much vitamin C in its food. It is able to live and reproduce without this accessory food element. Our experiments showed that rats on a cereal diet (deficient in C) were quite immune to anaphylaxis. Not satisfied with this result, we sought to determine whether a diet deficient in vitamin C or superabundant in C would effect anaphylaxis in the guinea pig, an animal very susceptible to anaphylaxis, and also easily becoming scorbutic in the absence of C. We therefore compared the anaphylactic shock in these animals when they were on (a) a complete diet; (b) when they had injections of orange juice sterilized and neutralized, in addition to a complete diet; and (c) when they were on a scorbutic diet.

It is well known that young guinea pigs gain weight, remain healthy and active, reproduce, etc., on a diet of rolled oats, dry timothy hay, water, and small amounts of butter, if sufficient amounts of any of the following substances are added, *i. e.*, orange juice, raw cabbage, tomato juice, or carrot tops But if one of these substances, which contain vitamin C, is not added, the guinea pigs develop scurvy and die within three to four weeks, depending upon the age of the guinea pig, the previous diet, etc.

Twenty-five healthy guinea pigs, about six to eight weeks old, were weighed, and placed upon a complete diet for one week, from July 23, 1923, until July 30, 1923, and then weighed again. They had all gained in weight and were apparently in good health. The diet given was that noted, with the addition of carrot tops as a source of vitamin C, and small amounts of butter. This week of preparation insured, as nearly as possible, that the guinea pigs were healthy, and that they had about the same amount of vitamin C to start with.

1. *Anaphylaxis in guinea pigs upon a complete diet (controls):* Group A (guinea pigs 1-5) were then sensitized by intraperitoneal injection of 1 c.c. of 1% solution of egg albumin, on July 30, 1923, were kept on a complete diet for two weeks, and on August 13, 1923, were again injected intraperitoneally with 1 c.c. of 10% solution of egg albumin. Within less than thirty minutes all five were dead. They showed all the symptoms of anaphylaxis, *i. e.*, scratching, sneezing, choking, spasm, and death.

2. *Effects of the injection of orange juice upon anaphylaxis:* Group B (guinea pigs 6-10) were sensitized by intraperitoneal injection of 1 c.c. of 1% solution of egg albumin July 30, 1923. They were kept for two weeks on the complete diet (same as Group A), but in addition were given daily by intraperitoneal injection 5 c.c. sterilized, neutralized orange juice. On August 13, 1923, they were injected intraperitoneally with a shock does of 1 c.c. of 10% solution of egg albumin. This injection was given nine hours after the final injection of the orange juice; so that any immediate effect of the orange juice injection should have worn off. For a period of from twenty minutes to one hour and ten minutes (see Table I) these guinea pigs showed symptoms of anaphylaxis, *i. e.*, sneezing, coughing, etc. *But none of these guinea pigs died.*

The orange juice that was used for injection was prepared in the following manner: Each evening

about 30 c.c. of juice from Florida oranges were expressed and placed in the ice box to filter. The next morning exactly 25 c.c. of the filtered juice was measured out into a flask and quickly brought to boiling on an electric hot plate. This was done to sterilize it. It was allowed to boil for one minute. Then to neutralize the free acid, which was too irritating for intraperitoneal injection, 10 c.c. of 0.5 M solution of Na_2CO_3 were added to it, and it was rapidly cooled to body temperature under running water. For these oranges it was found by indicators that this gave a PH between 7 and 8. Although this was nearly neutral, and was close to 37° C, the immediate effect after intraperitoneal injection was irritating. This irritation lasted from one to two minutes.

3. *Effect of scorbutic diet for sixteen days upon anaphylaxis:* Group C (guinea pigs 11-15) were sensitized with 1 c.c. of 1% solution of egg albumin on July 30, 1923, and were placed on the scorbutic diet After sixteen days on this diet they were given a shock dose, 1 c.c. of 10% solution by intraperitoneal injection. Guinea pigs Nos. 11, 12, 14 and 15 died within less than one-half hour. (See Table I.) Guinea pig No. 13 showed irritation symptoms, but did not die. The period elapsing between injection and death of those which died was plainly shorter than in Group A, the controls. The average in the controls being twenty-four minutes and in those on a scorbutic diet eighteen minutes. (See Table I.)

The diet given during these sixteen days consisted of rolled oats, dry timothy hay, small amount of butter and water, all they would eat.

Effect of a longer scorbutic diet (twenty-one days) upon anaphylaxis: Group D (guinea pigs 16-25) were sensitized July 30, 1923, by intraperitoneal injection of 1 c.c. of 1% solution of egg albumin, and kept on the scorbutic diet for twenty-one days. They were then given the shock dose, *i. e.*, 1 c.c. of 10% solution of egg albumin intraperi-

TABLE I.

Numbers of Guinea Pigs	Sensitization Period*	Time between the second dose and death		Duration of symptoms in pigs which did not die	
	Days	Hrs.	Minutes	Hrs.	Minutes
Group A. (Complete Diet.)					
1	14	....	28.	....	....
2	14	....	20.	....	....
3	14	....	21.5	....	....
4	14	....	28.5	....	....
5	14	....	21.5	....	....
Group B. (Complete Diet Plus Orange Juice Intraperitoneally.)					
6	14	....	....	....	59.
7	14	....	....	1	10.
8	14	....	....	....	20.
9	14	....	....	....	37.
10	14	....	....	....	44.
Group C. (Scorbutic Diet for 16 Days.)					
11	16	....	13.	....	....
12	16	....	17.	....	....
13	16	....	....	....	24.
14	16	....	26.	....	....
15	16	....	14.	....	....
Group D. (Scorbutic Diet for 21 Days.)					
16	21	5	10.	....	....
17	21	....	15.5	....	....
18	21	....	10.	....	....
19	21	....	....	....	10.5
20	21	....	14.	....	....
21	21	....	21.	....	....
22	21	....	35.5	....	....
23	21	....	....	....	18.
24	21	....	12.5	....	....
25	21	....	....	....	11.

*By the period of sensitization is meant the period between the first and the second injections of foreign protein.

TABLE II.

Weight Chart of Guinea Pigs.

Guinea Pig No.	July 23,'23 grams	July 30,'23 grams	Aug. 6,'23 grams	Aug. 13,'23 grams	Aug. 20,'23 grams
1	340	361	376	427	....
2	305	310	342	385	....
3	328	343	369	408	....
4	345	359	378	400	....
5	330	343	378	415	....
6	320	324	325	355	....
7	300	311	318	354	....
8	361	272	285	320	....
9	255	272	291	315	....
10	338	254	347	375	....
11	305	317	337	348	....
12	260	264	372	279	....
13	350	362	374	390	....
14	343	358	357	366	....
15	250	260	259	274	....
16	278	281	300	299	238
17	314	325	350	341	302
18	275	274	299	310	257
19	386	395	426	448	379
20	305	307	316	332	275
21	343	345	360	361	313
22	314	300	315	310	264
23	360	365	373	376	330
24	309	320	298	316	265
25	269	280	245	279	244

toneally. Six guinea pigs, Nos. 17, 18, 20, 21, 22 and 24, died very promptly of anaphylactic shock. Three guinea pigs, Nos. 19, 23 and 25, did not die, but did, however, show symptoms of anaphylaxis, *i. e.*, irritation symptoms. The period elapsing between injection and death of those which died was shorter than the controls, except in pig No. 16, which died in five hours and ten minutes. (See Table I.) The average of the six dying promptly was eighteen minutes. The weight chart of the guinea pigs is given in Table II.

CONCLUSIONS.

The foregoing results are quite consistent and show clearly that vitamin C is an important factor in anaphylaxis in the guinea pig; the lack of this factor making the symptoms more acute; and the subcutaneous injection of orange juice, which contains vitamin C, protected the pigs, so that none of those receiving this in addition to their diet died. While it has not been certainly proved that C vitamin is the protective substance in the orange juice, since control experiments of injection of orange juice in which vitamin C has been destroyed have not been tried, yet it is probable that C vitamin is the active agent. The greater susceptibility of guinea pigs on a scorbutic diet indicates this. The fact that one or two pigs survived, even though they had been on a scorbutic diet, is probably without significance, since always in a large group of pigs a certain number will usually survive.

It is possible that C vitamin may be more effective in desensitizing the pigs when given parenterally than by the mouth.

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Chemical Basis of Immunity.

IV. A Preliminary Report of Vitamin B Exhaustion by Low Grade Infections.

By

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Read before the Daniel Drake Society, November 6, 1924.

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Chemical Basis of Immunity.
IV. A Preliminary Report of Vitamin B Exhaustion by Low Grade Infections.

By Paul E. Wedgewood.

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The old idea that bacteria may be harmful to their host by consuming certain essential substances, such as internal secretions, vitamins, and so forth, has hitherto had little or no experimental basis. Nevertheless this is an attractive idea and worthy of investigation. Studies prompted by this idea may yet enlighten many perplexing problems of etiology of diseases such as diabetes insipidus, diabetes mellitus, myxoedema, progeria, infantalism, various dementias, rheumatic fever, etc. It would not be difficult for us to imagine that some such process is depleting insulin faster than it is produced in the case of diabetes mellitus. In many of these diseases the picture of exhaustion is accompanied by chronic infections or perhaps a history of such infections, which might well be the cause

of this exhaustion.

However, the discovery and study of the action of vitamins have added probability to this idea. A great many symptoms common to disease, such as asthenia, malaise, cachexia, pain, neuritis, fever, anemias, decreased resistance, hemorrhage, to say nothing of paralysis, skeleton deformation and death, have been produced by deficiencies of vitamins. If a low grade chronic focal infection were consuming vitamins faster than supplied by food intake, it would be reasonable to assume that various symptoms of avitaminosis would be produced depending upon the degree of such deficiency.

There is much evidence in the literature in favor of this idea. That certain bacteria require water soluble vitamin B in their nutrition is well known (5). Thus S. Hosoya and M. Kuroya (1) after many extended and carefully controlled experiments were able to classify many pathogenic bacteria according to their water soluble vitamin B requirements, e.g. streptococcus hemolyticus, pneumococcus, meningococcus, and B. diphtheriae would not grow on vitamin-free media, but would readily grow on the same media if vitamin B was added. Out of many other bacteria examined, only four kinds - B. coli, cholera vibrio, B. pyocyaneus and B. anthracis - were able to flourish continuously, thru about seventy

generations, for a year upon vitamin-free media. The more common bacteria such as B. typhoidus, B. paratyphoidus (type A), B. dysenteriae and pyogenic staphylococcus, which were capable of faint growth during the first generation, upon vitamin-free media, failed to give continuous growth after two to three generations. The addition of yeast extracts, oryzanin, and Tsukie's vitamin B greatly favored their growth. The favorable influence of vitamins, especially water soluble B upon bacteria has also recently been shown by T. Shiba (2), S. H. Ayers and C. S. Mudge (3), J. Broadhurst (4), and many others.

It is also well known (6)(7)(10) that vitamin B is comparatively scanty in the animal body except in the highly cellular organs (9) such as liver, kidney, pancreas, thymus, and heart muscle (13). The relative richness of vitamin B in fresh endocrine glands (9), especially those most important to developmental processes, suggests to us a possible impairment of function of these glands, in case these glands were constantly drained of vitamin B by some low grade chronic infection. For Mayerstein (12), and also H. Gotta (14), have shown that the sex organs suffer especially from a vitamin deficiency. However, it is probable, that the less active tissue would be first to suffer, and that impairment of function of the more important tissues would

result only from a severe avitaminosis. H. Steenbock and his co-workers (11) have called attention to the necessity of preventing the animals, (upon which vitamin B experiments are being tried) from eating their own excreta. Therefore estimates of vitamin B content of animal tissues obtained without this precaution are probably too high. In any case, the margin of safety between the vitamin B stores and intake on the one hand, and the requirements on the other, is probably very small on the average diet. So that an abnormally large demand might result in hypovitaminosis.

That resistance to infection is greatly lowered by a diet deficient in vitamins is well known (8) (15) (17) (18). The importance of vitamin B to resistance of pneumococcal infection has recently been shown by Findlay (16). But it has not been so clearly shown that infection augments avitaminosis, and brings about an early onset of its symptoms. McCarrison (8) made the following statement when discussing the relations between vitamin deficiencies and infections, which shows that he appreciated this bi-modal relation. "This important factor (infection) operates in two ways: it may precipitate the onset of symptoms due to food deficiency, or it may impart new clinical

features to the food deficiency syndrome." He found that subcutaneous inoculation of B. suipestifer, an organism which complicated his results, into well fed pigeons, rabbits and fowls produced paralysis of the ~~birds~~ limbs, and that the clinical picture simulated closely that due to alimentary polyneuritis columbarum. When pigeons fed on Rangoon rice were congregated together, the organism spread to every one of them causing polyneuritis and death more rapidly than did the deficient food alone in isolated birds. Control birds on the other hand similarly exposed almost completely escaped its effect. He also reports a similar case in monkeys.

Thus, in the literature there is considerable evidence which suggests and supports the idea that infection raises the vitamin B requirement. Now, since the margin of safety between intake and stores on the one hand and requirement on the other, is small we should not be surprised if we found an exhaustion of vitamin stores, by chronic infection.

We (19) began our studies upon this problem by studying first the relation of vitamins to a single factor of disease, namely anaphylaxis. The general basis of the work is that given by Professor A. P. Mathews (20), in his paper entitled "The Nature of Disease and Its Natural

Therapy". In that paper Professor Mathews developed the theory that the symptoms of infectious disease of all kinds were due to a hypersensitivity of certain tissues of the body and in particular the nervous tissues, produced by their sensitization through the partially digested bacterial proteins. Whether this hypothesis be true or not, it puts anaphylaxis as a cardinal process in infectious disease, where it has also been placed by numerous other writers, and for this reason we began our study on the relation of vitamins to this process.

In the first series of experiments we chose young white rats. It is impossible to produce either active or passive anaphylaxis in ~~an adult~~ white rat when the latter is on a complete diet. Such rats show no effects whatever on the second or third parenteral injection of a foreign protein. We (19) have found, however that young white rats may be sensitized and will die upon the second or third parenteral injection of a foreign protein (anaphylaxis) if they are kept on a diet deficient in vitamin B. ~~This effect could not be explained by variation in salt intake.~~ Therefore a deficiency of vitamin B predisposes to sensitization or increases the effects of sensitization in these animals.

We might also mention here that an excess of vitamin C (21) protects guinea pigs from an anaphylactic

death. We have been unable to explain this effect by the salts present in the orange juice which was used as a source of vitamin C.

In the course of these experiments, a series of rats were found to be susceptible to sensitization in spite of the fact that they were on an adequate diet. That is, they were killed by subsequent protein injections. On examining these rats, post mortem, it was found that in every instance they were suffering from a mild chronic lung disease. The fact that these rats behaved exactly like those with a vitamin B deficiency suggested that the lung infection had in some way caused a vitamin B deficiency. The body thus infected might require more of this vitamin than normal, or the vitamin stores of the body might be depleted. This at once suggested that the power of sensitization could be used as a measure of their deficiency of vitamin B. Experiments were planned to see whether or not other low grade infections would produce a similar increase in power of sensitization.

We divided forty rats into four groups of ten each. The first group were simultaneously sensitized and diseased. This was accomplished by subcutaneous injection of suspensions of staphylococcus albus at the time of the injection of the sensitizing doses of protein. The second group were only sensitized, i.e. injected with small doses of protein. The

third group were only diseased. And the fourth group were left untreated. After two weeks on an adequate diet all forty of the rats were injected with a shock dose of protein.

This series of experiments showed that a pyogenic disease (local abscess produced by subcutaneous injection of staphylococcus) greatly increased the severity of the anaphylactic shock. This disease had also acted like the deficiency of vitamin B, i.e. it had increased the sensitization, so that the shock dose of protein had either caused death or a very severe anaphylactic shock, in the sensitized rat. They also confirmed our previous findings: first, that healthy rats on a complete diet could not be sensitized, and second that the one dose of protein was not the cause of death. Thereby again showing that we were dealing with anaphylaxis.

Furthermore these experiments showed a totally unexpected result. The pyogenic disease caused a state of hypersensitivity to protein without previous sensitization to this protein so that on the first injection of protein the rats either died or showed a severe anaphylactic like shock. In other words we had here some clear cut evidence that a focal infection brought about a non-specific hypersensitivity. This opens up a large number of possibilities which we are now investigating. It is well known that in

humans we often find cases of multiple hypersensitivities, i.e. one individual may be hypersensitive to twenty or more different proteins. It is hardly reasonable to suppose that this individual has been accidently sensitized to each one of these. Furthermore it is also well known that these individuals often show evidences of focal infections such as large tonsils, hypertrophy of mucous membranes, leukocytosis etc. The cause of this multiple hypersensitization may be chronic low grade infection. We plan to repeat this work making careful examination of the blood for signs of infection and of hypersensitivity, i.e. septicemia, leukocytosis, eosinophilia, sensitization to various proteins etc. We are as yet at loss for a satisfactory explanation of this curious but extremely interesting phenomenon.

However we do have some rather definite results which tend to show that bacteria may be harmful to their host by consuming some essential substance, i.e. vitamin B, (1) Healthy rats (rats in which no signs of infection could be found) may be sensitized to proteins if their diet is deficient in vitamin B. Other variations such as number of times of injection, size of dose, inorganic salts in diet etc. did not produce this change. (2) Disease also has this power of acting like a vitamin B deficiency, i.e. causing rats to become sensitized to protein.

of extracts of normal, diseased and vitamin B deficient tissues upon beri beri pigeons.

In conclusion the author wishes to express his appreciation for Professor A. P. Mathews' guidance in this work.

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Chemical Basis of Immunity.

V. The Probable Exhaustion of the Body's Store of Vitamin B by a Low Grade Chronic Infection. and its Influence on sensitization.

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It is an old idea that bacteria may harm the body by consuming some necessary substance; but so far there has been very little or no evidence of the truth of this idea. Most bacteria act either by the production of toxin or by sensitization; that they may injure by destruction of some essential constituent, such as one of the internal secretions, is, however, an attractive idea and one worthy of investigation. The discovery of vitamins has added some probability to this view; for vitamins altho present in very small amount are of vital importance to health. It is certain that when these substances are supplied in too small amounts various derangements of the body are brought about, including fever, neuritis, anemias, asthenia, malaise, cachexia and so on. Attention can be directed particularly to vitamin B

in this regard, for it is certain that this substance is stored in very small amounts in the body and is of vital importance to the nervous system. If a low grade focal infection ~~might be~~ were consuming vitamin B faster than supplied by the food, symptoms of avitaminosis might be produced and particularly symptoms of derangement of the nervous system. Something of this sort might be occuring in dementia praecox, in which the picture of disease is that of an exhaustion. Such a patient impresses ~~me~~ one as lacking some substance or substances vitally important to the proper irritability of his nervous system. We were at loss for a long time to know just how to attack this problem, until a chance observation opened a possibility which we investigated and which gave the interesting results reported here.

In the first series of experiments the influence of a lack of vitamin B on anaphylaxis was studied. It is well known that rats are extraordinarily difficult to sensitize to a foreign protein. We have never been able to sensitize a healthy rat when he was on a proper diet, i.e. one which contained sufficient amount of vitamin B. It was found, however, that young rats could be sensitized and killed by subsequent protein injections, provided they were fed on a diet deficient in vitamin B. These results have already

been published.(1) In the course of this experiment a series of rats were discovered which were on a diet containing vitamin B in what was supposed to be adequate amount but which were found to be susceptible to sensitization and which were killed by a subsequent protein injection. On examining these rats post mortem it was found that in every instance they were suffering from a mild disease of the lungs. No normal rat could be sensitized; but diseased rats could be. This observation appeared to us to be very suggestive. The rats with lung disease behaved exactly like rats with deficient B vitamin in the respect that they became susceptible to anaphylaxis and acquired the peculiarity of being sensitized.

This led to the suggestion that the lung infection had in some way led to a vitamin B deficiency either because the body thus infected required more vitamin than normal; or because the vitamin stores of the body were directly or indirectly depleted by the infectious agent. This at once suggested that the power of sensitization of rats might be used as a measure of their defectiveness in vitamin B and experiments were planned to see whether other low grade infections would produce a similar increase in power of sensitization.

The author then attempted to test this possibility, but as is so often the case it was a difficult task to demonstrate what appeared to be self evident. In the first place it was necessary to produce a disease in healthy rats which would not of itself be fatal during the experimental period. Secondly, the disease must cause gross

pathological changes which are readily observable. The organism which produced the peculiar lung disease would obviously be the one to use, but here as yet insurmountable difficulties have presented themselves. While this organism produces gross pathological changes in the lungs, spreads like wildfire through a colony of animals if it once gets a foothold, and while it can be readily demonstrated in stained smears as a diplococcus, it does not grow readily upon the ordinary culture media and results at isolation of pure cultures have so far been negative. For these and other reasons other organisms were tried. The organisms experimented with were: Bacillus Pestis Caviae, Para Typhoid Bacillus from mice, Bacillus Bronchosepticus from guinea pigs and Staphylococcus albus from a case of ethmoiditis. Of these the staphylococcus albus appeared to be best suited for the experiment since it grew readily upon agar slants, produced an abscess from which it could easily be isolated in pure cultures, and did not itself kill the animal. This organism also frequently produced a congestion of the lungs.

However, during the course of these bacteriological studies, it became apparent that injections of broth cultures of bacteria in itself produced a state of

hypersensitivity to egg albumin. Since egg white had been used to clarify the broth media, it could not be concluded that this state of hypersensitivity was due to disease process for it might well be that an amount of protein sufficient to sensitize had remained in the broth after coagulation. In order to test out the possibility of a disease process producing a state of hypersensitization and to eliminate sources of error, only saline suspensions of organisms grown upon agar slants were used.

Experimental Part.

That disease not only alters the course of anaphylaxis but also produces the condition is shown by the following experiments.

Non-specific hypersusceptibility: Ten healthy white albino rats (Group I, Nos. 1 to 10) were injected subcutaneously with 1 c.c. of a suspension of staphylococcus albus on June 17, 1924. This suspension was prepared by washing the twelve hour growth of staphylococcus albus from an agar slant with 10 c.c. of sterile sodium chloride solution (.9%). The colonies of bacteria were broken up by shaking the suspension with sterile glass beads. One week later (June 23, 1924)

these injections were repeated. Two weeks later at 1.20 P.M., July 7, 1924, intraperitoneal injections of 3 c.c. of 1% egg albumin solution were made.

The effects of the shock dose of protein may best be shown by describing the whole picture and then pointing out how nearly complete this picture is in each case.

Behaviour of the rats to the injection of protein may be divided into four more or less distinct stages, namely, (1) latent or prodromal (2) irritability (3) depression (4) convulsions. The first stage lasted from three to five minutes. The rats behaved quite normally. They played about the cage, ate their food, and were quite tame. The second was quite a marked change. The rats became very irritable, they bit at each other, hovered together in far corner of cage, either showed fight if molested or as was more often the case would beat against the sides of the cage in an effort to avoid capture. The fur was no longer smooth but stood on end. This stage lasted from one-half to one hour and gradually changed into the third stage. This stage may be called the depression stage, and because of its long duration, three to twelve hours, is most easily observed. In fact it is so prominent that unless the rats are closely observed all

other stages will be missed. This stage either terminates in a more or less gradual return to normal or in convulsions (fourth stage). The stage of convulsions preceded death about twenty or thirty minutes. During the stage any unusual noise will cause spasms. In fact one can easily pick out the rats that have reached the fourth stage by simply striking the cage or whistling shrilly. Those rats that have reached this stage will go into convulsions. Spasm of the extensor muscles usually predominate; these are of short duration, i.e., ten to fifteen seconds. As this stage progresses there is a marked decrease in the rate of the heart. Shortly before death, when convulsions could no longer be induced, the rate was between ten to fifteen beats per minute. While these stages have been described as separate stages, the reaction varies considerably in the different rats.

All ten of the rats of this group showed the first three stages. The fourth stage could be demonstrated only in rats Nos. 4, 5 and 10. Rats Nos. 4 and 5 died at 4.30 P.M., i.e. three hours and ten minutes after the injection of egg albumin. Rat No. 10 died at 7.45 P.M., i.e. six hours and twenty-five minutes after the injection.

Autopsy of rats.

Nos. 4, 5 and 10. These rats showed a fairly well localized abscess with small amount of inflammation

in the surrounding tissue. Fluid pus was present at the center of the abscess. The axillary and superficial inguinal glands were enlarged. The inflammatory reaction of the surrounding tissue was much more marked in rat No. 5 than in the other two.

Nos. 1, 2, 3, 6, 7, 8, 9. These rats were observed for one week and were chloroformed and autopsied. With the exception of rat No. 8 they were all healthy, i.e. no gross pathological changes were found. At the site of the injection of bacteria there was usually found a small fibrous nodule, i.e. a healed abscess. Rat No. 8, however, had a diffuse congestion of the lungs.

The pathological picture presented by this rat is that which is seen about three days after exposure or innoculation. It is therefore believed that this rat was healthy at the time of the protein injection, and that the protein injection caused the latent disease to become active.

In spite of the fact that previous work had suggested the possibility of infection producing a non-specific sensitization ~~anaphylactia~~, these results were rather unexpected. It is difficult to conceive of the protein of bacteria being so closely related to that of egg albumin that one may be substituted for the other in a phenomenon so specific as anaphylaxis. It seems more reasonable to assume that the body's means of defense is the same in both cases, i.e. sensitization is primarily a mechanism of defense not only against foreign protein invasion but also against bacterial invasion. If this assumption be true then the difference between prophylaxis (immunity) and anaphylaxis is only one of degree. ~~Another~~ Cellular hypersensitiv

according to Metalinkow[2] and many others[3], is considered to be the basis not only of anaphylaxis but also of immunity. Another possible explanation is that the tissue necrosis, produced by the infection, sets free into the blood or lymph the body proteins or their decomposition products. These then act as sensitizing substances. However this explanation explains only by assuming something that is not only harder to explain, but also something that appears opposed to facts, i.e. sensitizing a body to its own proteins or its protein split products. Whatever the explanation of this curious phenomena may be, if the fact that a focus of infection can render the body hypersensitive to one or more foreign proteins is substantiated by future work then we may consider the etiology of allergies and multiple sensitizations from a new view point.

A control experiment ~~was now tried to~~ showed that coincident with the others a single injection of one large dose of egg albumin intraperitoneally was not the cause of death.

Ten white albino rats (Group II, Nos. 11 - 20) were injected intraperitoneally at 1.25 P.M., July 7, 1924, with 3 c.c. of a 10% solution of egg albumin. Previous to this injection they had been kept for three weeks under conditions as nearly as possible identical to those of the preceding group.

They showed no immediate effects, and one week after the injection appeared to be in perfect condition.

They were then killed and no abnormal or pathological changes could be found in any of them. This showed that the death of the rats in the preceding group was not due to the single large dose of albumins, but was an anaphylactic phenomenon.

Having thus shown that an active infection caused rats to become hypersusceptible to egg albumin, an experiment was tried at the same time which showed that ordinary sensitization, i.e. injection of small doses of protein, superimposed upon infection greatly increased this susceptibility.

Ten white albino rats (Group XII, Nos. 21 - 30) were placed under conditions of diet and surroundings as nearly as possible identical to those of Groups I and II. They were injected subcutaneously on June 17, 1924, and again June 23, 1924 with 1 c.c. of 2% solution of egg albumin and also with 1 c.c. of staphylococcus suspension. Two weeks later at 1.30 P.M., July 7, 1924, they were injected intraperitoneally with 3 c.c. of a 10% solution of egg albumin.

They all showed the first three stages, and six of them (Nos. 22, 23, 24, 25, 27 and 28) showed the fourth stage. All six died. Time of injection and death are tabulated below.

Table I.

No. of rat	Injected at	Died at	Time between injection and death
22	1.30 P.M.	5.30 P.M	4 hrs. 00 min.
23	1.30 "	8.00 "	6 " 30 "
24	1.30 "	8.15 "	6 " 45 "
25	1.30 "	6.00 "	4 " 30 "
27	1.30 "	4.30 "	3 " 00 "
28	1.30 "	6.00 "	4 " 30 "

Autopsy of rats Nos. 22, 23, 24, 25, 27 and 28. These rats died shortly following the injection of the large dose of egg albumin. An active more or less localized abscess was found in all of them. This abscess contained pus and was surrounded by inflammation. The inflammatory process extended into the surrounding tissue from 3 m.m. to about 3 c.m. The axillary and superficial inguinal glands were markedly enlarged. The lungs were congested in all but two cases (Nos. 23 and 27). The spleen usually had a very dark color. In rat No. 23 the peritoneum was inflamed.

Autopsy of rats Nos. 21, 26, 29 and 30. One week after the injection these rats appeared to be in perfect condition. They were killed and no pathological changes could be found except a small fibrous nodule at the site of the subcutaneous injection of bacteria. This nodule was about the size of a grain of wheat and represented a healed abscess.

Thus it is shown that sensitization plus infection doubles the mortality, i.e. markedly increases the susceptibility of rats to egg albumin. Furthermore it should be noted that sensitization also increases the susceptibility to infection. This is shown not only by the greater number of rats actively infected, but also by the greater severity of the infection.

Altho this experiment can not be said to prove that the infection has exhausted the vitamin B reserves of the body, it strongly suggests this possibility. For it has been shown (1) that the susceptibility of rats to anaphylaxis is also greatly increased by a deficiency of vitamin B. these rats had an adequate supply of vitamin B in the diet for purposes of health, but nevertheless reacted as if the supp was deficient.

A control experiment was tried at the same time which showed that healthy rats, of the same lot under identical conditions of diet and surroundings, did not become hypersensitive to egg albumin; altho they were given two small subcutaneous, sensitizing doses of egg albumin.

Ten white albino rats (Group IV, Nos. 31 - 40) were injected subcutaneously with 1 c.c. of a 2% solution of egg albumin on June 17, 1924 and again on June 23, 1924. Two weeks later at 1.37 P.M. July 7, 1924, they were injected intraperitoneally with 3 c.c. of a 10% solution of egg albumin. No effects of this injection were seen except in rat No. 39. Rat No. 39 showed all the various stages of anaphylaxis. It died at 6.40 P.M., five hours and three minutes after the injection.

Autopsy of Rat No. 39. Axillary, inguinal, and other superficial lymph glands were only slightly enlarged. However intestinal and intra-abdominal lymphatic structures were markedly enlarged. The intestines were inflamed. The abdominal cavity contained about 5 c.c. of bloody fluid. A small congested area was found at the apex of each lung.

Autopsy of other rats of Group IV. These rats showed no immediate effects and one week after the injection appeared to be in perfect condition. They were killed and no pathological changes could be found in any of them.

This shows then that healthy rats under the conditions of this experiment cannot be made hypersusceptible to egg albumin by injection of sensitizing doses of protein alone. There are at least two other possible means of completing the production of hypersensitization.

If rats are deprived of vitamin B (1) they then may be made hypersensitive to egg albumin by two or more injections of small doses of protein. But this vitamin B starvation in itself does not produce hypersensitization, but must be supplimented by protein injections. In this respect it differs from the second method.

An active abscess will not only complete this process of sensitization, but it can by itself produce a state of hypersensitivity to egg albumin.

The diet given during this experiment consisted of whole wheat flour 1½ parts, yellow corn meal 1 part, oat meal 1 part; linseed meal, bone meal, and McCollum's salt mixture* ½ part. This had plenty of vitamin B, but no vitamin C, and rats grow normally on it.

* McCollum's salt mixture used here consisted of NaCl 17.3 gms.,

(Footnote continued)

mono-sodium phosphate 34.7 gms., calcium phosphate 51 gms., anhydrous $MgSO_4$ 26.6 gms., di-basic potassium phosphate 95.4 gms., calcium lactate 130 gms., and ferric citrate 11.8 gm

The weights and sex of the rats thus used in the preceding experiments are given in Table II.

Table II.

Weight chart of rats in grams.

Group I.

Date	Rat 1 Male	Rat 2 Female	Rat 3 Female	Rat 4 Male	Rat 5 Male	Rat 6 Female	Rat 7 Female	Rat 8 Female	Rat 9 Female	Rat 10 Female
6/17/24	75.2	75.	78.	74.5	84.5	69.	56.	58.2	54.	44.5
6/23/24	82.	82.5	84.	84.5	104.	75.	64.	66.5	63.5	53.
7/7/24	91.	93.5	87.	94.	140.	96.5	71.	78.	74.	57.5

Group II.

Date	Rat 11 Male	Rat 12 Female	Rat 13 Female	Rat 14 Female	Rat 15 Male	Rat 16 Male	Rat 17 Female	Rat 18 Male	Rat 19 Female	Rat 20 Female
6/17/24	96.	101.	113.5	79.7	85.5	72.	62.	64.5	69.5	49.5
6/23/24	113.5	110.	125.	82.5	105.	87.	55.5	77.	72.5	63.
7/7/24	124.	120.	137.	109.	121.	105.5	70.	84.	87.5	73.5

(Table II continued)

Group III.

Date	Rat 21 Male	Rat 22 Female	Rat 23 Male	Rat 24 Female	Rat 25 Male	Rat 26 Female	Rat 27 Female	Rat 28 Female	Rat 29 Female	Rat 30 Female
6/17/24	88.5	72.5	102.	53.5	50.	65.2	66.	76.5	58.5	63.
6/23/24	88.5	75.	103.5	66.	62.	68.	68.	79.	61.5	72.
7/7/24	94.	107.5	118.5	74.	76.5	92.5	94.5	105.	68.	90.5

Group IV.

Date	Rat 31 Male	Rat 32 Female	Rat 33 Male	Rat 34 Male	Rat 35 Male	Rat 36 Male	Rat 37 Female	Rat 38 Female	Rat 39 Male	Rat 40 Female
6/17/24	88.	123.	97.	91.	109.	88.3	82.5	69.5	86.	67.5
6/23/24	106.	130.	100.	107.5	127.	98.5	93.	79.	91.	75.
7/7/24	112.5	136.5	104.5	108.	147.5	135.5	101.	96.5	119.	94.

Discussion.

These results show that anaphylaxis in the white rat is dependent upon disease. It suggests a possible etiological factor in multiple sensitizations. When these results are interpreted in the light of previous work(1) which showed that vitamin B starvation predisposes to anaphylaxis, they strongly suggest the possibility of disease exhausting the vitamin B stores of the body. But disease must do more than this, for vitamin B starvation alone does not produce hypersensitivity to egg albumin. It is difficult to imagine just how infection is able to bring about this state of hypersusceptibility to egg albumin. It must at least consist of two separate processes. First disease must make the rats susceptible to sensitization, and secondly it must sensitize them. The first process we believe is brought about by an exhaustion of vitamin B, because this according to our previous work (1) will cause rats, which are normally quite resistent to anaphylaxis, to lose this natural resistance. Altho we have not made a special study of the manner in which vitamin B ~~occurs~~ causes rats to be naturally resistant to anaphylaxis, we may make an assumption which is based on the work of Longcope (4). He found that altho normal rats could not be sensitized that their ~~tissues~~ nevertheless reacted to the foreign proteins as shown by the production of precipitins. The author also found that the serum of normal rats which had been sensitized to egg albumin would react with egg albumin to

produce a precipitate of egg albumin even in high dilutions, and that this mixture of egg albumin and rat serum was highly toxic to normal guinea pigs. As yet we have not had an opportunity to repeat this work in sufficient number of cases to draw any definite conclusions but since it is in agreement with Longcope's observation we feel that we can at least make a temporary assumption as to the nature of the action of vitamin B in this natural resistance. Now if the appearance of precipitins can be taken to mean anything it can surely be taken to indicate that the foreign protein has reacted with the tissues of the animal. Therefore we believe that vitamin B protects against sensitization of the nerve ends perhaps by preventing the combination of the protein split substances with these cells. Or that it acts to prevent shock by preventing the toxic protein split products from stimulating the nerve ends. This explanation is in accord with Dr. Isaac's results(5) on desensitization by extracts of plants. It is also in accord with our results(6) on desensitization of guinea pigs by injections of orange juice.

A plausible explanation of the second process here involved is not so easy. For we do not know the chemical nature of these substances which sensitize the cells. These sensitizing substances must not be the original unchanged protein for the incubation period of active anaphylaxis is much longer

than passive anaphylaxis. These substances must exist in an uncombined state in the serum of an immunized animal for the serum of an immunized animal is more effective in passively sensitizing a second animal than the serum of an anaphylactic animal. These sensitizers possess an affinity for the tissue of both immunized and normal animals for Dale[7] has shown that the isolated uterus of an immunized guinea pig is highly anaphylactic. Altho we do not know whether these substances are formed from the foreign protein or from the bodies tissues, it nevertheless seems most reasonable to look upon these sensitizers as relatively simple chemical compounds perhaps of a toxic nature, which sensitizes the cells by altering their respiration. Now it may be that these sensitizing groups may be present in both egg albumin and bacteria or it may be that either bacteria or egg albumin may be able to initiate their production in case the sensitizers are of somatic origin. That is these substances may be formed by tissue necrosis or dissolution.

Footnote:

After preparing this paper for publication there appeared a report of a case of sensitization to bread [Sensitization to Bread, Pasteur Vallery-Radot and A. R. Barrieu. Bull. de la Societe Medicale des Hospitaux, Paris, 48: 1364, Oct. 10, 1924 J. A. M. A. Vol. 83, No. 21, Nov. 22, 1924, p. 1719)] These authors report that a boy aged 11 was sensitized to many foods

(Footnote continued)

which resulted in urticaria. Attempts at desensitization failed but the whole wheat bread of war time checked the condition. They assumed that bran contained an urticaria - preventing vitamin. We believe that this is a clinical example similar to our results showing the role of vitamin B in preventing desensitization. G. A. Hartwell (The Biochemical Journal Vol. XVIII, No. 5, 1924, p. 784) has recently shown that there is a quantitative relation between vitamin B and protein intake in the diet of mother rat, a heavy protein intake is harmful unless accompanied by high vitamin B and unless this ratio is correct that disturbances occur in the young nursing rats.

Summary.

1. An active pyogenic disease (local abscess produced by staphylococcus) produces in white rats a state of hypersensitivity to egg albumin.
2. The injection into healthy normal rats, of one large dose of egg albumin was without effect, therefore it could not be the cause of the symptoms and death that followed similar injections into diseased rats.
3. The severity of the anaphylactic symptoms are greatly increased, if rats are simultaneously injected with sensitizing doses of protein and suspensions of bacteria. Simultaneous injections of egg albumin greatly ~~lowered the resistance~~ increased the severity of the reaction of rats to staphylococcus albus injections.
4. Healthy rats are not made hypersensitive to egg albumin by injection of small doses of protein alone.

Conclusions.

From these experiments we may conclude that a disease process (local pyogenic abscess) greatly modifies the anaphylactic response in white rats. Since disease causes rats to act ~~like~~ as if they were suffering from a deficiency of vitamin B, we believe that it acts by exhausting vitamin B

and thereby renders the nerve ends hypersensitive to foreign protein split products.

We may also conclude that a local pyogenic abscess sensitizes white rats to egg albumin.

It is with great pleasure that the author expresses his indebtedness to Dr. William B. Wherry, of the Department of Bacteriology of this University for his advice and assistance which made the bacteriological part of this work possible; ~~and~~ to Miss Agnes H. Grant for assistance in planning and carrying out the dietary part of this work; and to Professor A. P. Mathews under whose direction this work was carried out.

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The Chemical Basis of Immunity.

VI. The Anti-neuritic Content of Normal, Diseased and Beri-beri Rats.

By

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(Merrell Fellow in Biochemistry)

From the Department of Biochemistry, University of Cincinnati, Cincinnati, Ohio.

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Since we had found certain indirect experimental evidence which had led us to make the suggestion that a chronic low grade infection might bring about the exhaustion of the body's stores of vitamin B, so that the body suffers from an avitaminosis; our next step was to make an attempt to measure the vitamin B content of the bodies of (a) normal rats, (b) diseased rats, and (c) beri-beri rats.

At this time, we were quite firm in our opinion that the substances which relieved the beri-beri convulsions, prevented the onset of the nervous symptoms, and the vitamin B (growth vitamin) were identical. Therefore the pigeon seemed to us to be the animal best suited to serve as a test animal in these determinations.

Twenty-four pigeons were fed upon the anti-neuritic deficient diet until they developed the typical nervous symptoms, i.e. the retraction of the head (opisthotones) or

convulsive seizures during which the pigeon would turn somersaults backwards. They were then observed for a short time to be sure that symptoms were actually present, and to determine as nearly as possible the severity of the attack. If they were judged to be suitable for the test they were then given various amounts of the different extracts which were to be tested. Since these extracts were in a fluid state it was, with a little practise, found to be a simple matter to introduce a pipette into the esophagus and give the desired amount. Over twenty cubic centimeters of fluid can be given in this way without any danger to the pigeon or any fear of having the fluid lost by vomiting.

The rats (whose bodies were to be examined for their antineuritic content) were prepared as follows: Thirty-six (36) white albino rats, about four weeks old, were divided into three goups of twelve each. Group (I) were fed upon a complete (thought to be a complete diet at this time but we have since found certain deficiencies in the diet used here) diet for fourteen days. Group (II) were fed upon the same diet for a similar period, but in addition they were diseased by injecting subcutaneously three doses of 1 cc. of a staphylococcus aureus suspension at intervals of one week. This suspension was prepared by growing the bacteria staphylococcus aureus from infected antrum, upon agar slants for twelve hours, and then washing off the growth with sterile (0.9% NaCl) salt

solution (10 cc. per tube). The suspension was then shaken with sterile glass beads in order to break up the clumps of bacteria and make the distribution as even as possible. Group (III) were fed upon the diet deficient in the anti-neuritic substance. This diet was the same as that given to the pigeons. The diets used were as follows:

Food	Complete Diet %	Antineuritic Deficient Diet %
Rice ****	62	66
Casein	16	16
Butter*	7	7
Crisco**	7	7
Salt mixture***	4	4
Rice polishings	4	0

* Only the butter fat was used. It was purified by melting it, allowing the salts, water, and other organic matter to settle out. The yellow fluid fat was decanted, and washed with distilled water.

** Crisco is a partially hydogenated vegetable fat.

*** McCollum's salt mixture. (see Part I, page 175)

**** The rice was a highly polished grade of rice. It was carefully purified by washing for several hours under running water (hot water for 2 hrs. or cold water for 10 hrs.).

Preparation of the extracts:

One extract was made from each group of rats. The rats were killed with chloroform. The skin adn the large and small intestines were removed. The stomach and the duodenum were split open and carefully washed. The bodies (including stomach, duodenum, bones, etc.) were ground to a fine paste in a meat chopper. The wet weight of the combined tissues of the different groups were: Group I = 515 gms., Group II = 560 gms., and Group III = 425 gms. This paste was then extracted with 1000 cc. of redistilled ethyl alcohol (70% by weight) which contained 10 cc. of glacial acetic acid. After 20 hours the solids were filtered off. They were re-extracted for 4 hours with 500 cc. of redistilled ethyl alcohol (sp. g. 0.80 at 20° C.). The solids were again removed by filtration. The filtrates had a clear light brown color. The filtrates were then evaporated under reduced pressure, first at room temperature and later at 60° C., to dryness. The residue was taken up in 200 cc. of distilled water (which contained 1 cc. of glacial acetic acid as a preservative). The extract was then allowed to stand in the ice box overnight. It was filtered the next morning. The material insoluble in the dilute acid was discarded. The extracts were kept in the ice box except when actually in use. The extracts were of such a strength that 4 cc. of extract No. I = approximately 10 gms. of rat tissue;

5.5 cc. of extract No. II = approximately 10 gms. of rat tissue; and 4.7 cc. of extract No. III = approximately 10 gms. of rat tissue.

These extracts were then tested on the beri-beri pigeons, the curative dose being used as a measure of the relative antineuritic power of the various extracts. These results are tabulated and also given in detail in the following tables I, II, and III, and protocols.

The weights of the rats during their preparation are given in table IV. The weights of the pigeons during this experiment are given in table V.

In order to show that the acetic acid contained in the extract had no curative power in itself, pigeons No. 84 and 94 were given acetic acid solutions of a similar strength.

Pigeon No. 89 is representative of the behavior of a pigeon when treated with an extract which has a high antineuritic content.

Pigeon No. 98 is included because it shows that in order to use the pigeons as a test animals they must be carefully selected before the test is carried out for, if they have become too weak they cannot be revived even with extracts of high antineuritic content. As a rule the more active and violent the nervous symptoms the better suited is the animal for the test.

Table IV.

Weight Chart of Rats.

Group I. (Normal Rats)

Date	Number of rat											
	1	2	3	4	5	6	7	8	9	10	11	12
/3/24 **	51.	36.	58.	58.2	59.2	52.	31.3	60.	33.	47.7	31.4	60.2
/10/24	58.2	41.2	68.	68.2	74.	58.3	32.5	72.5	35.	59.5	35.6	64.6
/17/24 ***	61.	44.	70.	74.5	84.	61.	34.	83.	34.3	64.	32.	66.

Group II (Diseased Rats)

	13	14	15	16	17	18	19	20	21	22	23	24
/3/24	44.5	45.5	43.8	55.5	53.4	46.5	58.	64.2	56.6	40.	41.7	56.
/10/24	55.2	54.4	52.5	66.2	70.	52.4	60.	78.5	71.	43.	48.	64.
/17/24	55.5	61.8	54.	76.	77.3	59.	59.2	89.	80.	41.7	50.	65.5

Group III (Beri-beri Rats)

	25	26	27	28	29	30	31	32	33	34	35	36
/3/24	45.2	54.7	56.	45.2	47.	48.5	53.2	62.4	54.5	36.	32.2	29.
/10/24	45.5	56.4	62.	48;	50.	48.7	51.	72.	59.7	41.3	32.	31.3
/17/24	49.	53.	62.5	47.	47.	51.7	54.	75.	67.	40.	30.	D *

* Died with symptoms of beri-beri.

** Experiment begun on this date.

*** Extracts made from the rats on this date.

Weight Chart of Pigeons.

Number of Pigeons	Date 12/3/24	12/10/24	12/17/24	12/24/24	12/31/24	1/ 7/25	1/14/25	1/21/25	1/28/25	2/4/25	2/11/25	2/18/25	2/25/25	3/ 4/25	Remarks
81	310	303	282	230	240	237	240	260	284	294	314		300	315	Normal *
82	315	270	225												12/18/24 **
83	316	284	260	243	220	228									1/13/24 **
84	360	386	400	353	330	340	322	306	314	320	390		369	396	Normal *
85	306	290	287	270	256	260	263	221	250	255			257	273	Normal *
87	340	324	300	283	267	308	266	228	220	215					2/7/25 **
88	361	316	294	266	270	256									1/13/25 **
89	378	250	304	250	251	260	247	250	264	269	316		277	310	Normal *
90	337	326	307	280	284	288	244								1/17/25 *
91	380	358	303	288	276	254	228	227	256	244	240		350	390	Normal *
92	378	399	390	380	370	365	372	345	293	380	420		369	435	Normal *
93	340	314	309	294	282	280	220								1/14/25 **
94	350	316	298	280	280	280	270	254	230	200					2/8/25 **
95	368	340	326	312	296	260									1/13/25 **
96	265	246	208												12/18/24 **
97	350	327	324	291	285	300	276	294	241	312	345		340	344	Normal *
98	306	250	230	220	206	198	183								1/21/25 **
100	350	308	290	297	280	327	330								1/20/25 **
42		343	330	330	305	304	298	254							1/26/25 **
43		359	371	333	290	294	294	235							1/23/25 **
44		417	400	340	315	320									1/20/25 **
45		370	383	345	310	326	282								1/17/25 **
46		373	370	335	315	320	326	286	280	342	405				Normal*
47		316	340	299	286	277	272	257							1/26/25 **

*This was the condition of the pigeon at the termination of the experiment.

**This is the date of the death of the pigeon.

N.B. Pigeons Nos. 42, 43, 44, 45, 46 and 47 were placed upon the refined beri-beri diet on Dec. 10, 1924. The rest were placed upon this diet on Dec. 3, 1924.

Testing of the extracts on beri-beri pigeons.

Pigeon No. 81.

Extract of hormal rats.

ate	Time	Observation and treatment.
25/24	10:00 A.M.	First symptoms of beri-beri appear.
	11:00 A.M.	Head retracted, 6 cc. of extract No. 1 given.
	4:30 P.M.	Appeared normal had eaten some food, 6 cc. of extract. No. 1 given.
26/24	10:30 A.M.	Normal, could fly well.
27/24	11:00 A.M.	" " " "
23/24	10:00 A.M.	" " " "
29/24	1:00 P.M.	Return of symptoms of beri-beri.
	1:30 P.M.	Head retracted, 6 cc. of extract No. 1 given.
	4:00 P.M.	Much improved, no convulsions, 6 cc. of extract No. 1 given.
	7:00 P.M.	Appears to be normal, could fly about 4 ft., still a bit week.
30/24	1:00 P.M.	Normal, had eaten about 100 gms. of food, could easily fly to the end of a 20 ft. string.
31/24	3:30 P.M.	Normal.
7/25	1:00 P.M.	"
8/25	11:00 A.M.	Return of convulsions.
	11:30 A.M.	Convulsions continue, 4 cc. of extract No. 1 given.
	3:00 P.M.	Appears to be normal, but somewhat weak.
	7:00 P.M.	Normal.
9/25	3:00 P.M.	Normal.
	9:30 P.M.	Normal. Able to fly quite well, sense of direction good, still a bit weak.

Date	Time	Observation and treatment
10/25	9:30 A.M.	No convulsions, too weak to fly, sense of direction poor.
	4:00 P.M.	Spasms return. Marked increased in weakness. 4 cc. of extract No. 1 given.
	10:00 P.M.	Quite weak, but no convulsions.
11/25	10:00 A.M.	Very weak.
	12:00 noon	Convulsions return.
	12:15 P.M.	Pigeon now looked tooweak to withstand further experimentation so 10 cc. of extract of rice polishings (= 50 gms. of the dry polishings) was given.
	4:00 P.M.	Appears normal, eating heartily.
	5:45 P.M.	Appears to be normal, walks with a steady gait, too weak to fly more than 5 to 6 ft., sense of direction good.
	9:00 P.M.	Appears to be normal, able to fly fairly well.
12/25	9:15 A.M.	" " " " " " very " .
	7:00 P.M.	Normal.
19/25	11:00 P.M.	Still normal. 10 cc. of extract of rice polishings = 50 gms. of dry polishings given. Returned to normal diet.

Testing of the extracts on beri-beri pigeons.

Pigeon No. 44.

Extract of normal rats.

Date	Time	Observations.
9/25	12:00 noon	First symptoms of beri-beri appear, with marked head retraction and vomiting.
	4:00 P.M.	Convulsions continue. 4 cc. of extract No. 1 given.
	4:30 P.M.	Head retracted.
	9:30 P.M.	No convulsions, but too weak to fly, sense of direction poor.
10/25	9:30 A.M.	Mild spasms occur after two attempts at flying.
	4:00 P.M.	Head retracted. Mild spasm present.
	5:00 P.M.	Head retracted. 4 cc. of extract No. 1 given.
	10:00 P.M.	Dead.

Testing of extracts on beri-beri pigeons.

Pigeon No. 88.

Extract of normal rats.

Date	Time	Observations and treatment.
9/25	3:00 P.M.	First convulsions occur.
	4:30 P.M.	Convulsions very severe. 4 cc. of extract No. 1 given.
	10:00 P.M.	Very weak, no spasms.
9/25	12:45 P.M.	Appears to be normal, flutters backwards if tossed into the air, too weak to fly.
	6:00 P.M.	Appears to be normal, too weak to fly.
	9:00 P.M.	" " " " " " " "
9/25	9:15 A.M.	Looks rather droopy, when disturbed it shows symptoms of beri-beri, i.e. head retraction and convulsions.
	11:30 A.M.	Convulsions continue. 4 cc. of extract No. 1 given.
	7:00 P.M.	Sits with feathers ruffled up. No spasms. Too weak to fly.
	10:00 P.M.	Appears to be normal, too weak to fly.
9/25	10:00 A.M.	Sits with feathers ruffled up. Spasms return after attempt at flying.
	11:00 A.M.	Convulsions continue. 4 cc. of extract No. 1 given.
	8:00 P.M.	Dead.

Testing of the extracts on beri-beri pigeons.

Pigeon No. 90.

Extract of Normal No. I.

Date	Time	
2/25	7:30 P.M.	First symptoms of beri-beri.
	10:00 P.M.	Convulsions continue. 4 cc. of Extract No. 1 is given.
3/25	10:15 A.M.	Normal.
	8:00 P.M.	Looks normal, walks with unsteady gait.
	10:00 P.M.	Head retracted.
	10:15 P.M.	" " . 4 cc. of Extract No. 1 is given.
4/25	11:45 A.M.	Normal.
	7:30 P.M.	"
5/25	7:25 P.M.	Looks normal, but after attempts at flying, there is a slight retraction of head. Walks in a circle with unsteady gait turning always to the right.
6/25	5:00 P.M.	Normal.
7/25	8:30 A.M.	Return of convulsions during the night.
	11:30 A.M.	Looks too weak to withstand treatment. 4 cc. of Extract No. 1 given.
	1:30 P.M.	Dead.

Testing of the Extracts on beri-beri pigeons.

Pigeon No. 46.

Treated every day

with Extract of normal rats, No. I.

ate	Time	
19/25	8:00 A.M.	First spasms occur.
	11:00 A.M.	Spasms continue. 4 cc. of Extract No. 1 given.
	2:00 P.M.	Sits with feathers ruffled up. No convulsions.
	6:00 P.M.	Looks normal.
	11:00 P.M.	Normal.
20/25	8:00 A.M.	"
	5:00 P.M.	"
	10:00 P.M.	" 4 cc. of Extract No. I given.
21/25	11:00 P.M.	" " " " " " " .
22/25	1:00 P.M.	"
	9:30 P.M.	" " " " " " " " .
23/25	10:30 P.M.	" " " " " " " " .
24/25	6:00 P.M.	" " " " " " " " .
25/25	7:00 P.M.	" " " " " " " " .
26/25	5:30 P.M.	" " " " " " " " .
27/25	10:00 A.M.	"
	8:00 P.M.	" " " " " " " " .
28/25	1:30 P.M.	"
	10:00 P.M.	" " " " " " " " .

te	Time	
9/25	10:00 A.M.	Normal.
	5:00 P.M.	" 4 cc. of Extract No. 1 given.
	5:30 P.M.	Return of convulsions.
	10:00 P.M.	Convulsions continue very severe.
	10:30 P.M.	" " " " . 10 cc. of Extract of rice polishings given.
0/25	8:30 A.M.	Normal.
	5:00 P.M.	" .
	11:30 P.M.	"
1/25	10:00 A.M.	" . Returned to good diet.

Testing of the Extracts on beri-beri pigeons.

Pigeon No. 47.

Extract of Normal rats, No. I.

Date	Time	
23/25	4:00 P.M.	First attack of beri-beri; head retracted and convulsions.
	7:30 P.M.	Convulsions continue. 8 cc. of Extract No. I given.
	11:00 P.M.	Much improved. No convulsions; slight retraction of the head if disturbed.
24/25	8:00 A.M.	Looks normal, but quite weak.
	12:00 A.M.	" " " " "
	5:00 P.M.	Convulsions.
	6:00 P.M.	" continue. 8 cc. of Extract No. I given.
25/25	2:00 P.M.	Normal.
	6:00 P.M.	" .
26/25	8:00 A.M.	Return of convulsions.
	10:00 A.M.	Convulsions continued. 8 cc. of extract No. 1 given.
	2:00 P.M.	Normal.
	3:00 P.M.	Return of convulsions.
	5:00 P.M.	Appears too weak to withstand treatment, therefore given 10 cc. of rice polishings extract.
	7:00 P.M.	Dead.

Testing of the extracts on beri-beri pigeons.

Pigeon No. 83.

Extract of diseased rats, No. II.

Date	Time	
/28/24	11:00 A.M.	First symptoms of beri-beri occur.
	1:30 P.M.	Head retracted. 6 cc. of extract No. II given.
	4:00 P.M.	" " " " " " " "
	7:00 P.M.	Symptoms of beri-beri relieved, could walk, but was quite weak. Could not fly 4 ft.
/30/24	1:20 P.M.	Looked normal but shivered as if cold. Room temp. 22° C. Had eaten only about 20 grams of food. After second attempt at flying it fell to the floor in convulsions.
	7:00 P.M.	Convulsions still present. 12 cc. of extract No. II given.
/31/25	3:30 P.M.	Had eaten about 10 grams of food. No spasms present, but it sat hovered up as if cold. It could fly fairly well.
/ 1/25	2:00 P.M.	Normal.
/ 2/25	2:00 P.M.	" .
/3 /25	3:00 P.M.	" .
/ 4/25	2:00 P.M.	" .
/ 5/25	2:00 P.M.	" .
/ 6/25	3:00 P.M.	" .
/ 7/25	1:00 P.M.	" .

Date	Time	
8/25	1:00 P.M.	Normal.
9/25	9:30 P.M.	" .
10/25	9:00 A.M.	" .
	4:30 P.M.	" .
	10:00 P.M.	" .
11/25	12:25 P.M.	" .
	6:00 P.M.	" .
	10:00 P.M.	" .
12/25	9:15 A.M.	Return of convulsions and unable to fly.
	11:30 A.M.	Head retracted. 3.5 cc. of extract No. II given.
	7:00 P.M.	Head retracted, too weak to walk. Convulsions.
13/25	9:30 A.M.	Dead.

Testing of extracts on beri-beri pigeons.

Pigeon No. 93.

Extract of diseased rats, No. II.

Date	Time	
9/25	3:00 P.M.	First symptoms of beri-beri occur. Photographed.
	4:00 P.M.	Convulsions continued. 3.5 cc. of Extract No. II given.
	9:30 P.M.	Looks droopy, but too weak to fly. Sense of equilibrium very poor.
10/25	9:30 A.M.	Looks droopy and drowsy. Too weak to fly.
	5:00 P.M.	Too weak to fly; equilibrium very ppor.
	10:00 P.M.	Flutters weakly backward if thrown into the air.
11/25	12:30 P.M.	Makes no attempt to fly when thrown into the air. Walks with unsteady gait.
	5:45 P.M.	Severe beri-beri spasms.
	6:00 P.M.	Spasms continued. 3.5 cc. of extract No. II given.
	9:00 P.M.	Convulsions.
12/25	9:25 A.M.	" .
	11:30 A.M.	" .
	7:00 P.M.	" ; head constantly retracted.
	10:00 P.M.	" .
13/25	10:00 A.M.	" ; and very weak. 10 cc. of rice polishings given.
	8:00 P.M.	Normal.
14/25	11:45 A.M.	Looks normal, but makes no attempt to fly when tossed into the air.
	5:00 P.M.	Dead.

Testing of extracts on beri-beri pigeons.

Pigeon No. 42.

Extract of diseased rats, No. II.

Date	Time	
/11/25	10:00 A.M.	First symptoms of beri-beri occur.
	12:35 P.M.	Spasms continued, head retracted.
	12;55 P.M.	3.5 cc. of extract No. II given.
	4:00 P.M.	Head retracted.
	5:45 P.M.	" " ; spasms.
	9:00 P.M.	In severe convulsions.
/12/25	9:15 A.M.	Looks normal. When tossed into the air it flutters backward to the floor and then goes into convulsions.
	7:00 P.M.	Looks normal. Able to fly fairly well. Walks with unsteady gait. After an attempt at flying it goes into convulsions.
/13/25	10:00 A.M.	Looks normal. Able to fly but a short distance.
	8:00 P.M.	" " . Too weak to fly.
/14/25	11:45 A.M.	" " . Walks with unsteady gait. Able to fly about 6 ft.
/15/25	7:15 P.M.	Looks normal. Walks with unsteady gait. Flies about 6 ft.
/16/25	5:00 P.M.	Looks normal. Walks with unsteady gait.
/17/25	11:15 A.M.	Normal.
/18/25	3:00 P.M.	" .
/19/25	11:00 P.M.	" .
/20/25	5:30 P.M.	" .
/21/25	11:00 P.M.	" .

ate	Time	
2/25	1:00 P.M.	Looks normal, but weak. Able to fly but 10 ft.
	9:30 P.M.	" " " " " " " " " " "
3/25	10:30 P.M.	Looks normal, but too weak to fly over 5 ft.
4/25	6:00 P.M.	" " " " " " " " " " "
/25	6:60 P.M.	Return of convulsions.
	7:00 P.M.	Convulsions continue.
	11:00 P.M.	No improvement. Nearly dead, too weak to get up.
		3.5 cc. of extract No. II given.
6/25	8:00 A.M.	Dead.

Testing of extracts on beri-beri pigeons.

Pigeon No. 43.

Extract of diseased rats, No. II.

Date	Time	
14/25	5:00 P.M.	First symptoms of beri-beri occur. Head retracted.
	7:15 P.M.	Head retracted, spasms. 3.5 cc. of Extract No. II given.
	10:00 P.M.	Slight retraction of head.
15/25	10:00 A.M.	No convulsions, weak.
	7:30 P.M.	Normal.
16/25	5:00 P.M.	" .
17/25	11:45 A.M.	" .
18/25	3:00 P.M.	" , except too weak to fly.
	8:00 P.M.	Sits with feathers ruffled up. Walks with unsteady gait. Too weak to fly.
19/25	8:00 P.M.	Convulsions.
	11:00 P.M.	" continue. 3.3 cc. of extract No. II given.
20/25	8:00 A.M.	Head retracted.
	5:30 P.M.	Convulsions.
	10:00 P.M.	" continue. Nearly dead. Since there was no relief 10 cc. of rice polishings extract given.
21/25	8:00 A.M.	No convulsions present, but too weak to fly.
	11:00 P.M.	Normal.
22/25	1:00 P.M.	Normal.
	9:20 P.M.	" .
23/25	10:30 A.M.	Quite weak, droopy and drowsy.
	2:30 P.M.	Dead.

Testing of extracts on beri-beri pigeons.

Pigeon No. 85.

Date.	Time	Observation and treatment.
/19/25	6:00 P.M.	First symptoms of beri-beri occur (spasm).
	8:00 P.M.	Spasms continue.
	11:00 P.M.	" " . 3.5 cc. of extract No. II given.
/20/25	8:00 A.M.	Mild convulsions.
	5:30 P.M.	Sits with feathers ruffled. Walks with unsteady gait. Too weak to fly, no convulsions.
	10:00 P.M.	Same as before, i.e., 5:30 P.M. 3.5 cc. Extract No. II given.
/21/25	11:00 P.M.	Head retracted, mild convulsions, general appearance bad, unable to fly. Walks with unsteady gait. 3.5 cc. Extract No. II given.
/22/25	1:00 P.M.	Sits with feathers ruffled. Makes no attempt to fly when tossed into the air. Walks with unsteady gait.
	9:30 P.M.	Condition the same. 3.5 cc. Extract No. II given.
/23/25	10:30 P.M.	Sits with feathers ruffled up. Shivers as if cold. Unable to fly. 3.5 cc. Extract No. II given.
/24/25	6:00 P.M.	Sits with feathers ruffled up. Shivers as if cold. Makes no attempt to fly when tossed into the air. 3.5 cc. of extract No. II given.
/25/25	7:00 P.M.	Sits with feathers ruffled up. Shivers as if cold. Able to fly a short distance. 3.5 cc. of Extract No. II given.

Date	Time	Observation and treatment
26/25	5:30 P.M.	Normal. 3.5 cc. of Extract No. II given.
27/25	10:00 A.M.	" .
	8:00 P.M.	" . 3.5 cc. of Extract No. II given.
28/25	1:30 P.M.	" .
	10:00 P.M.	" . 3.5 cc. of Extract No. II given.
29/25	10:00 A.M.	" .
	5:00 P.M.	" . 3.5 cc. of Extract No. II given.
30/25	8:30 A.M.	" .
	5:00 P.M.	" .
	11:00 P.M.	" . 3.5 cc. of Extract No. II given.
31/25	10:00 A.M.	" .
	5:00 P.M.	" . 3.5 cc. of Extract No. II given.
1/25	8:30 A.M.	" .
	1:30 P.M.	" .
	6:30 P.M.	" . 3.5 cc. of Extract No. II given.
2/25	6:30 P.M.	" . 3.5 cc. of Extract No. II given.
3/25	7:00 P.M.	Sits with feathers ruffled up. 3.5 cc. of Extract No. II given.
4/25	8:00 P.M.	Sits with feathers ruffled up. 3.5 cc. of Extract No. II given.
5/25	8:30 A.M.	Looks normal.
	5:30 P.M.	" " . 3.5 cc. of Extract No. II given.
6/25	10:00 A.M.	Normal.
	6:00 P.M.	" .
7/25	9:30 A.M.	" . 3.5 cc. of Extract No. II given.
	8:30 P.M.	" . 3.5 cc. of Extract No. II given.

Date	Time	Observation and treatment.
8/25	1:30 P.M.	Sits with feathers ruffled up.
	5:30 P.M.	" " " " ". 3.5 cc. of Extract No. II given.
9/25	5:30 P.M.	Normal. 3.5 cc. of Extract No. II given.
10/25	5:30 P.M.	" " " " " " " ".
11/25	8:00 P.M.	" " " " " " " ".
12/25	8:00 P.M.	Pigeon does not fly to roost. Walks with unsteady gait. Can fly only a few feet. Sits with feathers ruffled up. 3.5 cc. of Extract No. II given as last dose because preparation was exhausted.
1/25	7:00 P.M.	Severe convulsions return. 10 cc. of Extract of Rice Polishings given.
	10:30 P.M.	Spasms continue, perhaps a bit milder.
13/25	8:00 A.M.	No spasms, but still a bit weak.
	7:00 P.M.	No convulsions, but still a bit weak. 10 cc. of Extract of Rice Polishings given.
	10:00 P.M.	Sits with feathers ruffled up. No convulsions, walks with a steady gait. Too weak to fly.
13/25	12:30 P.M.	Sits with feathers ruffled up. Still quite weak.
	8:30 P.M.	Sits with feathers ruffled up. Able to fly fairly well. 10 cc. of Extract of Rice Polishings given. Returned to normal diet.

Testing of Extracts on beri-beri pigeons.

Pigeon No. 92.

Extract of diseased rats, No. II.

Date	Time	
25/25	10:00 P.M.	First occurrence of beri-beri spasms.
	10:45 P.M.	Convulsions continue. 7 cc. of extract No. II given.
26/25	8:30 A.M.	Much improved, no convulsions. Makes no attempt to fly when tossed into the air.
	3:00 P.M.	Return of convulsions.
	5:00 P.M.	Convulsions continue. 7 cc. of extract No. II given.
	8:00 P.M.	" " .
	10:30 P.M.	" " , perhaps a slight bit milder than before.
27/25	8:00 A.M.	Looks much better, walks with a steady gait. Too weak to fly.
	8:00 P.M.	Unable to fly. Walks with unsteady gait.
28/25	1:30 P.M.	Return of convulsions.
	3:00 P.M.	Convulsions continue. 10 cc. of rice polishing extract given.
	10:00 P.M.	Normal.
29/25	10:00 A.M.	" .
	3:00 P.M.	" , returned to normal diet.

Table II

Showing the results of treating beri-beri pigeons with an acid-alcohol extract of beri-beri rats.

Number of Pigeons	Time on deficient diet before the onset of opisthotonos	Time between the onset of opisthotonos and treatment	Treatment	Time between the treatment and the relief of the beri-beri spasm	Time between the treatment and return to normal	Time between treatment and the return of opisthotonos	Time between the return of the opisthotonos and the second treatment	Second treatment	Time between 2nd treatment and the relief of the beri-beri spasm	Time between the second treatment and the return to normal	Time between the second treatment and the return of opisthotonos	Time between the return of opisthotonos and the 3rd treatment	Third treatment	Time between the 3rd treatment and the relief of the beri-beri spasms.	Time between the 3rd treatment and the return to normal.	Final results and remarks.
	Days	Hrs. Min's.		Hrs. Min's.	Days Hrs. Min's.	Days Hrs. Min's.	Hrs. Min's.		Hrs. Min's.	Days Hrs. Min's.	Days Hrs. Min's.	Hrs. Min's.		Hrs. Min's.	Hrs. Min's.	
95	33	1 15	Ext. No. 3 = 26 gms. of rat tissue	7 15	No complete recovery	5 4 45	none	Ext. No. 2 = 20 gms. of rat tissue	5 30	no complete recovery	No return of spasms but increasing weakness		Ext. of rice polishings = 50 gms. of the dry material		No complete recovery	After a partial recovery this pigeon gradually grew weaker and died 65 hrs. and 30 min's. after 3rd treatment.
91	37	20	Ext. No. 3 = 10 gms. of rat tissue	19	4	27	30	Ext. No. 3 = 10 gms. of rat tissue	5	1 8 30	6 10 30	30	Ext. of rice polishings = 50 gms. of the dry material	2	12	Was normal 2 days and 13 hrs. after treatment. Returned to normal diet.
45	33	3	Ext. No. 3 = 10 gms. of rat tissue	12	1 12	5 1 15	none	Ext. No. 3 = 10 gms. of rat tissue	No relief	No recovery						Died about 11 hours after 2nd treatment.
100	46	3 30	Ext. No. 3 = 10 gms. of rat tissue	5	No complete recovery	5 hrs. to 19 hrs.	13	Ext. No. 3 = 10 gms. of rat tissue	No relief	No recovery						Died 13 hours and 30 min's. after 2nd treatment.
87	47	3	Daily doses of Ext. No. 3 = 10 gms. of rat tissue	24	3	17		No treatment in addition to daily doses	3 hrs. to 15 hrs.	No complete recovery						Convulsions return about 13 days later and pigeon dies.
97	54	2	Ext. No. 3 = 20 gms. of rat tissue	4	No complete recovery	5	2 30	Ext. No. 3 = 20 gms. of rat tissue	2 hrs. 30 min's to 5 hrs.	10 30	3 30		Ext. of rice polishings 50 gms. of dry material	4 30	4 30	Remained normal for three days then returned to normal diet.

Testing of the extracts on beri-beri pigeons.

Pigeon No. 95.

Extract of beri-beri rats, No. III.

Date	Time	
5/25	10:30 A.M.	First symptoms of beri-beri occur. Photographed.
	11:45 A.M.	Head retracted. 12 cc. of extract No. III given.
	7:00 P.M.	Much improved. No convulsions. Too weak to fly.
6/25	10:00 A.M.	No convulsions, quite weak. Was able to fly about 10 ft.
7/25	10:00 A.M.	No convulsions. Pigeon too weak to fly. Lost sense of direction.
8/25	7:00 P.M.	Feathers ruffled up. Nearly too weak to walk. Unable to fly more than 6 ft.
9/25	9:30 P.M.	Too weak to fly. Appears droopy.
10/25	9:00 A.M.	Looks droopy. No sense of direction in flights flies against wall.
	4:30 P.M.	Severe convulsions return. 12 cc. of extract No. III given.
	10:00 P.M.	Much improved. No convulsions.
11/25	12:25 P.M.	Normal. Flied fairly well except for sense of direction.
	6:00 P.M.	Normal except that he flies into the wall.
	9:00 P.M.	Normal.
12/25	9:15 A.M.	" .
	7:00 P.M.	" , except quite weak.

Date	Time	
/12/25	10:00 P.M.	Very weak and drowsy.
/13/25	9:45 A.M.	So nearly dead that the only sign of life was pupilary reflex and very slight respiratory reflex. 10 cc. of rice polishings given.
	10:00 A.M.	Dead.

Testing of extracts on beri-beri pigeons.

Pigeon No. 91.

Extract of beri-beri rats, No. III.

Date	Time	
1/ 9/25	10:00 P.M.	First convulsions appear.
	10:20 P.M.	Head retracted, convulsions present.
		4.7 cc. of extract No. III given.
1/10/25	9:00 A.M.	Looks droopy. Flies backward. Mild spasms occur after two attempts at flying.
	5:00 P.M.	Too weak to fly. Flutters backward if thrown into the air. Mild spasms present.
	10:00 P.M.	Quite weak. No spasms.
1/11/25	12:45 P.M.	Looks normal, flies fairly well, but lacks sense of direction.
	6:00 P.M.	Same as before.
	9:00 P.M.	Too weak to fly. Walks with unsteady gait.
1/12/25	9:25 A.M.	Appears droopy. Sits with feathers ruffled up. Too weak to fly. Walks with unsteady gait.
	7:00 P.M.	Looks normal, but is too weak to fly. Walks with unsteady gait.
1/13/25	10:00 A.M.	Normal.
	8:00 P.M.	" .
1/14/25	11:45 A.M.	" .
1/15/25	7:15, P.M.	" .
1/16/25	11:10 A.M.	" .
1/17/25	10:00 A.M.	" .
1/18/25	3:00 P.M.	" .

Date	Time	
1/20/25	5:30 P.M.	Normal.
1/21/25	11:00 P.M.	" .
1/22/25	1:00 P.M.	" .
1/23/25	10:30 P.M.	" .
1/24/25	6:00 P.M.	" .
1/25/25	7:00 P.M.	" .
1/26/25	6:30 P.M.	" .
1/27/25	10:00 A.M.	" , but looks a bit sick.
	8:00 P.M.	" .
1/28/25	1:30 P.M.	" .
	10:30 P.M.	" .
1/29/25	10:00 A.M.	" .
	5:15 P.M.	" .
	10:00 P.M.	" .
1/30/25	8:00 A.M.	" .
	5:00 P.M.	" .
	11:45 P.M.	" .
1/31/25	10:00 A.M.	" .
	5:00 P.M.	" .
2/ 1/25	8:30 A.M.	" .
	1:30 P.M.	" .
	6:30 P.M.	" .
2/ 2/25	6:00 P.M.	" .

Date	Time	
2/ 3/25	7:30 P.M.	Sits with feathers ruffled up.
2/ 4/25	7:00 P.M.	" " " " ", and too weak to fly.
		Walks with unsteady gait.
2/ 5/25	8:30 A.M.	Convulsions return.
	9:00 A.M.	" continued. 4.7 cc. of extract No. III given.
2/ 5/ 25	2:00 P.M.	Looks normal, walks with a bit unsteady gait. Too weak to fly.
	5:30 P.M.	Normal.
2/ 6/25	10:00 A.M.	" .
	6:00 P.M.	" .
2/ 7/25	9:30 A.M.	" .
	8:30 P.M.	" .
2/ 8/25	1:30 P.M.	" .
2/ 9/25	5:00 P.M.	Looks normal, walks with unsteady gait. Too weak to fly.
2/10/25	6:00 P.M.	Sits with feathers ruffled up. Much weaker.
2/11/25	9:00 A.M.	" " " " " " " .
	7:30 P.M.	Retraction of head after being disturbed.
	8:00 P.M.	Same. 10 cc. of rice polishings extract given.
	9:30 P.M.	Very severe convulsions.
	9:45 P.M.	Milder convulsions.
	10:00 P.M.	Very weak, but no spasms or head retractions.
2/12/25	8:30 A.M.	Normal.
/13/25	8:30 A.M.	" .
	9:00 P.M.	" . Returned to normal diet.

Testing of the extracts on beri-beri pigeons.

Pigeon No. 45.

Extract of beri-beri rats, No. III.

Date	Time	
1/12/25	7:00 P.M.	First attack of beri-beri spasms. Head retracted.
	10:00 P.M.	Spasms continue. 4.7 cc. of extract No. III given.
1/13/25	10:00 A.M.	Looks normal, too weak to fly. Slight retraction of head and mild convulsions.
	8:00 P.M.	Looks normal, too weak to fly.
1/14/25	11:45 A.M.	Normal.
1/15/25	7:20 P.M.	" .
1/16/25	5:00 P.M.	" .
1/17/25	11:15 A.M.	Return of convulsions. 4.7 cc. of extract No. III given.
	2:00 P.M.	Convulsions continue.
	10:00 P.M.	Dead.

Testing of extracts on beri-beri pigeons.

Pigeon No. 100.

Extract of beri-beri rats, No. III.

Date	Time	
1/18/25	11:30 A.M.	First attack of beri-beri.
	3:00 P.M.	Spasms continue. 4.7 cc. of extract No. III given.
	8:00 P.M.	Sits with feathers ruffled up. Too weak to fly or walk. No convulsions.
1/19/25	10:00 A.M.	Spasms return.
	6:00 P.M.	" continue.
	11:00 P.M.	" " ; nearly dead. 4.7 cc. of extract No. III given.
1/20/25	8:00 A.M.	Just barely alive.
	12:30 P.M.	Dead.

Testing extracts on beri-beri pigeons.

Pigeon No. 87.

Extract of beri-beri rats, No. III.

Date	Time	
1/19/25	8:00 P.M.	First attack of beri-beri.
	11:00 P.M.	Convulsions continue. 4.7 cc. of extract No. III given.
1/20/25	8:00 A.M.	Quite weak. No convulsions.
	5:30 P.M.	Mild convulsions and retraction of the head.
	10:00 P.M.	No convulsions, but quite weak. 4.7 cc. of extract No. III given. Shortly after treatment there is a retraction of the head and mild convulsions.
1/21/25	11:00 P.M.	Walks with unsteady gait. Too weak to attempt flying. No convulsions. 4.7 cc. of extract No. III given.
1/22/25	1:00 P.M.	Looks normal, too weak to fly more than 3 ft.
	9:30 P.M.	Normal. 4.7 cc. of extract No. III given.
1/23/25	10:30 P.M.	" " " " " " " .
1/24/25	6:00 P.M.	" " " " " " " .
1/25/25	7:00 P.M.	" " " " " " " .
1/26/25	5:30 P.M.	" " " " " " " .
1/27/25	10:00 A.M.	" " " " " " " .
1/28/25	1:30 P.M.	" " " " " " " .
1/29/25	10:00 A.M.	" " " " " " " .
1/30/25	8:30 A.M.	" " " " " " " .

Date	Time	
/31/25	10:00 A.M.	Normal. 4.7 cc. of extract No. III given.
/ 1/25	8:30 A.M.	" " " " " " " " .
/ 2/25	6:30 P.M.	" " " " " " " " .
/ 3/25	7:00 P.M.	Sits with feathers ruffled up. 4.7 cc. of extract No. III given.
/ 4/25	8:00 P.M.	Same as before only much weaker. 4.7 cc. of extract No. III given.
/ 5/25	8:30 A.M.	Sits with feathers ruffled up. Makes no attempt at flying if tossed into the air. No convulsions. Weaker than yesterday.
	5:15 P.M.	Same as before. 4.7 cc. of extract No. III given.
	5:30 P.M.	Following last treatment this bird developed convulsions.
	8:00 P.M.	Convulsions continue quite severe.
/ 6/25	10:00 A.M.	Looks normal, but too weak to fly.
	6:00 P.M.	" " " " " " " " .
/ 7/25	9:30 A.M.	Return of convulsions.
	12:00 noon	Dead.

Testing of extracts on beri-beri pigeons.

Pigeon No. 97.

Extract of beri-beri rats No. III.

Date	Time	
1/26/25	8:00 A.M.	First symptoms of beri-beri occur.
	10:00 A.M.	Convulsions continue. 9.4 cc. of extract No. III given.
	2:00 P.M.	Looks normal. No convulsions, but makes no attempt to fly when tossed into the air.
	3:00 P.M.	Severe convulsions return.
	5:00 P.M.	" " continue.
	5:30 P.M.	" " " . 9.4 cc. of extract No. III given.
	8:00 P.M.	Looks quite normal, but when disturbed shows mild spasms.
	10:20 P.M.	Appears quite normal, but too weak to fly. No convulsions.
1/27/25	10:00 A.M.	Normal.
	8:00 P.M.	" .
1/28/25	1:30 P.M.	" .
1/29/25	10:00 A.M.	Appears quite droopy.
	5:00 P.M.	Return of convulsions.
	5:30 P.M.	Spasms continue. 10 cc. of rice polishings extract given.
	10:00 P.M.	Normal.
1/30/25	8:30 A.M.	" .
	5:00 P.M.	" .
	12:00 noon	" .

Date	Time	
1/31/25	10:00 A.M.	Normal, returned to normal diet.

Testing of extracts on beri-beri pigeons.

Pigeon No. 84.

Controls (1% glacial acetic acid)

Date	Time	
1/31/25	10:00 P.M.	First symptoms of beri-beri. Slight retraction of head. Does not even flutter when tossed into the air, but falls like a rock to the floor.
	10:30 P.M.	Head still retracted. 5 cc. of 1% acetic acid given.
2/ 1/25	8:30 A.M.	No convulsions. Walks with unsteady gait. Makes no attempt to fly when tossed into the air.
	1:30 P.M.	Same as before.
	6:00 P.M.	Marked increase in weakness. Slight retraction of the head.
	6:30 P.M.	Retraction of the head quite marked. 10 cc. of rice polishings given.
	10:30 P.M.	Much improved. No convulsions. Walks well. Flutters to floor when tossed into the air. Equilibrium good, but too weak to fly.
2/ 2/25	8:30 A.M.	Normal.
	6:30 P.M.	" ; returned to normal diet.

Testing of the extracts on beri-beri pigeons.

Pigeon No. 89.

Extract of Rice Polishings.

Date	Time	
3/ 2/25	6:00 P.M.	First spasm of beri-beri occurs.
	7:00 P.M.	Mild spasms still present. Head retracted. 10 cc. of extract of rice polishings given.
	10:00 P.M.	No convulsions. Able to fly fairly well.
3/ 3/25	12:00 P.M.	Normal.
	8:30 P.M.	Normal. Returned to normal diet.

Testing of extracts on beri-beri pigeons.

Pigeon No. 98.

Rice polishings Extract.

Date	Time	
1/21/25	3:00 P.M.	This pigeon has shown no convulsions, but is too weak to stand. Has been droopy for the past 5 days.
	7:30 P.M.	No convulsions, no head retractions, but too weak to stand. Looks nearly dead. 10 cc. of rice polishings given.
	8:30 P.M.	Dead.

Vitamin B content of normal, diseased, and beri-beri rats; as shown by curative power of extracts of their bodies upon polyneuritis columbarum.

Autopsy of Pigeons.

Pigeon No. 88. Marked atrophy of muscles, stomach filled with a brilliant green fluid, lungs have a healthy pink color.

Pigeon No. 90. Medium atrophy of muscles, otherwise appears to be healthy.

Pigeon No. 83. Marked " " " " " " " " .

Pigeon No. 44. Slight " " " " " " " " .

Pigeon No. 93. Marked " " " , blood clot in the right side of the cerebellum, stomach and the intestines filled with a brilliant green fluid, liver has a dark greenish discoloration.

Pigeon No. 95. Marked atrophy of muscles, otherwise appears to be healthy.

Pigeon No. 45. Appears to be in fairly normal condition except for congestion of the anterior part of left lung.

Pigeon No. 42. Appears to be in fair condition except that the whole of the left lung is congested. Both lungs show considerable anthracosis.

Pigeon No. 47. Same as No. 42 except that there is a diffuse congestion in both lungs.

Pigeon No. 43. Slight atrophy of muscles, lungs show a large amount of anthracosis but no congestion; stomach filled with a brilliant green fluid.

Pigeon No. 100. Medium atrophy of muscles, some anthracosis, no congestion.

Pigeon No. 94. Marked atrophy of muscles, 12 round worms about 40 m.m. long, and 1 mm. thick were found in the intestines. One was free in the abdominal cavity.

Pigeon No. 98. Marked atrophy of muscles. Upper part of right lung congested; some anthracosis of both lungs.

Pigeon No. 87. Marked atrophy of muscles, marked anthracosis of both lungs, but no congestion.

Discussion:

While these results are not strikingly positive, they are, when viewed in the light of our recent studies, quite suggestive if not definitely positive. Our recent studies of the diet used in these experiments have shown that the diet that we supposed to be complete when we made these studies is really deficient in several respects. One of these deficiencies is vitamin B.

Conclusion:

We may conclude that these experiments are certainly not opposed to the conclusions which we deducted from our studies of the relation of disease to anaphylaxis. On the other hand we are inclined to interpret them as positive evidence which show that vitamin B is less plentiful in diseased tissue than in normal, i.e. a chronic staphylococcus infection brings about an exhaustion of vitamin B.

The Chemical Basis of Immunity.

VII. A Preliminary Report of Various Studies upon the Chemical Basis of Immunity.

By

Paul E. Wedgewood.

(Merrell Fellow in Biochemistry.)

From the Department of Biochemistry, University of Cincinnati, Cincinnati, Ohio.

Contents.

A. Relation of Disease, Food Deficiencies and Sensitization to Anaphylaxis.

B. Studies of the Diets used in our Experimental Studies.

C. The Effects of the Anti-neuritic Substance upon Anaphylaxis as manifested in the Guinea Pig.

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VII. A Preliminary Report of Various Studies upon the Chemical Basis of Immunity.

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This paper, as the title signifies, is a brief report of some of the recent studies carried out during the past few months.

(A) Our experiments dealing with the effects of disease and of vitamin B upon anaphylaxis have been repeated. The results were in accord with our previous findings, but due to certain changes were complicated by dietary deficiencies which tend to obscure the facts that it was hoped that they would reveal. We sought to determine by these studies whether or not vitamin B and disease were the only factors which modified the anaphylactic shock of the white rat. If these two factors were the only ones concerned in this phenomenon, then by sensitizing rats, which were

suffering from a deficiency of vitamin B and an infectious disease, we should expect to get nearly 100 % anaphylaxis. However the results were not 100 % and it is therefore probable that there are other factors that must be controlled. Staphylococcus aureus was used in these studies in place of staphylococcus albus. This organism produced an abscess which quickly broke down and discharged to the exterior, before a well defined abscess was formed in most of the cases. This may have been a factor which interfered with this experiment. Another difference between this experiment and the former ones was that in this experiment the rats were older, about one to two months older. The important point that was revealed by these experiments was that the diet that we supposed to be a complete one was really deficient in one or more factors. This discovery was made more or less accidently, due to the fact that other experiments were in progress it was necessary to keep the rats used in these experiments for five weeks before we began our experiment upon them. They were kept for this period upon the following diet: polished rice 62%, casein 16%, crisco 7%, butter 7%, McCollums salt mixture 4%, and rice polishings 4%. After about seven weeks upon this diet several of the rats developed xerophthalmia, blepharitis, cataract, etc. Instead of gaining weight they either began to lose weight or failed

to gain. They lost their appetite and became very drowsy, so much so that they took no interest in feeding preparation. Their fur either became very rough, dirty, dry, and shabby, or, as was more often the case, they became partially or completely bald. It therefore was quite evident that we were dealing with a food deficiency of some sort. For this reason, a careful study was made of the diets which had been used.

Boas (1) described a similar condition in rats when egg white was sole source of protein. She used marmite as a source of vitamin B and varied the protein. She was able to cure the condition by giving caseinogen, serum albumin, or spinach. From her experiment and the work of Harris (2) she suggest that there may be some amino acid such as cysteine deficient in the diet.

Later J. A. Hartwell (3) describes a similar condition and from her experiments concludes that the loss of fur in growing rats is closely correlated with quality or quantity (or both) of the dietary protein. She does not, however, feel that vitamin B has any direct influence upon the condition. In this paper we describe a similar condition which occured when the diet was rich in casein and we have cured it by varying the vitamin content of the diet.

(B) Study of the Diets used in our Experiments.

The following diets were carefully studied to determine what factors were responsible for the above mentioned symptoms.

Food	Diets A %	B %	C %
Polished rice[1]	62	58.5	66.
Casein[5]	16	15.1	15.1
Butter[3]	7	6.6	6.6
Crisco[4]	7	6.6	6.6
Cod liver oil	0	1.9	1.9
Rice polishings	4	7.5	0.0
Salt mixture[2]	4	4.0	4.0

Diet Food	parts
Whole wheat flour	1½
Yellow corn meal	1
Oat meal	1
Linseed meal	1/6
Bone meal	1/6
Sodium chloride	1/6

* This rice was carefully washed under running water for several hours (hot water 2 hrs., cold water 10 hrs.).

**McCollum's salt mixture: NaCl 4.71%, NaH_2PO_4 9.46%, $Ca_3(PO_4)_2$ 13.90%, $MgSO_4$ 7.25%, K_2HPO_4 26.01%, calcium lactate 35.45% and iron citrate 3.2%.

***Butter fat only was used.

****Crisco is an hydrogenated vegetable fat.

*****This casein is purified casein, in some of our work we further purified it by extracting with boiling alcohol.

The addition of 1 - 2% of cod liver oil to diet A improved the general condition of the rats somewhat. It cured and prevented xerophthalmia, but it did not have any marked beneficial effect upon the fur. It only slightly and temporarily improved the blepharitis. It did not greatly improve the weight. It did not prevent or cure the cataract.

On diet B similar conditions developed except there was no occurrence of xerophthalmia. The addition of one-half a gram per day per rat of hydrolyzed horn (which was rich in organic sulphur compounds) to this diet (B) failed to bring about any improvement in the fur of the rats. It did however temporarily stimulate their appetite and they temporarily gained weight.

The addition of orange juice, one cubic centimeter per day per rat, failed to bring about any noticeable improvement in the fur. It however did temporarily stimulate their appetites and they gained a bit.

The addition of dried yeast, one gram per day per rat, to diet B greatly improved their weight (doubled or trebled in two or three weeks), stimulated their appetites, increased their activity, caused them to become interested in their surroundings, improved their blepharitis and cured it in most cases, and stimulated their sexual activities. It also had a marked effect upon their fur. A new coat of fur started

to grow at once; in two weeks a complete new coat had developed so that it was nearly impossible to map out the formerly bald areas.

We are at present testing out the effects of large amounts of rice polishings (diet B plus 2 gms. of rice polishings per day per rat) upon these conditions. This has given very little, if any, improvement in three weeks.

By adding one gram of yeast per day per rat to diet C the rats improved more slowly. It took about three weeks for an improvement equivalent to that noted above (diet B plus 1 gm of yeast per day per rat).

By completely changing the diet to diet D we got an improvement. This improvement was not nearly as complete as in the former cases. The new coat of fur came in very slowly. After about six or seven weeks the formerly bald areas could still be mapped out. The gain in weight was much more gradual and has not even yet (after nine weeks) reached that gained in three weeks by the ratswhich were were fed upon diet B plus yeast. Therefore it would be unreasonable to assume that the addition of rice polishing introduced any toxic factor into the diet.

We have also studied the effects of these diets upon pigeons. On diet C pigeons slowly develop beri-beri. If the casein is further purified they develop beri-beri much

sooner. If, however, pigeons are fed upon diet B they do not develop beri-beri. For a time they lose a little weight but after a while they become accustomed to the change of diet and eat it well, they behave quite normally. At present we have several pigeons upon this diet; they have been on it for some time, about eight weeks; they look quite normal; build their nests; lay eggs; and are gaining weight.

Before leaving this discussion of diets, two conditions which have been noted should be mentioned. Some of the rats upon either diet A or B developed either cataract or a nervous lesion. Neither of these have cleared up when the general condition of the animal improved. The nervous lesion referred to has to do with the equilibrium of the animal. It is probably a lesion of the semi-circular canals or of the cerebellum. It may be either a right or left sided lesion. It causes the animals to act as if one side of the cerebellum had been destroyed.

(C) The Effects of the Injection of Large Doses of Anti-Neuritic Substance (extract of rice polishings) upon Sensitization as Shown in the Guinea Pig.

An extract of rice polishings was prepared. It was tested and found to be very powerful in its curative action of beri-beri of pigeons. The power of this preparation to modify guinea pig anaphylaxis was then tested as follows: Twenty-four guinea pigs were divided into four groups of six

each. Group I were sensitized and given the shock dose in the usual manner as controls. Group II were sensitized, but were given interperitoneally an injection of 5 cc. of rice polishing extract (equivalent to 25 gms. of dry rice polishings) two hours before the shock dose. Group III were given a similar injection two hours before the sensitizing dose. Group IV were given two similar injections, one, two hours before the sensitizing dose of protein and the other two hours before the shock dose. The result of this treatment on the anaphylaxis is shown in the following table. (see table I)

It is therefore quite apparent from those results that the anti-neuritic substance exerts an important influence upon the anaphylaxis as manifested in the guinea pig. It appears to suppress the shock, prolong it or prevent sensitisation.

Table I.

Effect of anti-neuritic substance upon anaphylaxis.

Number of Guinea Pig	Time between second injection and death		Duration of severe anaphylactic symptoms.			
			In the guinea pigs which die		In the guinea pigs which did not die.	
	Hrs.	Min's.	Hrs.	Min's.	Hrs.	Min's.
Group I. (Controls)						
1	1	20	1	20		
2					1	30
3						55
4	1	32	1	32		
5					1	54
6		59		59		
Group II (extract before shock dose)						
7	3		no immediate symptoms			
8					no early	symptoms*
9					" "	"
10					" "	"
11					" "	"
12	10		no immediate symptoms			
Group III (extract before sensitizing dose)						
13					1	53
14					practically no symptoms	
15					no symptoms	
16					1	52**
17					1	51
18	8	21	1	30		
Group IV (extract both before sensitizing and shock dose)						
19	30		practically no symptoms			
20						42
21	7			7		
22					1	12
23	18		practically no symptoms			
24	10		2			

* These animals were carefully observed for the first two hours and after that time at frequent intervals.

** Symptoms were very mild.

References.

1. Boas, M.A.: The Bioch. J. Vol. XVIII, No. 2, 1924, p. 422.

2. Harris, : Proc. Roy. Soc. Lond. B., 94, 441.

3. Hartwell, G. A.: The Bioch. J. Vol. XIX, No. 1, 1925, p. 75.