

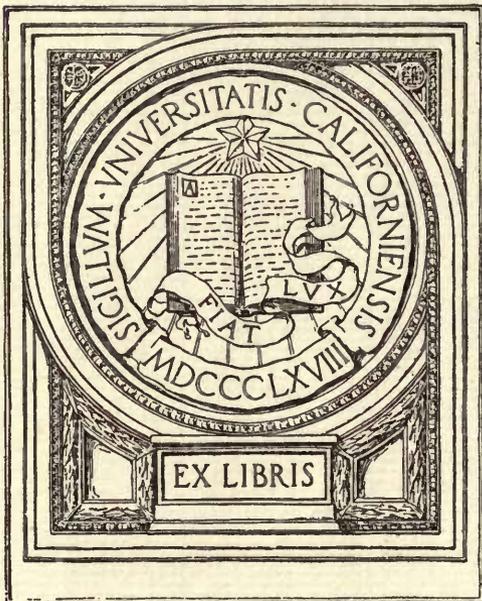
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THE CROONIAN LECTURES  
ON  
THE CHEMICAL CORRELATION OF  
THE FUNCTIONS OF THE BODY

DELIVERED BEFORE  
THE ROYAL COLLEGE OF PHYSICIANS OF LONDON  
ON JUNE 20TH, 22ND, 27TH & 29TH, 1905

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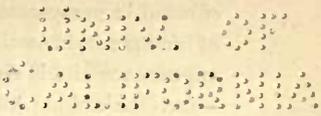


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## LECTURE I.

*Delivered on June 20th.*

### THE CHEMICAL CONTROL OF THE FUNCTIONS OF THE BODY.

MR. PRESIDENT AND GENTLEMEN,—From the remotest ages the existence of a profession of medicine, the practice of its art, and its acceptance as a necessary part of every community, have been founded on a tacit assumption that the functions of the body, whether of growth or activity of organs, can be controlled by chemical means; and research by observation of accident or by experiment for such means has resulted in the huge array of drugs, which form the pharmacopœias of various civilised countries and the common armamentarium of the medical profession throughout the world. The practice of drugging rests on the supposition that the functions of the body can be influenced in a normal direction by such means. I propose in these lectures to inquire how far such a belief is consonant with our own knowledge of the physiological workings of the body; how far, that is to say, the activities and growth of the different organs of the body are determined and coördinated among themselves by chemical substances produced in the body but capable of classification with the drugs of the physician. If a mutual control, and therefore coördination, of the different functions of the body be largely determined by the production of definite chemical substances in the body, the discovery of the nature of these substances will enable us to interpose at any desired phase in these functions, and so to acquire an absolute control over the workings of the human body. Such a control is the goal of medical science. How far have we progressed towards it? How far are we justified in regarding its attainment as possible?

I hope to be able to vindicate to you the assumption which is at the basis of medical practice, and to show that the activities of, at any rate, the large majority of the organs of the body are coördinated among themselves by the production and circulation of chemical substances. The results of physiological researches up to the present justify us in the faith that, within a reasonable space of time, we shall be in the possession of chemical substances which are normal physiological products, and by means of which we shall be in a position to control not only the activities but also the growth of a large number of the organs of the body.

In man and the higher animals, the marvellous adaptations effected by means of the central nervous system are so much in evidence, that physiologists have been tempted to ascribe every nexus between distant organs to the intervention of the nervous system; the more so because by this means an adaptation to changes, internal or external, can be

effected in many cases within a fraction of a second. But in the evolution of life upon this earth, this method of adaptation is of comparatively late appearance and is confined almost entirely to one division of living beings—*i.e.*, the animal kingdom. In the lowest organisms, the unicellular, such as the bacteria and protozoa, the only adaptations, into the mechanism of which we can gain any clear insight, are those to the environment of the organism, and in these cases the mechanism is almost entirely a chemical one. The organism approaches its food or flies from harmful media in consequence of chemical stimuli; it prepares its food for digestion or digests it by the formation of chemical substances, toxins or enzymes. In the lowest metazoa, such as the sponges, there is still no trace of any nervous system. The coördination between the different cells of the colony is still determined by purely chemical means. The aggregation of the phagocytic cells round a foreign body is apparently due to the attraction exerted on them by the chemical substances produced in the death of the injured tissues.

With the appearance of a central nervous system or systems in the higher metazoa, the quick motor reactions determined by this system form the most obvious vital manifestations of the animal. But the nervous system has been evolved for quick adaptations, not for the abolition of the chemical correlations which existed before a nervous system came into being. A study of the phenomena of even the highest animals shows that the development of the quick nervous adaptations involves no abrogation of the other more primitive class of reactions—*i.e.*, the chemical ones. Where the reaction is one occupying seconds or fractions of a second the nervous system is of necessity employed. Where the reaction may take minutes, hours, or even days for its accomplishment, the nexus between the organs implicated may be chemical. Already we are able, in many cases, to prove the existence of such a chemical nexus, and to employ it in artificially producing a state of growth or activity, which is in normal circumstances merely a phase in a complex series of physiological changes.

The chemical reactions or adaptations of the body, like those which are carried out through the intermediation of the central nervous system, can be divided into two main classes—(1) those which are evoked in consequence of changes impressed upon the organism as a whole from without; and (2) those which, acting entirely within the body, serve to correlate the activities, in the widest sense of the term, of the different parts and organs of the body.

The first class of adaptations includes the reactions of the body to chemical poisons produced by bacteria or higher organisms, and represents one of the most important means by which the body maintains itself in the struggle for existence. The complicated phenomena involved in the formation of antitoxins, of cytolytins, of bactericidal substances, and such like means of protection, have been the subject of much study of recent years, and their immediate interest to the practical physician renders it unnecessary for me to devote any time to their discussion,

especially as the subject is one to which I have not given any personal attention. The investigation of the second class, that of the correlation of the activities of organs, has by reason of its greater obscurity, or of the greater difficulty of its practical application in medicine, fallen largely to the province of the physiologist, and I therefore propose to deal almost exclusively with those members of this class of reactions which have so far been definitely ascertained.

Before, however, entering into details of any particular correlation, it may be profitable to consider what we may expect to be the nature of the substance which will, in any given case, act as a chemical nexus between different organs. We are dealing here with a question of general pharmacology. As Ehrlich has pointed out, the chemical substances which act on the body or parts of the body, producing physiological or pharmacological effects, can be divided largely into two main groups. Ehrlich's conception of the first group is bound up with his conception of the nature of the living protoplasmic molecule as a living nucleus with side chains of various descriptions. Assimilation of food-stuffs consists in the linking on of the food molecule as a fresh side chain to the central nucleus. The common feature among the substances of the first class is their close resemblance to an assimilable substance or foodstuff. All these substances acquire a close attachment to, or even identification with, the living protoplasm, and as a rule their effects are apparent only after sufficient time has elapsed for their building up into the protoplasmic molecule. To this class belong the numerous bodies, closely allied in their chemical character to the proteids, which are designated as toxins. All are produced by the agency of living organisms. I need only adduce as examples the various products of the pathogenic bacteria, such as diphtheria and tetanus, the poisonous toxins of higher plants, such as ricin and abrin, and those formed as a weapon of offence by higher animals, such as the active principles of the various snake venoms. According to Ehrlich, these all resemble assimilable foodstuffs in that they possess a haptophore group, by which they can anchor themselves on to the living molecule, becoming thus part of its side chains. The toxophore group thus introduced into the living molecule upsets and disorganises its reactions, leading by disorder of one or more functions to the death of the animal. In most cases the toxophore group is specific for some definite tissue or type of cell. Thus tetanotoxin exercises its effect almost entirely on the peripheral sensory neurones. It is doubtful, however, whether the haptophore group is so specific, if we are to accept Ehrlich's conception of the mode of formation of antitoxins; since we may get formation of antitoxins in animals where the toxic effect is entirely wanting.

The idea that these toxins are the part in the protoplasmic molecule of an assimilable foodstuff does not involve as a necessary sequence the formation of antitoxins or antibodies to the normal foodstuffs. That the power of assimilation is independent of the power to produce antibodies has been shown by van Dungern in a research specially

directed to determine this point. This observer found that the proteids of crabs' blood could be injected into the blood stream of the rabbit and undergo assimilation. Being proteids foreign to rabbits' blood, their injection provoked the production in the latter of a precipitin for crabs' blood plasma, but the assimilation of the proteid and the production of the precipitin were found to be absolutely independent phenomena.

The first group of pharmacological substances may be defined as substances presenting many points of resemblance to proteids, potent like enzymes in infinitesimal doses, and giving rise, as a result of their introduction into the body, to a reaction consisting in the production of an antibody.

The substances belonging to Ehrlich's second group, which includes all our common drugs, probably act on the protoplasmic molecule or part of it by reason of their chemico-physical properties or their molecular configuration. It is difficult to give a more definite expression of their mode of action. We know that in many cases slight changes in the molecule, such as the introduction or withdrawal of an ethyl, methyl, or  $\text{NH}_2$  group into or from a drug or group of drugs, alter their physiological actions in a regular manner. We know, moreover, that substances of the most diverse constitution, such as the various anæsthetics, may have little more than their fat solvent powers in common. All these drugs, however, are more or less stable compounds, generally to be obtained in a crystalline form and not easily destroyed by heat. On introduction into the body, the incubation period of their physiological effects is generally determined only by the time necessary for their distribution to, and their diffusion into, the cells which they chiefly affect. Although repeated doses of them can set up a certain degree of tolerance, in no case is there any evidence of the formation of a physiological antidote or antitoxin to the poison.

To which of these two groups of bodies must we assign the chemical messengers which, speeding from cell to cell along the blood stream, may coördinate the activities and growth of different parts of the body? The specific character of the greater part of the toxins which are known to us (I need only instance such toxins as those of tetanus and diphtheria) would suggest that the substances produced for effecting the correlation of organs within the body, through the intermediation of the blood stream, might also belong to this class, since here also specificity of action must be a distinguishing characteristic. These chemical messengers, however, or "hormones" (from ὀρμάω, I excite or arouse), as we may call them, have to be carried from the organ where they are produced to the organ which they affect, by means of the blood stream, and the continually recurring physiological needs of the organism must determine their repeated production and circulation through the body. If they belong to the first class and are analogous to the toxins, the production of a given substance and its discharge into the blood stream must give rise to the formation of a specific antibody, which must increase in amount with each production of the substance in question and tend therefore to

neutralise its physiological effects. It might be suggested that, in the case of these chemical messengers, the formation of an antibody was a local one and limited to the organ affected and that, in fact, their physiological effect—*e.g.*, secretion—was actually a pouring out of the antibody to the chemical messenger. But, as we shall see later, experimental evidence is entirely against this view, which, moreover, is not supported by any known instance of a similar localisation of antibody as a result of injection into the organism of any of the substances which belong definitely to the toxin class. The formation of antibodies appears to be, not a process of value in the normal physiological life of the organism, but one which has been evolved as a chemical means of defence to prevent the spread of injurious substances from the spot originally affected or attacked.

We are therefore forced to the conclusion that, if the processes of coördination of activities among the organs of the body are carried out under physiological conditions to any large extent by chemical means—*i.e.*, by the despatch of chemical messengers along the blood stream—these emissary substances must belong to Ehrlich's second order of substances acting on the body and must fall into the same category as the drugs of our Pharmacopœia. Among these, indeed, specificity is not wanting and is the basis of their classification by pharmacologists. Thus we have drugs elevating or depressing the activity of the nervous system; we can excite secretion in all the glands of the body by pilocarpin; we can stimulate and finally paralyse the præganglionic nerve endings of the sympathetic system by the injection of nicotine, or arouse the anabolic mechanism of the heart by the administration of digitalis. In all these cases we are certainly interfering with normal processes. The methods however, which we employ, are not at variance with those made use of by the body itself in securing the harmonious coöperation of its various parts.

In discussing the internal chemical reactions of the body, it will be convenient to divide them into two classes—*viz.*, those which involve (1) increased activity of an organ, and (2) increased growth of a tissue or organ. In both cases we must assume that the reaction to the chemical stimulus is itself chemical, the first class including those changes which are chiefly katabolic or dissimilative and are always associated with activity, and the second class involving diminished katabolism and increased building up or anabolism. We might, in fact, speak of the two classes of chemical stimulants as augmentor and inhibitor.

#### REACTIONS INVOLVING INCREASED ACTIVITY OF ORGANS.

The most striking, because the simplest, of this class of reactions is that which determines in higher animals the adequate supply of a contracting muscle with oxygen and the removal of its chief waste product, carbon dioxide. The increased depth and frequency of respiration contingent on muscular exertion are familiar to everyone, and we know that

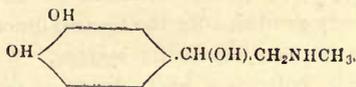
the physiological object of such changes is to secure the increased ventilation rendered necessary by the enormous rise of gaseous metabolism which accompanies muscular exercise. Even moderate work may raise the gaseous exchanges to between four and eight times their amount during rest. This increase in the respiratory movements is entirely involuntary and may, in its earlier stages, when affecting chiefly depth of respiration, be absolutely unnoticed by the subject of them. How is the respiratory centre aroused to an increased activity which is absolutely proportional to the increased metabolism of the distant muscles? A nervous path is at once excluded by the fact that hyperpnœa or even dyspnœa may be excited in an animal, after division of the spinal cord, by tetanisation of the muscles of the hind limbs. Zuntz and Geppert therefore came to the conclusion that the exciting agent in this increased activity was some acid substance or substances produced by the contracting muscles and transmitted from them through the blood stream to the respiratory centre. The subject has been lately investigated in this country by Haldane and Priestley. In a series of masterly experiments these observers show conclusively that the chemical messenger in this case is none other than carbon dioxide. The contracting muscle, when properly supplied with oxygen, takes up this gas and gives out carbon dioxide in direct proportion to the energy of its contractions. The carbon dioxide diffusing rapidly into the blood stream raises its percentage and, what is still more important, its tension in this fluid. The respiratory centre differs from the other parts of the central nervous system in having developed a specific sensibility to carbon dioxide. Its normal activity is determined by the normal tension of this gas in the blood and lymph bathing the centre. Diminution of the tension of this gas depresses the activity of the centre, causing slackening of respiration or even the total cessation of respiratory movements, known as apnœa.

This work by Haldane may be regarded as finally deciding a question which has been the subject of debate for nearly half a century. The dyspnœa, caused by the circulation of venous blood through the brain or by the deprival of the respiratory centre of the means of maintaining its normal gaseous interchanges, has been variously attributed either to oxygen starvation or to carbon dioxide intoxication of the centre. Haldane shows that the centre is very little sensitive to changes in the oxygen tension of the blood. The oxygen tension in the pulmonary alveoli may be altered from 20 per cent. to 8 per cent. without any increase in the depth or frequency of the respiratory movements. In these circumstances the heart or circulatory system may feel the deprivation of oxygen before the respiratory centre has responded to it. On the other hand, a rise of only  $\frac{1}{2}$  per cent. in the tension of carbon dioxide in the alveolar air, and therefore in the blood circulating round the respiratory centre, will increase the volume of air respired 100 per cent.

This simplest of all examples of a coördination of two widely separate organs by chemical means may, perhaps, give us a clue to the mode in which the more complex of such correlations have been evolved. In

this case the chemical messenger is a product of activity which is common to all protoplasm and must be excreted by the cell as a condition of its further activity. The adaptation in this case therefore is not the formation of a special substance which shall exert a specific influence on some distant organ, but the development in this distant organ of a specific sensibility to the common product of excretion of the first organ. We may, perhaps, assume that the more specialised messengers, which we shall have to consider in detail later, were at first accidental by-products of the selfish activity of the organ producing them, the first step in the development of a correlation being the acquisition of a sensibility to the substance in question by some distant organ.

The only other example of such a reaction, in which we know both the source and nature of the chemical messenger and the exact nature of the effects which it produces, is the suprarenal gland. Since the time of Addison we have known that atrophy of these glands in man leads to a disease characterised by the three cardinal symptoms of bronzing, vomiting, and extreme muscular weakness. Most of the attempts to reproduce this disease in animals have failed, owing to the fact that death follows the excision of both glands within 24 hours; the extreme muscular weakness is certainly produced, and this is attended by a profound fall in the general blood pressure. In 1894 Oliver and Schäfer showed that from the medulla of the suprarenals a substance could be extracted which, on injection into the circulation, caused marked rise of blood pressure and increased strength of the heart beat. Since the publication of these observations our knowledge concerning the nature and actions of this substance has progressed rapidly. The researches of Jowett in this country, and of von Fürth in Germany, have shown that the active substance is a definite chemical compound derived from pyrocatechin and having the formula—



Takamine, by the elaboration of a method for its preparation from the gland in a state of purity, has placed in the hands of the druggist a means of supplying the substance in bulk to the medical profession for therapeutic purposes. The exact knowledge of the constitution of adrenalin thus acquired has paved the way for the actual synthetic formation of this substance. Here, again, there has been a keen international rivalry, and the credit of its synthesis must be divided between this country and Germany. It is gratifying that the only original investigation of the subject which has yet been published as a contribution to science is the admirable account by Dakin of his synthesis, not only of adrenalin but of a whole array of substances, which are closely allied to this body in their chemical structure as well as in their physiological influence on the animal organism.

In order to comprehend the point of attack of adrenalin, the specific secretion of the medullary part of the suprarenal glands, we shall do well to go back to the mode of development of these organs. It was shown by Balfour that the suprarenals have in the fœtus a two-fold origin, the cortex being derived from the mesoblastic tissue, known as the intermediate cell-mass, while the medulla is formed by a direct outgrowth from the sympathetic system, and consists at first of an aggregation of neuroblasts. In some animals—*e.g.*, teleostean fishes—the two parts of the gland thus formed remain separate throughout life, but in the higher vertebrates the sympathetic outgrowth becomes surrounded by the cortex, and the cells rapidly lose all traces of resemblance to a nerve cell. But the medulla is genetically part of the sympathetic system, and its specific secretion, adrenalin, has an action which is apparently confined to the sympathetic system. In whatever part of the body we test the effects of adrenalin, we find that they are identical with the results of stimulating the sympathetic nerve fibres which run to that part. Thus, in all the blood-vessels of the body, adrenalin causes constriction; the contraction of the heart muscle is augmented, the pupil is dilated, while the intestinal muscle, with the single exception of the small ring of muscle forming the ileo-colic sphincter, is relaxed. The action of the sympathetic on the bladder differs, as shown by Elliott, markedly in various animals; but, whatever its effect, a similar one will be produced in the same animal by the injection of adrenalin. I have already mentioned that excision of the suprarenal bodies causes a profound fall of blood pressure, which continues until the death of the animal, and it has been stated that, when this fall is well established, it is impossible to raise the blood pressure by stimulation of the splanchnic nerve, or indeed to produce any effect at all on stimulation of the sympathetic nerve. Thus, not only does adrenalin excite the whole sympathetic system in its ultimate terminations, but its presence in the body as a specific secretion of the suprarenal bodies seems to be a necessary condition for the normal functioning, by ordinary reflex means, of the whole sympathetic system. We are dealing here with a problem which, betraying, as it does, an intimate relationship between nerve excitation and excitation by chemical means, promises by its solution to throw a most interesting light on the nature of the nerve process and of excitatory processes in general.

Our knowledge of certain other members of this group of chemical reactions is so shadowy that a mere mention of them will suffice. As an antithesis to the vaso-constrictor action of adrenalin, we find that every organ, when active, is supplied with more blood in consequence of a vaso-dilatation of the vessels which supply it. In certain instances Bayliss and I have found that boiled extracts of organs, when injected into the circulation, may evoke vaso-dilatation of the same organs of the animal under investigation, and we have suggested that the normal vaso-dilatation accompanying activity is brought about in consequence of the specific sensibility of the arterial walls to the metabolites of the organ which they supply. Too much stress, however, cannot be laid upon

these experiments, since a more extended series by Swale Vincent has failed to give a general confirmation of our results.

The severe diabetes which, as shown by Minkowski, can be produced in nearly all animals by total excision of the pancreas, has been held to denote the normal production in this organ of some substance which is indispensable for the utilisation of carbohydrates in the body. All efforts to obtain a more exact idea of the nature of this pancreatic substance or influence have so far proved in vain. Ordinary sugar, when placed in contact with extracts of muscular tissues, undergoes oxidation; and Cohnheim states that this process is much accelerated if an extract of pancreas be added to the extract of muscle. A repetition of Cohnheim's experiments by other observers has shown that the effect is so small as to be almost accidental; and we must therefore regard the nature of the pancreatic influence on carbohydrate metabolism and the causation of pancreatic diabetes as problems still to be solved.

So far (except in the case of the muscle—respiratory centre reaction) we have been dealing with isolated phenomena occurring in different parts of the body, in which the reaction affects a whole series of tissues. The chemical stimulus in these cases might be considered analogous to alterations in the composition of the surrounding medium, and the reaction lack that definite and localised character, which we have learnt to regard as distinguishing the adaptations or reflex actions brought about by the intermediation of the central nervous system. We have now to discuss the mechanism of a whole series of chemical reactions where this feature of a nervous reaction is not wanting, so that the reactions, until quite recently, were regarded as undoubtedly nervous in character. I refer to the chain of processes in the alimentary canal by which the secretion of one juice succeeds that of another as the food progresses along this tube. This series of reactions may well form the subject of a separate lecture.

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## LECTURE II.

*Delivered on June 22nd.*

## THE CHEMICAL REFLEXES OF THE ALIMENTARY TRACT.

MR. PRESIDENT AND GENTLEMEN,—With the first appearance of multicellular animals and the setting apart of a distinct layer of cells, the hypoblast, for digestion and assimilation, the activity of the cells is at first largely analogous to that of the previous unicellular organisms, each cell of the alimentary epithelium seizing and ingesting the food particles which reach the cavity of which the cells are a lining. The digestion is therefore at first largely intracellular, and the ingestion of food particles must be determined partly by chemical and partly by tactile or mechanical stimuli arising in the food particles themselves. In the lowest of metazoa, however, cells are found which exercise their influence on external organisms by the production and excretion of poisonous material, and a similar mechanism is soon evolved in the alimentary tract for the production of digestive fluids. An elementary type of digestive apparatus would therefore be a cavity lined with epithelial cells, which are stimulated to the secretion of digestive juices by the chemical and mechanical stimuli arising from food introduced into the cavity. The increase in these digestive cells, necessitated by the greater bulk and greater activity of the animal arising with the elevation in type, is procured in all the higher animals by the formation of outgrowths of cells from the lumen of the alimentary canal, outgrowths which give rise to the structures which we know as glands. The cells composing these glands are no longer subject to direct stimulation by any food present in the alimentary canal. Each act of secretion of digestive juices must therefore involve the transmission of a message of some sort or other from the lining epithelium of the alimentary canal to the gland in question—a transmission which is apparently analogous in all respects to the mechanism for the production of movements in response to external stimulation. In the higher animals all the motor reactions affecting skeletal muscles are carried out by reflex actions and involve the coöperation of the central nervous system. It is natural to suppose that the similar reactions, affecting the chemical activity of glands, should be carried out in the same way, and the earliest investigations of the influence of the nervous system on glandular activity corroborated in all respects this supposition. The taking of food into the mouth, for instance, is at once followed by a flow of saliva, due to an induced activity of the various salivary glands. This reaction was studied by Ludwig in the first half of the last century, and

the experiments of this investigator showed conclusively that the normal path for the message from mucous membrane of mouth to gland lay through the central nervous system. The nerves involved in the reflex secretion of saliva were determined, as well as their central connections. In such a case as this, where all kinds of material—dry, fluid, pleasant, or harmful—may be taken into the mouth, a rapid response of the salivary glands is necessary to provide for the speedy swallowing of the food or to facilitate its expulsion from the mouth. When the food has once undergone a preliminary warming and moistening in the mouth, and has been passed, so to speak, by the critical organs of taste and smell placed at the commencement of the alimentary tract, a rapid reaction of the other glands of the alimentary canal—*i.e.*, one occupying fractions of a second—would not seem to be absolutely necessary. The predominant part, however, played by the central nervous system in all the most direct reactive phenomena of the body led physiologists to search for similar reflex nervous mechanisms of secretion through the rest of the alimentary canal, and the firm faith that such nervous mechanisms existed was in no way damped by the difficulties experienced in the attempts to determine their existence and their paths. Thus for many years the only known method of exciting gastric secretion was the introduction of irritant substances into the stomach, apart, that is to say, from the taking of a meal which would excite in physiological fashion a secretion of gastric juice. In the case of the pancreas Heidenhain observed on a few occasions a scanty secretion from stimulation of the medulla oblongata, but could not obtain this result at will. Succus entericus was obtained by some observers from intestinal fistulæ by mechanical stimulation of the mucous membrane, and the so-called paralytic secretion of succus entericus was produced in certain circumstances in a denervated loop of intestine.

These were the sole indications of any influence of the nervous system on the secretions below the mouth until the subject was taken up by Pawlow at St. Petersburg. This observer realised that the last word could not be said on any physiological question until experiments had been made on an animal in an absolutely physiological condition, *i.e.*, with normal blood pressure, free from fear or pain, and unpoisoned by anæsthetics. To effect this aim Pawlow has enriched physiology with a new technique. By the exercise of scrupulous asepsis, and aided by his great surgical skill, he has succeeded in making observations on the digestive process in animals by providing them with fistulous openings into different parts of the alimentary canal, or with culs-de-sac which were in nervous and vascular continuity with the rest of the canal, and whose activity could be taken as samples of the activity of the whole organ from which they were cut off. The results obtained by this physiologist seem at first to point to an extension throughout the alimentary canal of processes similar to those occurring in the mouth, and to prove that every gland in the canal is excited reflexly through the central nervous system by the presence of the foodstuffs in different stages of digestion at the appropriate part of the lumen of the canal.

Thus the mere sight of food or the taking of pleasant food into the mouth excites reflexly through the vagi a reflex secretion of gastric juice, and this secretion can be also evoked by artificial stimulation of the peripheral end of a cut vagus. The passage of acid chyme, of water, or oil, from the stomach into the duodenum excites the flow of pancreatic juice, the efferent channels, according to Pawlow, for the reflex being both the vagi and the splanchnic nerves, since secretion of pancreatic juice can be evoked by stimulation of either of these nerves. The secretion of succus entericus is less distinctly due to the intervention of a distant nervous system, since it occurs under two conditions—namely, (1) mechanical distension of the gut; and (2) introduction of inert pancreatic juice.

Previously to the publication of Pawlow's results, no physiologist had succeeded in obtaining an invariable secretion of either gastric or pancreatic juice as the result of stimulation of any nerves. A few years after the description by Pawlow of the production of pancreatic secretion by stimulation at some point in the long reflex path, *viz.*, from intestinal mucous membrane, through medulla and vagus or splanchnics to pancreas—it was shown by Wertheimer in France and by Popielski in Russia that the introduction of acid into the duodenum evoked a reflex flow of pancreatic juice, even after division of the vagi and splanchnics or complete destruction of the spinal cord. These observers, therefore, regarded the reaction as belonging to the type of peripheral reflexes, and located the centre for the reflex in the ganglion cells, somewhere in the wall of the intestine or in the pancreas itself. Previous observations by Bayliss and myself had shown that the normal peristaltic movement of the intestine was a peripheral reflex, the paths of which lay wholly within the walls of the alimentary canal; and we were therefore interested to determine the conditions of the similar supposed reflex affecting the glandular activity of the pancreatic outgrowth of the canal. A few experiments sufficed to show that the so-called peripheral reflex secretion of pancreatic juice, evoked by the introduction of acid into the duodenum or upper part of the small intestine, occurred absolutely independently of any nervous channels whatsoever, and that it was possible to isolate the pancreas from the duodenum, to divide all the nerves going to a loop of the small intestine, and then by injection of acid into this loop to evoke secretion of pancreatic juice. Further observations showed us that the reaction, instead of being nervous, was in reality a chemical one. The entry of acid into the duodenum or upper part of the small intestine causes the production in the mucous membrane of a chemical substance which we call secretin. Since, as I shall show later, there are other secretins, we may speak of this as the pancreatic secretin. This pancreatic secretin is rapidly absorbed into the blood and travels with the blood to the gland, the cells of which it excites to secrete.

In order to obtain secretin, the method we have always adopted has been to scrape off the mucous membrane of the duodenum and upper two feet of the small intestine, to pound this up in a mortar with sand with

the addition of 0.4 per cent. hydrochloric acid, and then to boil the mixture over a free flame, the liquid when boiled being neutralised with caustic potash. All the proteids of the decoction are precipitated and, if the mixture be thrown on a filter, a clear fluid filters through. This fluid contains the secretin. In order further to free it from traces of gelatin and proteid, it may be precipitated by absolute alcohol and ether; the secretin is not thrown down, and can be recovered from the alcohol-ether solution by evaporation of the latter. A few cubic centimetres of the original filtrate from the boiled mucous membrane or of a watery extract of the residue left on evaporating the alcohol-ether solution, on injection into the veins of an animal, evoke a plentiful secretion of clear pancreatic juice which can be collected by means of a cannula placed in the duct of the gland. The secretin can be obtained in this way from the upper part of the intestine of any member of the vertebrata, and a solution of secretin obtained from any animal will, on injection into any other animal, evoke a secretion in the latter of pancreatic juice. We have, therefore, in secretin a substance of the same general distribution as adrenalin, which, like this latter, is unaltered by boiling, is soluble in alcohol, and, as we have determined by direct experiments, is more or less diffusible. Although secretin has not been further isolated and we have therefore no clue as to its chemical characters, there can be little doubt that it belongs, like adrenalin, to the drug class of substances which exercise an influence on the physiological workings of the body. The limited seat of production of secretin and the definite response evoked by its injection determine a chemical reflex, which is as adapted to the needs of the organism as are the muscular reflexes carried out by the central nervous system. The acid chyme entering the duodenum excites the formation of secretin in the mucous membrane, which in its turn causes a flow of alkaline pancreatic juice. The formation and absorption of secretin will proceed until the chyme is exactly neutralised by the alkaline juice. As soon as this neutralisation occurs, the pyloric sphincter, which remains firmly closed so long as the duodenal contents are acid, opens and allows the entry of a fresh portion of acid gastric contents which, in their turn, will, through the secretin mechanism, call forth a secretion of an exactly corresponding amount of pancreatic juice. It is thus contrived that the further digestion of the foodstuffs in the small intestine will proceed in a medium which is approximately neutral, and is at any rate free from any trace of mineral acid.

We may next inquire into the manner in which the contact of acid can give rise to the production of secretin in the mucous membrane. It seems most probable that the action of acid is one of hydrolysis, since, although various acids can be used for the preparation of secretin, their efficacy is more or less proportional to their strength or rather to their acidity, a weak acid such as boracic having no influence on the production of secretin. Bayliss and I have suggested therefore that the epithelial cells lining the upper part of the small intestine contain a substance, pro-secretin, from which by hydrolytic agents secretin can be split off. This precursor of secretin is quite insoluble, since we have been

unable to obtain secretin by the action of acids on filtered extracts of the mucous membrane. It is not destroyed by the death of the cell, since secretin may be obtained by the action of acids upon boiled mucous membrane or upon mucous membrane which has been repeatedly extracted with absolute alcohol and then dried. On the other hand, if the process by which secretin is formed is hydrolytic this change must be able to be effected by the living cells in the absence of such strong hydrolytic agents as dilute hydrochloric acid. Thus Fleig has shown that a secretin similar in all respects to that described can be obtained by the action of soaps on the mucous membrane; and there is no doubt that a similar substance is produced in the mucous membrane and absorbed into the blood, when such substances as ether, chloral, or oil of mustard are introduced into the lumen of the gut. It is difficult to imagine that any of these substances can exercise a direct hydrolytic effect; and it is certainly impossible by their means to extract secretin from a mucous membrane which has been already killed.

That the secretin formed in the mucous membrane actually reaches the pancreas by way of the blood stream has been shown by Wertheimer. This observer led the blood from the intestinal veins of one dog (A) into the blood stream of a second dog (B) and found that injection of dilute acid into the intestine of dog A evoked a secretion of pancreatic juice in dog B. Although the view given above as to the origin of secretin is most probably correct, our failure up to the present to isolate pro-secretin in any way from the mucous membrane forbids us to accept the theory as definitely established. Délézenne has found that the digestion of a solution of secretin with a fresh extract of intestinal mucous membrane deprives the secretin of its efficacy. He has therefore advanced the suggestion that secretin is really preformed in the wall of the gut, but is accompanied by another body, its physiological antagonist, which he denotes by the name of anti-secretin. He imagines that the action of acid is to destroy the anti-secretin, thus unmasking the action of the secretin. The fact, however, that secretin, which is extremely soluble both in water and fairly strong alcohol, is not extracted in any appreciable quantity by these fluids from the mucous membrane until acid has been added, is difficult to reconcile with Délézenne's suggestion; moreover, the general characters of secretin, its resistance to proteid-precipitating agents, and so on, point to it as belonging to a class of bodies which would not give rise to antibodies when introduced into the organism.

As to the mechanism by which secretin arouses the activity of the pancreatic cells, the expulsion of its preformed ferment or pro-ferments, together with the building up of new protoplasm and ferments, which always accompanies normal activity, we have little or no conception. Early in our researches the suggestion was made to us by Dr. A. Walker that the secretion of pancreatic juice was of the nature of the formation of an antibody to the secretin. That this explanation will not hold is shown by the fact that secretin is equally efficacious when it is mixed with a large quantity of fresh pancreatic juice. If, however, the pancreatic juice be activated by the addition of succus entericus so that its

trypsinogen is converted into trypsin, it has the power of destroying the secretin. Moreover, as I mentioned in my last lecture, such a localised formation and excretion of an antibody has no analogue among the facts hitherto brought to light by bacteriologists as to the formation of antibodies in general. We are inclined to think that the action of secretin is that of a specific drug—that just as pilocarpin acts on all the glands of the body, including the pancreas, so secretin acts on the pancreas with, perhaps, one or two other glands which are associated with the pancreas in their functions. Its action is not, however, identical with that of pilocarpin. The latter drug induces in the pancreas the secretion of a thick viscid juice containing from 7 to 8 per cent. of solids. Its action is entirely abolished by the injection of a small dose of atropin. On the other hand, secretin produces a juice containing about 3 per cent. of solids, which resembles in every particular the juice obtained from an animal with a pancreatic fistula after it has received food. Its action is not altered in any way by the previous injection of, at any rate, moderate doses of atropin.

The discovery of a chemical reflex, which is sufficient to explain the correlation of activities between the mucous membrane of the small intestine and the pancreas, must cause us to inquire how far these results are to be reconciled with the previous results obtained by Pawlow. According to Pawlow's original idea the reflex secretion of pancreatic juice was entirely nervous. The question now arises whether both mechanisms function in normal circumstances or whether it is possible to explain all Pawlow's results by the chemical mechanism which I have described. Although we cannot at the present time give a definite answer to this question, we are inclined to believe that the chemical mechanism is the only one involved in the secretion of pancreatic juice, and that in all Pawlow's experiments, where secretion was excited by the stimulation of nerves such as the vagus or splanchnics, the effect on the pancreas was really a secondary one, due to movements of the stomach arising as a result of the nerve stimulation, and squeezing some of its acid contents into the first part of the small intestine. Other workers, however, such as Fleig and Wertheimer, believe that both mechanisms are at work—namely, that through the mucous membrane of the intestine the pancreas can be excited to secrete by both nervous and chemical means. We ourselves have never been able by nervous means to obtain secretion of pancreatic juice, provided that we excluded all possibility of entry of acid into the upper part of the small intestine.

The discovery of this simple chemical nexus between alimentary canal and pancreas suggests at once the possibility of other mechanisms of the same description taking part in the complex chain of events involved in the digestion of our foodstuffs. Investigations on this point, carried out partly in my laboratory and partly by independent observers, have shown this belief to be justified, and I propose in my third lecture to deal with the facts at present ascertained which point to a whole chain of such chemical reflexes throughout the alimentary tract.

## LECTURE III.

*Delivered on June 27th.*

## THE CHEMICAL REFLEXES OF THE ALIMENTARY TRACT.

MR. PRESIDENT AND GENTLEMEN,—It is not until the food arrives at the stomach that any use is made by the organism of the more primitive chemical method of adjusting the secretion of the digestive juices to the presence of food at various sections of the canal. We have searched in vain for evidence of the production of a secretin in the mouth or its mucous membrane, as the result of the taking of food into this cavity, which might serve as a stimulus to the salivary glands, and the processes in the first part of the alimentary canal are so closely associated with consciousness and volition that one would expect their complete subordination to the central nervous system. In the stomach, however, the researches of Pawlow have already pointed to the possibility of some chemical mechanism being involved in the secretion of gastric juice. This observer has shown that gastric secretion begins even before the entry of food into the stomach, as a result either of stimulation of the nerves of taste or of the mental reproduction of sensations of smell and taste. This immediate secretion is therefore largely psychical and is so designated by Pawlow. The efferent channel of the nervous process is represented by the vagus nerves, and Pawlow has shown that division of both vagus nerves absolutely prevents this immediate secretion of gastric juice. There can be no doubt that the psychical secretion is the more important factor in the production of gastric juice in normal circumstances. The dependence of normal digestion in the stomach upon the free play of these nervous influences is shown by the indigestion which is a familiar result of mental disturbance. Gastric secretion has, however, two stages. In the first stage there is the rapid secretion due to the psychical stimulation. Two or three hours after the taking of food into the stomach a second rise in the secretion of juice may be observed, and this second rise is apparently dependent, not on the taste of the food or mental condition of the animal, but on the nature of the stomach contents. Pawlow states that this second stage occurs even when both vagus nerves are divided, and the possibility is at once suggested that this stage is not due to nervous processes at all, but is determined by some chemical mechanism similar to that which we have studied in the case of the pancreas. Pawlow is apparently of opinion that the secondary secretion is due to local reflexes in the wall of the viscus, but recent experiments by Edkins have shown that it is unnecessary to invoke the aid of any such obscure mechanism.

The stomach consists functionally of two parts—namely, the fundus and the pyloric end. In the fundus of the stomach are found the glands which secrete an acid juice. In the pyloric end the glands are devoid of oxyntic cells and the mucous membrane is much more closely adherent to the subjacent muscular coats. After a full meal the food forms a mass lying in the fundus of the stomach, the pyloric portion being at first quite empty. The movements, which occur from 20 to 30 minutes after the taking of food, involve only the pyloric half of the stomach, the fundus gradually contracting on its mass of food, so that the portions which are already partially digested are squeezed into the pyloric mill, where thorough admixture of the food with the juices takes place. It seems that such absorption as occurs in the stomach takes place solely at its pyloric end. If, then, there be a gastric secretin, we should expect it to be formed in the cells of the pyloric mucous membrane under the influence of the acid or food passing to this end of the stomach from the fundus. Injection of an extract made by treating pyloric mucous membrane with the products of gastric digestion should provoke a secretion of gastric juice. On such lines Edkins made his observations. Extracts of the pyloric mucous membrane were made by rubbing this up with 5 per cent. dextrin, with dextrose or maltose, or with peptone. A dog's stomach, which had been previously washed out, was filled up with normal salt solution under slight pressure. No absorption of salt solution takes place through the stomach, so that at the end of an hour the salt solution could be recovered unchanged. If, however, the extracts made, as just described, were injected in repeated small doses into the veins of the animal under observation, the salt solution removed from the stomach at the end of an hour was found to contain hydrochloric acid as well as pepsine, showing that secretion had been excited in the stomach. Injections of dextrin, maltose, etc., by themselves were without effect, so that the secretory results must be due to the injection of some substance produced in the pyloric cells under the influence of these digestive products. This substance, which Edkins calls *gastrin*, but which would be better named *gastric secretin*, is produced only from the pyloric mucous membrane, extracts made by rubbing up digestive products with fundus mucous membrane being without influence on the gastric secretion. Edkins has shown that the substance is not destroyed by boiling, so that it evidently belongs to the same class of bodies as the pancreatic secretin described in my last lecture.

It is an old theory, as propounded by Schiff, that certain constituents of the food have a special influence in promoting the secretion of gastric juice. These substances, among which Schiff placed dextrin, were called by him peptogenous, and it seems from Edkins's researches that this term is justified and that our future definition of peptogenous substances will be such bodies as can by their action on the pyloric mucous membrane give rise to the production of gastric secretin.

The movements of the pyloric mill have the effect of squirting into the first part of the duodenum, at intervals of a few minutes, a small

quantity of strongly acid chyme containing the products of gastric digestion of the foodstuffs. The pylorus opens to admit the passage of a few cubic centimetres of chyme at a time, and then closes and remains closed so long as the contents of the duodenum continue to be acid. As soon as the duodenal contents are neutralised, the pylorus opens again and allows the propulsion of a further portion of semi-digested chyme into the duodenum. As we have seen, the effect of this acid chyme is to give rise in the cells of the mucous membrane of the first part of the intestines to secretin which, carried by the blood-vessels to the pancreas, excites a flow of strongly alkaline pancreatic juice. We may take it that ten cubic centimetres of pancreatic juice would suffice to neutralise from 15 to 20 cubic centimetres of the acid chyme. Pancreatic juice, however, although it contains many ferments, is dependent for the full display of its digestive activity on the coöperation of the other juices which are poured into the intestines at the same time—namely, the bile and the succus entericus. Pancreatic juice contains preformed both a fat-splitting ferment and a starch-splitting ferment. The activity of both these ferments is, however, doubled or trebled if bile be simultaneously present. Moreover, the fat-splitting properties of pancreatic juice are of little avail to the organism unless bile salts are also present, which by their solvent power on fatty acids and soaps and by their effect on surface tension can enable the absorption of the products of fat digestion to take place. It is essential, then, that the secretion of bile shall take place at the same time as, and in proportion to, the flow of pancreatic juice. The simultaneous and correlated flow of these two juices is effected by one and the same mechanism. Bayliss and I observed that if a cannula were introduced into the bile duct, the cystic duct having previously been ligatured, intravenous injection of secretin evoked not only the flow of pancreatic juice but also an increased flow of bile. Most extracts of the mucous membrane of the small intestine contain small traces of bile salts which are themselves cholagogue and might, therefore, be responsible for any increase in bile secretion observed as the result of the injection of ordinary extracts of intestinal mucous membrane. The presence of bile salts in our extracts was, however, guarded against, since, in our experiments on the influence of secretin on bile, we used for the preparation of secretin only mucous membrane, which had been previously thoroughly extracted with boiling absolute alcohol and proved by special experiment to contain no trace of bile salts.

Still more important for the full display of the powers of the pancreatic juice is the coöperation of the succus entericus. It was shown in Pawlow's laboratory that the proteolytic effects of pancreatic juice obtained from a pancreatic fistula were enormously increased by the addition of a small trace of intestinal juice, and it was concluded that the pancreatic juice contains, besides a small amount of trypsin, a large amount of trypsinogen. This latter "proferment" has no action on proteids. The succus entericus contains another ferment, called by Pawlow enterokinase, which has the power of converting trypsinogen into trypsin. More lately it has been

shown by Délézenne, as well as by Bayliss and myself, that normal pancreatic juice as secreted contains no trypsin whatsoever. It has, indeed, a feeble proteolytic ferment, which is powerless to digest coagulated proteids or even solid gelatin and only slowly attacks un-boiled fibrin or caseinogen. The proteolytic ferment of fresh pancreatic juice is therefore no stronger than the ferments which can be extracted from almost any tissue of the body. In the presence of succus entericus, however, the pancreatic juice speedily develops a proteolytic power more marked than that of any other proteolytic ferment we are acquainted with. It dissolves proteids, whether or not coagulated, and rapidly carries them through the stages of hydration to their end-products of amino-acids, bases, and so on, effecting in this way a thorough destruction of the proteid molecule. Certain French observers, Délézenne, Dastre, and others, have imagined that the interaction of enterokinase and trypsinogen is of the same nature as the interaction between antibody and complement, which is necessary for the destruction of red blood discs by hæmolytic sera. Experiments by Bayliss and myself have shown that this view is untenable and have confirmed Pawlow's original statement that the activating effect of succus entericus on pancreatic juice is due to the presence of a body which acts like a ferment. Our results have been fully confirmed more recently by Hekma in Holland and by Falloise in Belgium.

Enterokinase is active in such minute quantities that the presence of the minutest trace of mucous membrane in a pancreatic juice will suffice to activate the latter. It is important that the activation of the proteolytic ferment should take place as speedily as possible after the entry of pancreatic juice into the intestine, and there must therefore be some means by which a flow of succus entericus containing enterokinase is evoked in direct proportion to the amount of pancreatic juice entering the intestine. That such a mechanism is present and is of a chemical nature there can be little doubt, although some discrepancy still exists between different observers as to the exact nature of the chemical mechanism. We have commenced to study the subject, but our experiments are not yet far enough advanced to enable us to decide between the rival views. It is necessary to bear in mind that very considerable differences may exist between the mechanism of secretion of succus entericus in the upper and lower parts of the small intestine. It has long been known that there is a gradual increase in the absorbing powers of the intestine as we proceed from its upper to its lower end, and every worker with intestinal fistulæ has noticed an inverse ratio between the secreting powers of the different parts of this tube, secretion being most abundant in the duodenum and least abundant in the lower parts of the ileum. It is possible, therefore, that the various mechanisms described by different observers are really all involved, but that their importance varies according to the part of the canal under investigation. Pawlow, as the result of experiments carried out on dogs with intestinal fistulæ, came to the conclusion that two main factors were involved in the secretion of

succus entericus—namely: (1) the mechanical distension of the intestinal canal; and (2) the presence of pancreatic juice. He found that a flow of succus entericus could be more easily evoked by the introduction of a small amount of fresh pancreatic juice into the intestine than by any other means, and work by his pupils seems to point to the richness of the juice in enterokinase being proportional to the amount of pancreatic juice introduced into the canal. If these observations be correct, they would point to the trypsinogen, or some associated substance in the pancreatic juice, as being itself the chemical stimulant for the glands of the intestinal wall. Whether this chemical irritant acts directly, or whether it reaches the follicles of Lieberkühn by way of the blood stream, is as yet undetermined. Since it is the trypsinogen which needs the presence of enterokinase, it would be natural to assume that this substance itself is the actual stimulant of the intestinal glands.

A somewhat different view of the exciting agent for the intestinal secretion has been put forward by Délézenne. This observer, working on dogs with intestinal fistulæ at various regions of the gut, obtained practically no secretion except from the uppermost section—namely, that including the duodenum. He finds that the secretion of what we may call duodenal juice, which must be succus entericus together with a small amount of the secretion of Brunner's glands, is excited simultaneously with the pancreatic juice and the bile by the injection of secretin into the blood stream. According to this observer, therefore, the secretion of the three coöperating juices in the upper part of the small intestine—namely, bile, pancreatic juice, and succus entericus containing enterokinase—is brought about by one and the same mechanism—namely, the production of secretin in the intestinal mucous membrane under the influence of the entry of the gastric contents. It is, however, only in the upper part of the gut that the chief rôle of the succus entericus can be regarded as adjuvant to the pancreatic juice, and it is only here that the intestinal juice contains any large quantity of enterokinase. Lower down in the gut a secretion of alkaline intestinal juice is still of importance in consequence of its content in (a) sodium carbonate for the neutralisation of the organic acids produced in the changes in foodstuffs; (b) the ferment crepsin which breaks down the products of gastric and pancreatic digestion of proteids, converting albumoses and peptones into the nitrogenous end-products, amino-acids, and nitrogenous bases; and (c) the ferments invertase and maltase which complete the digestion of the carbohydrates of the food. To these two ferments we must, in the case of milk-fed animals, add the ferment lactase, in the absence of which milk sugar is incapable of undergoing assimilation.

According to Frouin the secretion of succus entericus (apparently in the middle part of the small intestine) can be evoked by the intravenous injection of succus entericus itself or by an extract of the intestinal mucous membrane. It is difficult to see the teleological significance of such a mechanism, unless we assume that some constituent of the succus entericus, produced by the mechanical stimulation of the foodstuffs themselves,

is absorbed into the blood and provokes the secretion of more intestinal juice lower down in the canal, in preparation for the reception of the advancing mass of food.

We see that, from the entry of the food into the stomach until its passage through the ileo-cæcal valve, there is a continuous chain of chemical reflexes, and that the process in any section of the alimentary canal calls forth the activity of the digestive apparatus in the immediately following section. In the stomach this chemical mechanism or reflex is associated with, and probably subordinated to, a nervous reflex mechanism. In the rest of the alimentary canal the chemical mechanism seems sufficient to account for the secretion of all the digestive juices which are demanded by the food. Whether there is in addition a chain of nervous processes, the evidence at present before us is not sufficient to decide, though we ourselves have been unable to obtain any satisfactory evidence of their existence. The mechanisms I have described suffice to explain in large measure the adaptation of the digestive processes to variations in the quality of the food supplied. As a rule the more indigestible the foodstuff, the longer will it remain in the stomach; the greater, therefore, will be the secretion of acid gastric juice, which is the stimulus setting free the chain of processes below the pyloric sphincter. Increased secretion of gastric juice will be attended automatically with increased secretion of the other digestive juices.

There is, however, evidence of a more specific adaptation of certain of the digestive juices to the nature of the foodstuffs. Experiments carried out in Pawlow's laboratory have shown that the saliva poured into the mouth varies in consistence and other qualities, according to the nature of the food or other substances introduced into the mouth. Thus introduction of sand into a dog's mouth evokes profuse secretion of watery saliva; meat provokes secretion of thick, viscid saliva; bread of thin saliva, chiefly from the parotid gland, and so on. The same results can be evoked by showing the dog these substances—*i.e.*, by the psychic reproduction of previous actual stimulation of peripheral sense organs.

In the stomach the psychic secretion, which is so important in inaugurating the digestion of the food, is apparently unaffected by alterations in diet, any variations which may be caused being sufficiently explained by the different digestibilities and therefore different length of stay of the foodstuffs in the stomach. In the case of the pancreas, however, according to Walther and Vasilieff, there is an accurate adaptation of the composition of the juice to the nature of the food; the dog that has been on a bread diet secreting more amylopsin, while the dog on a proteid diet secretes a larger amount of trypsin or trypsinogen. These results have been controverted by Popielski, according to whom the composition of pancreatic juice is determined solely by the strength of the stimulus which the pancreas receives. Further experimental work is still required on the subject. If Walther's contentions are supported, it will be interesting to determine whether the adaptation of the pancreatic activity to the nature of the food is nervous in character as imagined by Pawlow, or

whether the mechanism in this case also is chemical. An apparent example of adaptation of the pancreatic activity to the nature of the food was described in 1901 by Weinland, who stated that, whereas the pancreatic juice of an ordinary adult dog contains no lactase and is therefore without effect on milk sugar, it is only necessary to feed the dog for some time, a week or more, with milk or with milk sugar in order to determine the appearance of lactase in the pancreatic juice. These experiments of Weinland were confirmed and amplified more recently by Bainbridge, who endeavoured also to determine the mechanism by which the adaptation was carried out, and especially to decide the question whether it were of a nervous or of a chemical nature.

(More recent work by Bièrry has thrown doubt on this adaptation of the pancreas to lactose. Dr. Plimmer has therefore reinvestigated the whole question, using more accurate methods than either Weinland or Bainbridge, and has proved conclusively that under no circumstances does the pancreas or pancreatic juice contain lactase. I am therefore inclined to accept Popielski's view as to the absence of any qualitative adaptation of the pancreas to the nature of the food—at all events until further experimental evidence has been brought forward for such adaptation. *Note added December 1st, 1905.*)

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## LECTURE IV.

*Delivered on June 29th.*

## THE CHEMICAL CORRELATIONS INVOLVING GROWTH OF ORGANS.

MR. PRESIDENT AND GENTLEMEN,—The chemical adaptations of the body, which I have discussed in my first three lectures, have been almost exclusively in the direction of increased activity of the responding organ. One cannot, however, draw a sharp line between reactions involving increased activity or dissimulation and those which involve increased assimilation or growth, since under physiological circumstances the latter is always the immediate sequence or accompaniment of the former. It is a well-known fact that the best method of producing hypertrophy of any organ is by increase of its physiological activity, and a good example of the coincidence of assimilative with dissimilative changes is afforded by the action of one of the hormones—namely, secretin—on the pancreas. The most obvious result of injection of secretin is secretion of pancreatic juice. If the animal is in bad condition, so that assimilatory changes are, so to speak, handicapped, this increased activity of the pancreas is attended by a total discharge of the zymogen granules, so that after a few hours the gland on microscopic section is a typical discharged gland. The discharge may go so far, as shown by Dale, that the ordinary secreting cells of the gland lose not only their zymogen granules but also the main part of their protoplasm and acquire the aspect and arrangement of the cells which are familiar as forming part of “Langerhans’ islets.” If, however, we are dealing with an animal in good condition and are using a preparation of secretin free from harmful admixtures, such as the depressor substance usually present in extracts of the intestinal mucous membrane, we may succeed in exciting a continuous flow of pancreatic juice for some hours without inducing any change in the aspect of the gland, microscopic sections at the end of four or five hours’ continual secretion presenting the typical aspect of a resting gland. In this case the stimulus exciting to activity has at the same time directly or indirectly called forth a corresponding building up of protoplasm and secretory granules. The division of the chemical adaptations which we have adopted must, therefore, relate solely to the primary effect of the excitation involved. In the cases hitherto studied the primary effect has been katabolic or increased activity. In the cases I shall bring before you to-day the primary effect of the chemical stimulus is anabolic. We must not, however, expect that the anabolic stimulus will diminish permanently

the activity of the responding organ; rather will its ultimate effects be, by building up the responding organ, to increase its activity.

The most familiar example of a chemical correlation evoking the building up of tissues is that presented by the thyroid gland, though the effects of the chemical substance formed by the thyroid are so widespread, and differ to such an extent according to the age of the animal employed, that a physiological analysis of its results is still difficult to give. In the growing animal the chemical substance secreted by the thyroid evidently influences the growth of tissues, among others of the bones, and it is a familiar fact that injection or administration of thyroid to cretins will result in a restoration of the child towards normal, in increased growth of bones, and in development of various functions, including those of the brain and central nervous system. On the other hand, in adults, the pronounced effect of injection of thyroid is increased activity of the chemical changes of the body, as instanced by the increased nitrogenous metabolism, and disappearance of all over-growth in the subcutaneous connective tissue such as is present in myxœdema. Although, therefore, the main result of thyroid treatment is to restore normal growth where such has been previously wanting, it is difficult to say whether its primary effect should be regarded as dissimilative or assimilative. The fact that the thyroid gland can be administered by the mouth shows that the active principle is not destroyed by the gastric juice, and would, therefore, remove this from the proteid class of bodies, and would diminish very largely any probability of the hormone furnished by this gland being of the nature of a toxin. Whether it is represented by the thyroiodin, the organic iodine compound extracted from the gland by Baumann, is still doubtful, and we can only conjecture that in all probability, when isolated, it will be found to belong to the drug class rather than to the toxin class. We are still quite without knowledge as to the conditions which determine the amount of active substance produced in the thyroid gland. All we know is that the activity of the thyroid, like that of the suprarenal gland, is essential to the normal development of the functions of the body. Whether we are dealing here with a constant process, or with a chemical reflex similar to those we have studied in the alimentary canal and evoked by some event affecting directly the thyroid gland, we cannot say.

The largest group of correlations between the activity of one organ and the growth of others is formed by those widespread influences exercised by the generative organs on the body as a whole and on parts of the body. The effects of removal of the testes in the male animal on the growth and disposition of the individual have been known for centuries, and marked additions to our knowledge of this subject have been made recently by Mr. S. G. Shattock. According to Ancel and Bouin, the interstitial cells of the testis provide an internal secretion, in the absence of which the sexual characters of the male are not developed. The experiments of Shattock and Seligmann show that the formation of the so-called secondary sexual characters must be due to chemical influences from the

gland, and not to metabolic changes set up by a nervous reflex arising from the function of sperm ejaculation.

Corresponding results have been obtained in the female by extirpation of the ovaries, double oöphorectomy before puberty not only preventing the onset of puberty and the occurrence of menstruation, but modifying the future growth of the whole body in the direction of the male character. It has been shown recently by Marshall and Jolly, in a paper read before the Royal Society, that the changes in the uterus which determine menstruation are due, not to ovulation, but to an internal secretion arising from the ovary. These observers suggest that the interstitial cells of the ovary may be the seats of manufacture of this internal secretion or hormone; and it is interesting to note that, in a research recently carried out by Miss Lane-Claypon, definite evidence is brought forward of the origin of the interstitial cells of the testis from the germinal epithelium and of the complete equipotentiality of those cells with those which are forming the ova and Graafian follicles. Many observations have been made on the effect of administering by the mouth preparations of ovaries, especially in cases where the morbid symptoms were presumably due to abolition of the ovarian functions, either by disease or in consequence of the operative removal of these organs. Although in many cases favourable results are said to have been attained—results which would appear to prove the resistance of the ovarian hormone to digestion in the alimentary canal—it is difficult in this, as in most clinical evidence, to judge how far the results were due to the treatment or to the expectation of the medical man or patient. More definite evidence of a direct influence of the ovary on the growth of the uterine mucous membrane has been furnished by the experiments of Fraenckel, as well as by those of Marshall and Jolly. At the suggestion of Born, Fraenckel removed the ovaries of rabbits from one to six days after copulation, in order to decide whether the ovary exercised any influence on the growth of the mucous membrane of the uterus and its preparation for the fixation of the ovum. In every case on subsequently killing the animal it was found that the extirpation of the ovaries had prevented the fixation of the ova. On the other hand, if the ovaries were removed on or after the fourteenth day of pregnancy, which in the rabbit lasts about 30 days, the animals went on to full time and healthy fœtuses were produced. The fact that the corpus luteum of pregnancy grows enormously during the first third of pregnancy and then diminishes in size, suggests that this hypertrophy and growth of cells are for the express purpose of influencing the mucous membrane; and Fraenckel states that destruction of the corpora lutea by means of the galvano-cautery is as efficacious as total removal of the ovaries in determining the end of pregnancy. The cells which form the corpora lutea are derived, not from connective tissue cells but from the interstitial cells lying immediately outside the Graafian follicles. Their origin is, therefore, identical with that of the interstitial cells of the ovary—*viz.*, from the primitive germinal epithelium.

These experiments of Fraenckel have been confirmed by Marshall

and Jolly, who conclude that the ovary is an organ providing an internal secretion, which is elaborated by the follicular epithelial cells or by the interstitial cells of the stroma. This secretion circulating in the blood induces menstruation and heat. In animals which have been deprived of their ovaries and in which the phenomena of heat are therefore absent, these phenomena can be reinduced by the injection of ovarian extracts. After ovulation the corpus luteum is formed. This organ provides a further secretion, the function of which is essential for the changes taking place during the attachment and development of the embryo in the first stages of pregnancy.

A still more striking example of growth in response to chemical stimulation from distant organs is afforded by the mammary glands. As is well known, at birth these glands are limited to a few ducts in the immediate neighbourhood of the nipple, continuous with those of the nipple and equal in extent in both sexes. At puberty in the human female there is growth of the breasts associated with some gland-growth, the main increase in size, however, being due to fat. With the occurrence of pregnancy a true hypertrophy of the gland begins at once and continues steadily up to birth. In the rabbit, in which we have studied the changes in the gland, it is extremely difficult to find in the virgin even a trace of mammary gland. The nipple is small and undeveloped and, on making serial sections through the nipple, the gland is found to be confined to a few ducts not extending more than a few millimetres outside the nipple. No trace of secreting alveoli is to be observed. With the occurrence of pregnancy a rapid growth of the gland appears to begin at once. Five days after impregnation, when it is still impossible to find the impregnated ovum with the naked eye in the enlarged uterus, the mammary glands are marked out as small pink patches about two centimetres in diameter just under each nipple. On microscopic section the gland is found to be made up chiefly of ducts, which, however, are undergoing rapid proliferation. The cells lining the ducts are about three deep and present numerous mitotic figures. At about the fourteenth day the whole of the front of the abdomen is covered with a thin layer of mammary tissue. Branching ducts with proliferating epithelium are still the predominant feature on section, but here and there, especially towards the margins of the gland, small secreting alveoli lined with a single layer of epithelium are to be seen. After this time the gland grows with ever-increasing rapidity, so that at birth, at the thirtieth day after impregnation, the mammary glands form a layer about half a centimetre thick over the whole of the abdomen. In the virgin rabbit it is impossible to obtain by expression any fluid from the nipples, but from the fifth to about the twenty-fifth day pinching the nipples results in the expression of a clear, colourless fluid. From the twenty-fifth day onwards this fluid becomes opalescent and during the second and third days immediately preceding birth the fluid obtained is typical milk. The appearance of milk is earlier in multiparous rabbits, and in animals where pregnancies succeed each other rapidly it may be possible to express milk throughout the whole

of pregnancy. In the primiparous rabbit termination of pregnancy at any time after the fifteenth day results in the appearance of milk in the mammary glands, a result which has also been observed in the human female under corresponding conditions. That this onset of lactation is not due to any stimuli, chemical or nervous, received by the mammary glands from the involuting uterus or ovaries is shown by the fact that it may be brought on by performing total extirpation of ovaries and pregnant uterus. The essential feature therefore seems to be in this case the removal of the growing foetuses.

These facts demonstrate the intimate connection between the growth and activity of the mammary glands and the growth of the foetus in utero. Many facts point to a close nervous connexion between the mammary glands and the uterus. I need only instance the production of uterine contractions on putting the child to the breast, and the occurrence of hypertrophy of the breast as a result of abnormal uterine conditions. It is, therefore, only natural that the growth of the mammary glands in pregnancy should have been regarded as determined reflexly through the central nervous system, and the nervous nature of the nexus between the generative organs and the mammary glands is still maintained by some writers, such as von Basch, who, however, locate the centres involved in the ganglia of the sympathetic system. There are many facts which militate against our acceptance of such a view. I need mention only some of the more striking of these. Ribbert transplanted in a guinea-pig a mammary gland to the neighbourhood of one ear. The occurrence of pregnancy in this animal was attended by enlargement of the transplanted gland, from which milk could be expressed at the termination of the pregnancy. A similar experiment was made by Pfister on the rabbit. Goltz and Ewald extirpated in the dog the whole of the lumbo-sacral cord. Pregnancy in this animal was attended by enlargement of the mammary glands, and the bitch suckled its pups normally. Von Basch suggests that in this case the nervous connexion could still be through the sympathetic system. Apart from the fact that there is no such connexion possible in the sympathetic system, which since Langley's researches is no longer the happy hunting-ground for speculative reflexes, von Basch's own experiments tell against such a hypothesis. Von Basch extirpated different portions of the abdominal sympathetic in the dog and rabbit and found that at the next pregnancy the glands showed their usual signs of activity. The only difference he observed between such animals and normal ones was a certain increase in the colostrum corpuscles, an unimportant difference which might have been occasioned by any trivial circumstance. Physiologists are therefore ready to believe that the nexus between generative organs and mammary glands is a chemical one, though opinions differ widely as to the seat of formation or origin of the chemical stimulus.

Pregnancy commences with changes in the ovary—*i.e.*, ovulation. The occurrence of fertilisation involves growth of the ovaries with their corpora lutea, growth of a foetus which soon enters in the mammal into close relationship with the maternal circulation, and growth of the uterine

mucous membrane and muscle. Moreover there is formed, partly from foetal and partly from maternal tissues, an organ of highly complicated structure—the placenta—the express object of which is the nourishment of the growing young animal. It is evident that the chemical stimulus or hormone for the growth of the mammary gland may be manufactured in any of these four organs—the ovary, uterus, placenta, or foetus—and that, in fact, two or more of these might coöperate in producing the effective stimulus for the mammary gland. The experimental solution of the question must therefore be a lengthy one. Even if we were certain of the seat of origin of the specific hormone, we should have to imitate the constant leakage of this substance into the maternal organism and to continue our experiments over a long period in order to produce artificially any growth of the mammary glands. It is impossible, in a short experiment, to prove the presence of a growth-compelling hormone in a fluid, as is so easily done in the case of substances, such as adrenalin or secretin, which increase the functional activity of any given tissue. There are a few clinical facts which, though not decisive, give an indication of the direction in which we may seek solution. Thus, in extra-uterine pregnancy, the growth of the mammary glands occurs as usual, although there is only a relatively slight hypertrophy of the uterus and its mucous membrane. If, under these conditions, death of the foetus occurs, growth of the mammary glands at once ceases, although no definite change has taken place in the uterus. We shall be therefore inclined to locate the origin of the hormone either in the foetus or in the foetal part of the placenta. The influence of the ovary on the growth of the mucous membrane of the uterus and on the attachment of the foetus has been already mentioned. This influence is apparently exerted by the luteal tissue. Since the corpora lutea atrophy during the latter half of pregnancy—*i.e.*, at a time at which the growth of the mammary gland is most rapid, it seems unlikely that the ovary will elaborate the specific hormone in question, though the possibility must be excluded by special experiments. For the past 12 months I have been engaged with Miss Lane-Claypon in an endeavour to determine the origin and nature of the specific stimulus which occasions the growth of the mammary glands during pregnancy. In a preliminary series of experiments one rabbit was injected every day for a fortnight subcutaneously and intraperitoneally with an emulsion prepared by grinding up the ovaries from pregnant rabbits. Another rabbit, also a virgin, received a similar emulsion prepared from the placenta and uterine mucous membrane from a series of pregnant rabbits. Each rabbit received about ten injections from as many pregnant rabbits. In no case, however, was any effect produced on the mammary glands.

These negative results caused us to try the influence of extracts made from the body of the foetus itself. The large size of the foetuses, however, rendered it impossible to adopt the method we had previously been using—*i.e.*, the injection of emulsions. The injection of emulsions of tissues, either subcutaneously or intraperitoneally, is, moreover, fraught with considerable risk of suppuration, even though scrupulous precautions

are taken with regard to asepsis. A large mass of material is introduced which can have no direct bearing on the experiment and in course of absorption must give rise to a large amount of plastic exudation. Proceeding on the hypothesis that the specific stimulus in this case must resemble the other members of the class of hormones in being a body of comparatively small molecular weight, diffusible and not a colloid, we determined in subsequent experiments to inject such constituents of the tissues experimented on as could be extracted by normal salt solution and filtered through a Chamberland filter. In all our later experiments the tissue, whether placenta, ovaries, or fœtus, has been pounded for one hour with sand so as to break up all the cells, and, after the addition of a little salt solution, mixed with Kieselgur; the resultant powder, which is almost dry, is then subjected in a Buchner's press to a pressure of 300 atmospheres. The whole of the fluid of the tissues, containing their soluble constituents, is pressed out. This fluid is sterilised by passage through a Berkefeld filter into a sterilised flask and can then be injected in any quantity either into the peritoneum or subcutaneously without fear of septic trouble.

The first experiment carried out with this improved technique was on a rabbit eight months old which had lived in a cage in the laboratory since it was 14 days old. This animal received 15 injections spread over a period of 17 days. The injections consisted of the fluid parts of the whole of the viscera of 66 fœtuses, varying in age from the fourteenth to the twenty-fifth day. The day after the fifteenth injection the rabbit was killed. It was found that fluid could be expressed from the nipples, which were distinctly enlarged, and on reflecting the skin of the abdomen all the mammary glands were seen to have grown to the size found in a rabbit about eight days pregnant. On microscopic section the gland was seen to consist entirely of proliferating ducts which were lined with an epithelium about three cells deep, presenting numerous mitotic figures. The aspect, in fact, both macroscopic and microscopic, was that of a gland incited to grow by the normal stimulus of pregnancy.

The question then arose whether one could not, by increasing the number of the injections and the duration of the experiment, carry on the hypertrophy of the gland to the point of formation of secreting alveoli. As the subject of our next experiment we had a rabbit which had been in the laboratory four weeks. The general aspect of the rabbit and the undeveloped condition of its nipples showed it to be a virgin. This rabbit received 24 injections, which were spread over five and a half weeks, an interval of ten days intervening at the end of the first week, during which the animal received no injections in consequence of the temporary failure of our supply of pregnant rabbits. During this time the animal received the fluid extract of the viscera of 160 fœtuses. Towards the latter part of the experiment the animal improved considerably in condition, the growth of hair on its abdomen became very active, and the nipples enlarged and yielded a colourless fluid when pressed. On killing the animal the day after the last injection the mammary glands

were found to form a thin sheet extending over almost the whole of the abdomen. Each gland was about five centimetres in diameter and consisted of branched ducts radiating from the nipple. The peripheral zone of the gland was swollen and vascular and on section was found to contain alveoli. Both in the alveoli and in the epithelium of the ducts, which was many-layered, many mitoses were seen, showing that cell proliferation was actively proceeding.

During the last 17 days of this experiment the rest of the bodies of the foetuses after the extraction of the viscera were finely minced and ground up and boiled with normal saline solution. While boiling the mixture was rendered slightly acid to precipitate the proteids and then filtered. The filtered extract was injected intraperitoneally into a rabbit which had been in the laboratory about two months but from the size of the nipples had evidently been previously pregnant. After the injection had been repeated for 10 or 12 days it was found that milk could be obtained by squeezing the nipples. On killing the animal the abdominal wall was covered with a well-marked layer of mammary tissue which contained many alveoli. In some of the ducts there was some indication of proliferation. Both alveoli and ducts contained fluid with fat granules and colostrum corpuscles. Although our failure to obtain a virgin rabbit for this last experiment rendered it impossible to be absolutely certain that the gland tissue had undergone hypertrophy as the result of our injections, the occurrence of secretion and the appearances observed in parts of the gland rendered it probable that we were really dealing in this case with a specified stimulus to the mammary gland and suggested that the specific hormone of the gland would therefore withstand boiling.

In our next series of experiments we employed four rabbits. Two were injected with the extract of the viscera, boiled and unboiled, and two with extracts of the rest of the foetus, also boiled and unboiled. For injection with the unboiled extract of viscera we took a rabbit which we knew to have been previously pregnant. The entire uterus had, however, been removed so as to avoid the possibility of any coöperation on the part of the uterus in the effects of our injections. For the injections of boiled viscera a rabbit was taken which, so far as we could judge, was nulliparous. Of the rest of the foetus the boiled extract was injected into a rabbit which had certainly borne young some time previously. But for the unboiled extract we obtained a rabbit which was nulliparous. Since in this series the foetuses were divided into four parts the injections were smaller than in the previous series. Each rabbit received 22 injections, spread over 31 days, obtained from 90 foetuses. The foetuses varied in age from the twentieth to the twenty-sixth day. The results of this last series were shortly as follows. In both the virgin rabbits there was hypertrophy of the mammary glands, due chiefly to duct proliferation, though not so marked as in the previous series. Towards the end of the injections a watery fluid could be obtained by squeezing the nipples of either of the two rabbits. In the two multiparous rabbits it was impossible to be certain that any actual growth of the gland had taken place.

But in each case from the ninth injection onwards typical milk could be obtained on squeezing the nipples.

During the last 17 days of this experiment two virgin rabbits were obtained and were used for trying the effects once more of placenta, uterine mucous membrane, and ovaries. The first received the fluid extract unboiled of 123 placenta mixed with the fluid extract of the mucous membrane of 14 uteri. The other received 13 injections composed of the fluid extract of 26 pregnant ovaries. In both cases at the end of the experiment the uteri of the animals which had received the injections were found to be somewhat enlarged and congested, but in neither case was there any trace of growth of the mammary glands.

A consideration of all these results brings us to the conclusion that the specific stimulus which determines the growth of the mammary glands in pregnancy is produced in the product of conception, *i.e.*, the fertilised ovum or foetus; that it is contained in all parts of the foetus; that in all probability it withstands boiling; that it passes through a Berkefeld filter, and is not retained to any appreciable extent by the Kieselgur in Buchner's method for the extraction of cell juices. This mammary hormone has apparently a two-fold effect, according as the animal experimented on is multiparous or primiparous. If the animal be a virgin, the effect of its injection is to produce hyperplasia of the gland, beginning, as in normal pregnancy, with proliferation of ducts and later leading to formation of secreting alveoli. In the multiparous animal, where there is already a considerable amount of partly involuted mammary tissue, the most striking result of the injection of this hormone is the production of secretion of milk.

The question arises whether these two results are to be ascribed to two substances or whether they represent phases in the action of one substance. A little consideration will show us that in all probability the latter is the true explanation. It has already been suggested by Hildebrandt that the specific hormone for mammary growth is a substance which inhibits autolytic processes in the gland. It is highly improbable that the process of secretion can be ascribed to autolysis. But the idea of an inhibitory substance as a stimulant to growth is a valuable one and consonant with our ideas of the inhibitory process in general. The stationary condition of any given cell is not the expression of inactivity but is the result of the equilibrium between two sets of processes, one set anabolic or assimilative, causing a building up of the cell, the other set katabolic or dissimilative, causing a breaking down of the cell. During activity of a muscle or gland, dissimilative processes predominate, causing a loss of material. During the subsequent period of rest, there is a swing back in the direction of increased assimilation, resulting in the making good of the loss during activity. The continued application to any tissue of a stimulus to dissimilation will finally result in its complete destruction and death. On the other hand, the continued application of an assimilatory stimulus, if such an expression may be allowed, results in a piling up of the sources of energy of the cell, with actual growth and probably

multiplication of the cells. The final result of this continued building up must be an increase in the functional capacity of the tissue, so that it will be ready to break down—*i.e.*, enter into activity, directly there is any diminution of the assimilatory stimulus. This is what happens in the case of the mammary glands. During the whole of pregnancy the mammary gland is receiving a continual inhibitory or assimilative stimulus from a substance produced in the fertilised ovum or foetus which, diffusing through the placental villi, circulates in the maternal blood-vessels. The result of this negative stimulus is a building up of a gland, proliferation of its cells, and formation of new secretory alveoli. As soon as parturition occurs, the source of the inhibitory stimulus is removed. As the substance which has already obtained entrance in the maternal organism is used up, the cells built up to a high state of activity begin to break down; the dissimilation which is the concomitant of activity in the mammary cells resulting in the production of milk. At any time during the course of pregnancy in an animal we can remove the source of the inhibitory hormone by excision of the foetuses or pregnant uterus. We have found that if extirpation of the pregnant uterus be carried out during the first half of pregnancy—*i.e.*, at a time when the mammary gland consists almost exclusively of proliferating duct tissue—the sole result is to bring about atrophy of the tissue already formed. After about the fifteenth day in the rabbit, extirpation of the pregnant uterus causes the appearance of milk in the gland alveoli, and this milk can be obtained within two days of the operation on squeezing the nipples. In the same way we may explain the secretion of milk which is observed on injecting multiparous rabbits with extract of foetus. The daily injection of a small amount of extract of foetus can be but a poor imitation of the continual leakage of the specific hormone into the maternal blood-vessels, which occurs during pregnancy. We must, in fact, imagine that, during the first few hours after the injection, we are really imitating the condition in pregnancy. The specific hormone will, however, be probably absorbed and used up long before the termination of the 24 hours which elapse between each injection. With the disappearance of the hormone the cells already built up beyond their normal point will tend to break down. There are, in fact, every day a temporary pregnancy and a parturition, and in all cases where there is a glandular epithelium present at the beginning of the experiment—*i.e.*, in all rabbits which have been previously pregnant—we shall have a tendency to formation of milk in the gland during a certain number of hours in every day of the experiment.

We must conclude, therefore, from these experiments that the growth of the mammary glands during pregnancy is due to the assimilatory or inhibitory effects of a specific hormone produced in the body of the foetus and carried thence through the placenta by the foetal and maternal circulations. The removal of this inhibitory stimulus at the end of pregnancy determines the spontaneous break-down of the built-up tissues—*i.e.*, activity which in those cells is expressed by the formation of milk,

Much work still remains to be done in the elucidation of various questions connected with the origin, conditions of formation, and mode of action not only of the mammary hormone but also of the other chemical messengers which I have mentioned in these Lectures. But the facts which I have been allowed to lay before you will, I trust, serve to convince you of the great part played by chemical processes in the coördination and regulation of the different functions of the body. If, as I am inclined to believe, the majority of the organs of the body are regulated in their growth and activity by chemical mechanisms similar to those I have described, an extended knowledge of the hormones and their modes of action cannot fail to render important service in the attainment of that complete control of the bodily functions which is the goal of medical science.

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*An Experimental Enquiry into the Factors which Determine the Growth and Activity of the Mammary Glands.\**

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[PLATE 19.]

The correlation between the mammary glands and the other organs concerned in generation presents perhaps the most striking example of the interdependence of the growth and activities of different organs of the body. Although the manner in which this correlation is brought about has been the subject of speculation for many years, especially among medical men, it is only quite recently that any attempt has been made to apply experimental methods to its explanation.

In the case of the mammary glands we have organs which are present in both sexes, and at birth are in the same immature condition. In both sexes there is frequently, during the few days after birth, an enlargement of the glands and an actual secretion of fluid, which is known as "witch's milk." This state of activity disappears at the end of the first or second week, and till puberty the glands remain in the same undeveloped condition. At this period in Man the first difference appears between the mammary glands of the two sexes, a rapid growth taking place in the female, and accompanying the commencement of the ovarian functions.

During adult life there is apparently at each œstral period a slight and

\* In this investigation the operations and inoculations were carried out by E. H. Starling, and the preparation of the extracts and the microscopic examination of the glands by J. Lane-Claypon,

temporary enlargement of the gland, and during the whole of adult life the glands remain in the female at the same state of development unless pregnancy occurs. The commencement of pregnancy acts as an impetus to a further great development of the gland substance, a development which, as we shall see shortly, differs in some respects from that which gave rise to the growth of the gland at puberty. This hypertrophy continues throughout the whole of pregnancy, being more rapid as pregnancy advances.

With cessation of pregnancy, whether abnormally or at the normal term, the growth of the glands at once comes to a stop, and two or three days after this cessation the activity which previously was spent on growth is now applied to the secretion of milk, a secretion which, if the gland is emptied periodically, lasts for many months.

This whole cycle of changes can be prevented by the removal of the sexual glands, namely, the ovaries, and these organs must therefore be regarded as primarily responsible for the growth of the mammary glands. We shall have to consider later on whether they are the immediate source of the impulses which induce the growth of the gland during pregnancy and its secretory activity after parturition. We must conclude, however, that there is some impulse or impulses arising in the ovaries, uterus, or product of conception, which exerts a direct influence on the mammary glands, and is responsible for their hypertrophy.

What is the nature of this connection? The well-known nervous connections between the mammary glands and the uterus, instanced by the occurrence of uterine contractions on stimulation of the nipples, has been taken to indicate that the impulses which cause growth of the glands are also nervous in character. Although this view is still sustained by certain physiologists, *e.g.*, K. Basch,\* there are many facts, experimental as well as chemical, which place such an explanation out of court. Thus in a goat Eckhard† cut all the nerves to the mammary glands without appreciably affecting the yield of milk from the denervated gland. Ribbert‡ transplanted in the guinea-pig one of the two mammary glands to the neighbourhood of the ear, thus dividing all its nervous connections. In a subsequent pregnancy this gland enlarged as usual, and milk could be obtained from it at the end of pregnancy.

Still more definite evidence is afforded by the well-known case in which Goltz and Ewald§ removed the whole of the lumbo-sacral cord in a pregnant

\* 'v. Ergebnisse der Physiologie,' vol. 2, 1, p. 325, *et seq.*, 1903; also Bibliography.

† 'Eckhard's Beiträge,' vol. 1, p. 18.

‡ 'Archiv f. Entwicklungsmechanik,' 1898.

§ 'Pflüger's Archiv,' vol. 63, p. 385.

bitch, thus severing all possible nervous connections between the pelvic organs and the mammary glands. This dog gave birth to puppies at full term. Pregnancy was attended with the normal hypertrophy of the mammary glands, and the bitch successfully suckled one of the puppies. A similar case has been recorded by Routh.\*

A woman when seven months pregnant was the subject of fracture of the spine at the sixth dorsal vertebra, completely crushing the spinal cord at this level. Parturition occurred two months later, and the woman was able to suckle the child normally.

Moreover we have experimental evidence that the correlating mechanism between the different parts of the generative system is not nervous. Thus Knauer† showed that, whereas extirpation of both ovaries puts an end to the periodical changes in the uterus which are responsible for the phenomena of "heat," both ovaries can be transplanted, thus dividing all their nervous connections, without undergoing complete atrophy and without abolishing the phenomena of "heat." We must conclude therefore that the connecting link in all these cases is chemical rather than nervous. It remains for us to determine the origin of the exciting substance or substances, as well as their mode of action.

In the case of the mammary glands we have really two questions to decide. We have to determine why the secretion of milk appears only at the end or after the termination of pregnancy, and in the second place the origin of the stimulus which, during pregnancy, is responsible for the hypertrophy of the mammary glands.

#### *The Causation of the Secretion of Milk after Parturition.*

In Man the secretion of milk commences two to three days after parturition. During the greater part of pregnancy a fluid can be expressed from the breasts, and this fluid can be obtained in greater quantity after birth; it is not true milk, but a watery fluid rich in proteids, which is called colostrum. In certain recorded cases where pregnant women have suckled during pregnancy, the fluid that has been obtained has also not been true milk, but of the nature of colostrum. In some animals, such as the rabbit, true milk can be obtained from the glands during the last two days of pregnancy. In each case, however, the factor which sets the secretory process going must be the same, and the question at once arises whether the secretion is due to a change in the metabolism of the uterus or ovaries, or to the absorption from the involuting uterus of products which may act as a special stimulus to the hypertrophied gland.

\* 'Obstetrical Society of London Trans.,' vol. 39, 1897.

† 'Archiv f. Gynäkol.,' p. 322, 1900.

Observations on Man show that the secretion of true milk cannot be due to a stimulus received by the gland at the time of conception, since the secretion occurs whenever pregnancy comes to an end, whether at the third month or at the ninth. A good account of the clinical evidence bearing on this point has been given by Halban.\* This observer points out that when premature death of the foetus occurs without its expulsion, as in cases of extra-uterine foetation, the breasts swell two or three days after the death may be presumed to have taken place, and a true secretion of milk may be obtained.

The same thing occurs when the death of the foetus happens *in utero*, but the result is not invariable. Thus Halban mentions certain cases in which the secretion of milk began two or three days after the presumed death of the child, but he also gives details of other cases in which the secretion occurred only after the expulsion of the dead foetus. Halban is inclined to ascribe the difference between these two classes of cases to differences in the placenta, and imagines that the appearance of milk in the breasts is determined, not by the removal or the death of the living foetus, but by the removal or death of the placenta.

The hypothesis that some special stimulating substance is formed in the involuting uterus or in the ovaries after parturition seems to be negatived by certain cases in which Porro's operation was performed without interfering with the subsequent onset of lactation. In order, however, to decide this point we have carried out a number of observations on pregnant rabbits. In these the whole uterus, uterine appendages, and ovaries, were removed at different stages of pregnancy. In the rabbit pregnancy lasts, on the average, 28 to 30 days. If in a primiparous rabbit Porro's operation be performed at the 10th day or at any day before the 14th, the development of the mammary gland at once ceases and gives place to retrogression. No milk, however, appears in the gland, and at the end of a couple of months the gland is little removed from that of a virgin animal. If, on the other hand, the operation be carried out on animals after the 14th day of pregnancy, within two days after the operation milk can be expressed from the nipples, showing that here, as in Man, the effective factor in determining the process of lactation is not the absorption of substances from ovaries or involuting uterus, but the *removal* of certain stimuli which normally proceed from the organs or foetus of the pregnant animal.

The most obvious explanation of these results is that lactation is due to the removal of the stimulus which during pregnancy occasions the hypertrophy of the mammary gland. Hildebrandt† has suggested that during pregnancy

\* 'Archiv f. Gynäkol.,' vol. 72, Heft 2, 1905.

† 'Hofmeister's Beiträge,' vol. 5, p. 413, 1904.

an impulse is exerted by the developing ovum on the mammary glands which acts as a stimulus to growth, and at the same time protects the cells of the gland from those autolytic disintegrative processes which occur to a large extent in the secreting gland. That the act of secretion can be ascribed to autolytic processes of the gland seems highly improbable. We have no evidence that the autolysis of the gland cells would give rise to the specific constituents which characterise milk, but Hildebrandt's idea of an inhibitory substance which excites hypertrophy of the gland, and whose removal leads to secretion, is a valuable one.

Our whole idea of inhibition involves the stopping of the normal dissipative processes in a tissue and the augmentation of the assimilative, so that the continued reception of inhibitory impulses must lead to an actual increase in the substance of the cells, with resulting division and growth of the gland. But, as Hering has pointed out, the more this process of assimilation is carried on, the greater is the tendency to autonomous dissimulation, and the removal at the end of pregnancy of inhibitory impulses, or the attainment towards the end of pregnancy of a maximum degree of hypertrophy, must result in an autonomous dissimulation, *i.e.*, activity of the tissue. The normal activity of the mammary gland is secretion, and it is entirely in accordance with the accepted principles of physiology that the stimulus, which during pregnancy gave rise to growth, should by its removal at the end of pregnancy be responsible for the act of lactation.

We may conclude then that lactation is due not to excitation of the gland by special substances produced in the other generative organs, but to the removal of the stimulus which during pregnancy was responsible for the growth of the gland.

*The Origin of the Substance or Substances by which the Hypertrophy of the Mammary Glands is determined.*

We have seen reason for regarding the development of the mammary glands, which occurs in the female at puberty, as being due to the production of some substance in the ovaries which must reach these glands by way of the blood. It is probable that the slight increase in size of these glands at each oestral period is also determined by the greater activity of the ovaries at these periods, an activity which has been shown by Marshall\* to be probably responsible for the changes which occur in the uterus. These changes are, however, insignificant as compared with the enormous hyperplasia which is associated with pregnancy. The question arises whether the greater growth during pregnancy is to be ascribed to an increased production of the specific

\* 'Phil. Trans.,' B, vol. 198, 1905.

chemical excitant by the ovaries, or to the changed metabolism of the uterus during pregnancy, or whether the new products of conception, namely, the foetus and placenta, represent the seats for the manufacture of the mammary hormone.

There is a large amount of clinical evidence which enables us to decide with some probability, though without certainty, between these various alternatives. Conception is followed by a great increase in the size of the ovaries, chiefly in consequence of the formation of the *corpus luteum*. This growth has been associated by Born, Franke<sup>\*</sup> and Marshall<sup>†</sup> with the production of a specific excitant for the uterine mucous membrane which determines the growth of this membrane and the attachment of the embryo. These observers have shown that extirpation of both ovaries at an early period in pregnancy, or even destruction of the *corpora lutea* by means of the cautery, causes the cessation of the pregnancy. We ourselves have found that extirpation of both ovaries in the rabbit if carried out before the 15th day is apt to cause abortion. Later on in pregnancy oophorectomy can be performed without interfering with the course of the pregnancy.

The growth of the ovaries is, however, chiefly marked in the first third or half of pregnancy. After this time the *corpora lutea* begin to diminish in size, and with them the whole ovaries. On the other hand, the growth of the mammary gland, although marked almost immediately after impregnation, becomes more and more rapid with the advance in pregnancy, and the growth during the last half of pregnancy is many times greater than that during the first half. Moreover, numberless instances in man show that extirpation of both ovaries, as in double ovariectomy, does not necessarily interfere with the course of pregnancy or with the growth of the mammary glands. Labour may come on at the usual time, and be followed by normal lactation.

The fact that the mammary glands undergo normal hypertrophy in extra-uterine foetation, where the growth of the uterus is only a small fraction of that occurring under normal circumstances, seems to point to the products of conception, *i.e.*, either the foetus or placenta, or both, as the seats of origin of the mammary hormone. This conclusion is borne out by the fact that in extra-uterine foetation, if the death of the foetus occurs, this event is followed within a few days by the appearance of milk in the hypertrophied gland. The same result occurs when the death of the foetus happens *in utero* some time before its expulsion. On the other hand, as mentioned in the earlier part of this paper, cases have been recorded in which the death of the foetus

\* 'Arch. f. Gyn.,' vol. 68, 1903.

† *Loc. cit.*

probably occurred a considerable time, a week or more, previous to its expulsion, and yet the appearance of milk in the gland has only taken place two or three days after the actual labour. Halban concludes, though, as it seems to us, on insufficient grounds, that the difference between the two sets of cases depends on whether the death of the placenta accompanies that of the foetus, and locates the seat of production of the specific hormone not in the foetus, but in the chorionic villi and placenta.

It is evident that no satisfactory solution of this question can be arrived at except by experiment. If any of the organs we have mentioned is the seat of production of a hormone which determines the growth of the mammary gland, it should be possible to obtain this hormone by extraction of the organ, and by its injection into a virgin animal produce the changes in the mammary glands which are characteristic of pregnancy. It is on these lines that we have proceeded in the present research, and have chosen rabbits as subjects of experiment since they can be obtained in large numbers, are easily handled, and breed fairly well in confinement. Before describing the experiments themselves we must say a few words as to the normal course of development of the mammary gland in this animal during pregnancy.

#### *The Natural Growth of the Mammary Glands.*

In a virgin rabbit of eight months to a year old, the average age of the rabbits used in our experiments, it is difficult with the naked eye to see any trace of the mammary gland in the tissue lying under the nipples. In order to bring the gland into view we have removed the skin with the subcutaneous tissue, dissected the latter away from the skin, stretched it on a ring of cork, and then, after hardening, stained it in a weak solution of hæmatoxylin. After staining, the subcutaneous muscle fibres were dissected away, leaving only connective tissue, with any mammary gland tissue which might be present, surrounding the nipple. In the spread-out specimen it is possible to see in the immediate neighbourhood of the nipple the ducts which form the mammary gland. As a rule they are limited to an area not more than 1 cm. broad. Plate 19, fig. 1, shows the largest extent of mammary gland we have ever observed in a virgin rabbit of this age. On section the gland is found to consist entirely of ducts, which are lined with a single layer of flattened epithelial cells, and terminate blindly. In no case is there any trace of the alveoli which are so characteristic of the fully-formed gland.

With the occurrence of conception a marked change begins in the gland. Four or five days after conception, when it is still impossible with the naked eye to discover any embryos in the swollen uterine horns, on reflecting the skin from the abdomen each mammary gland appears as a circular pink area,

about 2 to 3 cm. in diameter, surrounding the situation of each nipple (fig. 2). On section the gland is seen to consist still merely of ducts. These however are in an active state of proliferation, throwing out tree-like branches towards the periphery. The epithelial lining of the ducts is two or three cells thick; the cells are much more swollen than in the virgin gland, and numerous mitotic figures are seen in the epithelium. This growth proceeds rapidly, so that about the ninth day, on reflecting the skin from the abdomen, the whole is found to be covered with a thin layer of gland tissue. The margins of the glands are now almost contiguous, and the glands vary in diameter from 5 to 8 cm. (fig. 3). The appearance of the branching ducts in the stained specimen is also somewhat different, the ducts presenting, especially towards the periphery of the gland, cauliflower-like excrescences from their margins. The extreme periphery of the gland is generally somewhat thicker than the rest of the gland. On section it is found, especially at the periphery, that the formation of alveoli is commencing. This formation of alveoli proceeds henceforth rapidly, together with a continued growth of the ducts. At the twenty-fifth day the whole surface of the abdomen is covered with a layer of gland tissue, which may be 0.5 cm. thick, and on section is found to be formed for the greater part of alveoli. About this date, too, fat globules are forming in the cells of the alveoli. In the rabbit pregnancy lasts about 30 days. During the greater part of pregnancy, from the ninth day to the twenty-fifth, a watery fluid can be squeezed from the nipples. During the last two or three days of pregnancy this fluid becomes milky in character, so that immediately after parturition the mammary glands are already full of milk.

#### THE ARTIFICIAL PRODUCTION OF GROWTH IN THE MAMMARY GLANDS.

We may now proceed to an account of the experiments in which we tried to reproduce artificially some of the remarkable changes which are normally determined by pregnancy. It was evident to us, before we began our researches, that it would be difficult, if not impossible, to present any stimulus to the mammary glands which would be as effective as the normal one. For, wherever the mammary hormone is manufactured, the manufacture must be assumed to proceed continuously. There is therefore a constant leakage of the active substance into the blood, and it is probable that the amount of this substance produced increases with the duration of pregnancy. At no time will the mammary gland be set free from the influence of this specific stimulus. On the other hand, however, we might prepare our extracts of the tissues, we could not expect to get more than the amount residual in the tissue and caught, so to speak, in its progress through

the placenta into the maternal blood-vessels. This amount we might inject into our rabbits, but it would probably be taken up and absorbed into the circulation of the rabbit long before we were ready for our next injection, so that, whereas under normal circumstances the mammary glands are being continuously stimulated to hyperplasia, we could not expect to do more than give these glands a series of small shoves in the same direction.

#### *Methods of Experiment.*

Our object in the present research was to attempt to imitate the processes of pregnancy, by the injection of fluid extracts containing the soluble constituents of the various tissues, ovaries, uterus, placenta, or fœtus into a virgin rabbit. The extracts were prepared in various ways. In some of our earlier experiments an emulsion of the tissue in normal salt solution, prepared with aseptic precautions, was injected subcutaneously. This method limited us to the use of only small portions of tissue. Since the other hormones with which we are acquainted, such as secretin or adrenalin, are soluble and diffusible substances, in later experiments we used a much greater mass of tissue for the preparation of fluid extracts, which were made sterile and freed from solid particles by passage through a Berkefeld filter. In all cases the tissues were ground up thoroughly with sand. The resulting paste was then in some cases mixed with normal salt solution, allowed to stand for two or three hours, and centrifuged. The supernatant fluid was passed through a Berkefeld filter, and the resulting clear filtrate used for injection. In other cases the ground-up tissues were mixed with Kieselguhr, and the juices expressed in a Büchner press; the press-juice thus obtained was passed through a Berkefeld filter before injection. The injections—which were naturally made with aseptic precautions—were in the early experiments subcutaneous. As, however, we increased the bulk of the injection, we adopted the plan of injecting into the peritoneal cavity. A further advantage of injecting into the peritoneal cavity is that one not only obtains quicker absorption, but is free from the disadvantage attendant on subcutaneous injection, namely that the fluid introduced under the skin of the back tends to flow down to the subcutaneous tissue in the abdomen, so that the mammary glands are bathed in an aseptic fluid containing proteids, which might in itself serve as a stimulus to proliferation.

#### *Experiments on Injection of Tissue Extracts.*

Our experiments can be divided into the following groups according to the organs injected, namely injection of ovaries, of uterine wall or mucous membrane, of placenta, of placenta plus uterus, of fœtus, together with

placenta and membranes, or of the fœtus alone. Finally, we made certain control experiments in which the animals were injected either with emulsion of liver or with blood serum obtained from normal animals.

### I.—Ovaries.

*Experiment 1.* April 22, 1904.—Two ovaries from rabbit 15 days pregnant were implanted into peritoneum of second non-pregnant rabbit.

May 3.—Rabbit killed. No change in mammary glands; the implanted ovaries were necrosed and vascularised; uterus and vulva congested. On microscopic examination there was no proliferation of mammary gland. The uterine mucous membrane was typical of "heat."

The rabbit from which the ovaries were taken aborted immediately after the operation.

*Experiment 2.* May 31, 1904.—A rabbit which had been in the laboratory since January was injected with chloroform water extract of six ovaries from three rabbits, each about the fifteenth day of pregnancy. The rabbit received three injections in the course of nine days.

It was killed on June 13, four days after the last injection. No changes were found in the mammary glands, and there were no changes in the uterus.

*Experiment 3.* June 6, 1904.—A virgin rabbit was injected on six occasions during 19 days with an aseptic emulsion of 10 ovaries from rabbits between 11 and 14 days pregnant.

June 20.—Killed. Mammary glands invisible, uterus enlarged and congested, with proliferation of mucous membrane.

*Experiment 4.* May 16, 1904. Virgin rabbit received 13 injections of the saline extract of 26 ovaries between May 16 and June 1.

June 1.—Killed. The uterus slightly congested. No effect on mammary glands.

### II.—Uterus and Uterine Mucous Membrane.

*Experiment 5.* May 30, 1904.—Virgin rabbit, about six months old, injected with chloroform water extract of the mucous membrane of four pregnant uteri. During 14 days it received four injections.

It was killed on June 20. No changes, either of mammary glands or of uterus.

*Experiment 6.* October 12, 1904.—Two virgin rabbits received during a period of 19 days seven injections of the filtered saline extract of the mucous membrane of seven pregnant uteri. One rabbit (A) received the boiled extract, the other rabbit (B) received the unboiled extract. Rabbit A was killed on October 31. The uterus and ovaries were congested (the animal was in heat), but there was no growth of the mammary glands.

Rabbit B, killed on November 2. The mammary glands showed no change, but the uterus in this one was also congested.

Two other experiments were made on the injection of uterine mucous membrane, but with no result.

### III.—Injection of Placenta.

*Experiment 7.* May 15, 1904.—A virgin rabbit received the unboiled extracts (press juice) of the mucous membrane of 14 pregnant uteri and 123 placenta. These were given in 14 injections between May 15 and June 1. It was killed on June 2. The mammary glands were unaltered. The uterus was congested.

*Experiment 8.* October 12, 1904.—Two virgin rabbits, A and B.

A received on eight occasions during 19 days the boiled extract of 55 placenta. It was killed on October 31. No results either on uterus or mammary glands.



FIG. 1.

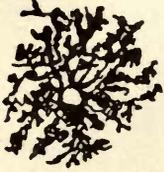


FIG. 2.



FIG. 3.

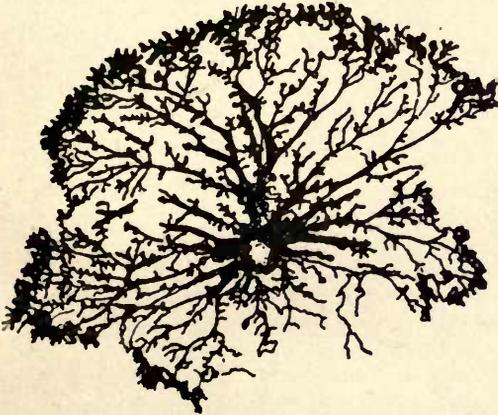


FIG. 4.

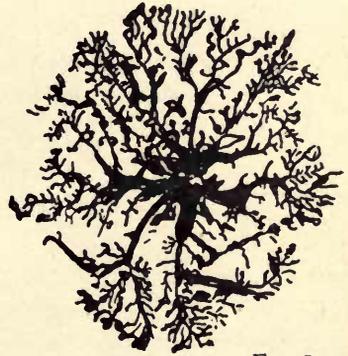


FIG. 5.



FIG. 6.

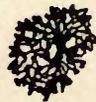
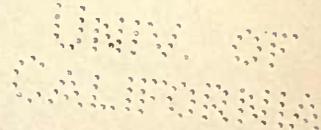


FIG. 7.





B received the unboiled extract of 55 placenta. It was killed on November 2. Mammary glands unchanged ; uterus slightly congested.

IV.—*Injection of Fœtus with or without Placenta and Uterine Mucous Membrane.*

The first three experiments on this point were also fruitless so far as regards any change in the mammary glands. In these cases the rabbits received from five to seven injections of extract of fetus. The following experiments, therefore, were conducted for a longer period of time, and a much larger number of injections were given, and in these positive results were obtained.

*Experiment 9.*—February 8, 1905.—A rabbit, eight months old, which had been in the laboratory since it was a fortnight old—virgin—received, *subcutaneously*, extracts made by rubbing up fœtuses and their placenta with sand, extracting with normal saline, centrifuging, and filtering through a Berkefeld. The injections were made as follows :—

February	8.	—	Half the extract of 7 fœtuses at the 20th day.
"	9.	—	" " " 7 " 20th "
"	10.	—	4 fœtuses at the 22nd day.
"	11.	—	5 " 14th "
"	13.	—	4 " 14th "
"	14.	—	3 " 22nd "
"	15.	—	4 " 20th "
"	16.	—	Half the extract of 8 fœtuses at the 20th day.
"	17.	—	" " " 8 " 20th "
"	18.	—	6 fœtuses at the 19th day.
"	20.	—	5 " 14th "
"	21.	—	Half extract of viscera of 6 fœtuses at the 25th day.
"	22.	—	" " " 6 " 25th "
"	23.	—	Viscera of 7 fœtuses at the 20th day.
"	24.	—	" 4 " 24th "

The rabbit was killed on February 25. There was marked hypertrophy of the mammary glands, which were surrounded with a certain amount of fluid remaining from the subcutaneous injections. The ovaries were large, the uterus congested, and the vulva showed signs of heat. On microscopic examination there was found to be marked hypertrophy of ducts, which were branching and lined with many layers of cells. The mitotic figures were numerous. Specimens of the mammary glands from this rabbit were shown at the meeting of the Physiological Society at University College in March, 1905.

*Experiment 10.* February 27, 1905.—Intraperitoneal injection of press-juice of the viscera of fœtuses, together with placenta and uterine mucous membrane.

A virgin rabbit received the following injections :—

February	27.	—	8 fœtuses, etc., at 24th day.
"	28.	—	Half of 9 fœtuses, etc., at 24th day.
March	1.	—	" 9 " 24th "
"	2.	—	8 fœtuses, etc., at 25th day.
"	3.	—	8 " "
"	4.	—	2 " "
"	6.	—	6 " "
"	7.	—	4 " "

Interval of 10 days, owing to failure of supply of pregnant animals.

March	17.—	7 fetuses, etc.,	at 16th day.
„	21.—	5	„ 26th „
„	23.—	8	„ 22nd „
„	24.—	14	„ 14th „
„	25.—	12	„ 20th „
„	27.—	5	„ 20th „
„	28.—	8	„ 22nd „
„	29.—	5	„ 21st „
„	30.—	7	„ 17th „
„	31.—	4	„ 20th „
April	1.—	9	„ 13th „
„	3.—	9	„ 18th „
„	5.—	5	„ 15th „
„	6.—	11	„ 16th „
„	7.—	6	„ 18th „

The rabbit, therefore, received the fluid extracts of the viscera of 160 fetuses. It was killed on April 8. On reflecting the skin of the abdomen, the mammary glands were seen to be markedly hypertrophied. The margins were almost contiguous, and were somewhat raised and pink, presenting, therefore, much the appearance which is seen in a pregnant rabbit of the eighth or ninth day. The general aspect of the stained gland is shown in fig. 4. On microscopic section not only was there marked duct proliferation with mitotic figures, but at the thickened border the formation of alveoli was just commencing. The whole of these injections had been made intra-peritoneally, or, on one or two occasions, under the skin of the legs, so that there was no infiltration of the connective tissue surrounding the mammary glands. This is the best result which we obtained.

*Experiment 11.* May 2 to June 1, 1905.—In this experiment we sought to determine whether the growth-producing substance is contained chiefly in the viscera or in the body, *i.e.*, muscles, skin, and bones, of the fœtus, and, moreover, whether it could be extracted from these tissues by boiling. Unfortunately, however, out of the four rabbits which we chose for this experiment, only two were definitely virgin, so that the results in the other two cases were equivocal.

Rabbit 1, not a virgin, received the pressed juice of the viscera.

Rabbit 2, a virgin, received the filtered boiled extract of viscera.

Rabbit 3, also a virgin, received the pressed juice of the bodies of the fetuses unboiled.

Rabbit 4, which was evidently multiparous, received the boiled extract of the bodies of fetuses. From this rabbit, at the commencement of the experiment, a small portion of mammary gland was taken as a control.

All four rabbits received portions of 182 fetuses of all ages between May 2 and May 31.

From Rabbits 1 and 4 milky fluid could be expressed from the nipples after the ninth injection.

In Rabbit 2 a watery fluid could be expressed from the nipples after the seventeenth injection.

Rabbit 3 showed traces of watery secretion after the twelfth injection.

All four were killed on June 1. Results were as follows:—

*Rabbit 1.*—Multiparous. Mammary glands well developed and showing many alveoli on microscopic section. The ducts were full of milk; they were, however, lined with only a single layer of epithelium, and it was impossible to say that any hypertrophy had taken place.

*Rabbit 2.*—Virgin. The mammary glands did not present much enlargement as judged from inspection. On microscopic examination, however, many branching ducts were

observed lined with two layers of cells, presenting the same appearance, but in a smaller degree, as those in the glands of the rabbit in Experiment 10.

Rabbit 3.—Virgin. Glands large, hypertrophied, containing a fair amount of watery fluid. Alveoli present and ducts showing proliferation.

Rabbit 4.—Not virgin. Mammary glands fully marked, and distended with milky fluid, but impossible to determine whether or not hypertrophied.

In order to be certain of the induction of growth in the mammary gland by the injection of extracts of fœtus, three more experiments were made. In the first of these, in which the rabbit received 16 injections of the pressed juice of the viscera of 138 fœtuses, the results were absolutely negative. In this experiment, however, we had been obtaining very small amounts of pressed juice from the tissues, and we thought that the absence of result might possibly be due either to retention of the active substance by the Kieselguhr or to insufficient destruction of the cells in the process of grinding. It is possible, too, that immaturity of the rabbit may have been in some measure responsible for the negative result.

In the next two experiments, therefore, we abandoned the Büchner method and, after grinding with sand and with normal salt solution, centrifuged and filtered the supernatant liquid through a Berkefeld candle before injection. Both these experiments gave positive results.

*Experiment 12.*—October 4 to 21. Virgin rabbit, full-grown. Received daily, intraperitoneally, the saline extract of the viscera of a number of fœtuses about the fifteenth to twentieth day of pregnancy. Killed on the 21st. It showed distinct growth of the mammary glands with duct proliferation (*vide* fig. 5).

*Experiment 13.*—October 4 to 21. Virgin rabbit. Received the saline extract, intraperitoneally, of the bodies and placentæ of the same fœtuses used in Experiment 12. Fifteen injections were given in the 17 days. Killed on the 21st. It showed marked growth of mammary glands with plentiful mitotic figures. The appearance of this gland in the stained specimen is shown in fig. 6.

#### *Discussion of Results.*

From the results just described, it will be seen that in six cases we succeeded in producing in virgin rabbits a growth of mammary glands similar to that occurring during the early stages of pregnancy, and consisting in the proliferation of the epithelium lining the ducts, with the multiplication of these ducts by branching into the surrounding tissues. In one of these (Experiment 10) where our injections were carried out during five weeks and the experiment lasted nearly seven weeks, there was an actual formation towards the periphery of the gland of secreting acini. In some of these cases, however, namely those in which the injections had been given under the skin of the back (*e.g.*, Experiment 9), the mammary glands were bathed for considerable periods of time in the injection, and it seemed to us possible that this might be a determining factor in producing growth.

We therefore carried out a control experiment on a virgin rabbit, in which normal rabbit's serum was injected, for the most part subcutaneously, for a period of three weeks. The serum, which was derived from non-pregnant animals, but contained much more nutrient material, *e.g.*, proteid, than the

fluids used in our previous injections, ran down in the subcutaneous tissue, so that during the whole duration of the experiment the abdominal wall was thickened and œdematous through the presence of the serum. On killing the animal at the end of three weeks the glands were little, if any, larger than those usually obtained from a virgin animal (fig. 7). On section, however, mitoses were present in the epithelium of the ducts, and there was apparently a certain amount of proliferation of the ducts.

We must conclude, therefore, that superabundant supply of nutrient material in the fluid surrounding the acini may lead to proliferation resembling in kind that which was produced by our injections. This result had not been produced in an earlier control experiment, in which we injected the saline extract of liver, and in view of the small results produced by the injection of the serum as compared with those produced by the injection of the extracts of fœtus much poorer in proteids, we are inclined to believe that it is impossible to explain our results in the other experiments as due to the infiltration of the tissues round the glands. This explanation, at any rate, could not hold for the growth in Experiment 10, in which there had been at no time any injection into the subcutaneous tissue of the back. It is interesting, from the general pathological point of view, to note that typical epithelial proliferation in the ducts can be produced by an abnormally large supply of proteid in their surrounding lymph, and the subject is worthy of further investigation.

A striking fact in all our experiments with a positive result is the smallness of the growth produced as compared with the quantity of material used for injection. In all the positive cases the material for injection was derived from fœtuses; in Experiments 11 (2) and 12 from the viscera only; in Experiment 11 (3) from the bodies only; in Experiments 9 and 13 from the fœtuses together with placenta; and in Experiment 10 from the fœtuses, placenta, and mucous membrane of uterus together. On the other hand, injection of extracts made from ovaries, uterus, or placenta alone had no effect on the growth of the gland. We are therefore justified in concluding that under normal circumstances the hormone which is responsible for the growth of the mammary gland during pregnancy is produced mainly in the growing embryo. This hormone, however, must be produced in minimal quantities. It is apparently not stored up in any of the tissues of the fœtus or of the placenta, so that, in injecting extracts of fœtus, we are simply injecting the small amount of material which is diffused through the juices on its way to the blood-vessels and into the maternal blood.

It is possible, of course, that the specific mammary hormone is produced from a precursor or mother-substance in some organ or other, and that future

research may reveal some method of splitting off the hormone in large quantities, and also of determining whether its production is diffused throughout all the tissues or is confined to one special organ of the body. Injection of extract of duodenal mucous membrane, for example, would give only minimal effects on the pancreas. We should not be justified in concluding from this absence of result that the duodenum was not the seat of origin of the chemical stimulus to the pancreas. Its peculiar relation to the pancreas is only brought into prominence when it is treated with acid, so as to liberate the secretin from its mother substance.

Our experiments, therefore, throw no light on the seat of production of the hormone in the foetus. Apparently the extent of the growth obtained is a function of the quantity of tissue used in preparing the extracts. The widespread occurrence of the substance in the body of the foetus points to its being extremely diffusible, as indeed we should expect from analogy with other hormones.

We can only say, therefore, that the hormone is produced by some or all the tissues of the fertilised ovum, whence it is carried off by the blood to the placenta, and so makes its way by diffusion into the maternal blood-vessels. Whether it is identical with the substances which are responsible for the production of the other changes associated with pregnancy, or whether there are distinct substances acting on each organ which is modified during this condition, our experiments do not show. But we have evidence that in the foetus itself the hormone or hormones of pregnancy have the same result as in the maternal organism. Thus there is increased growth of the mammary glands in the foetus during the last month of pregnancy, and also in the female an increase in the uterine mucous membrane, as has been shown by Halban. After birth the mammary glands may begin to secrete just as after pregnancy, and there are changes in the uterine mucous membrane similar to those associated with menstruation.

Are we to regard, then, the foetus as the only source of this hormone? The facts mentioned at the beginning of this paper show that such a conclusion is impossible. The growth of the mammary glands which occurs at puberty can only be ascribed to ovarian influence, and is absent if the ovaries have been previously removed, and Halban ascribes to this ovarian substance both the growth of the mucous membrane during each pro-œstrus and the swelling of the glands at each œstral period, which may in rare cases be attended or followed by the actual formation of milk. Halban explains in the same way those cases recorded by Heape and Kehrer, in which bitches, which had not been impregnated at the normal time, have, after two months, not only made a bed for their young, but have had swelling of the mammary

glands, with, in some cases, actual secretion of milk. He would regard this condition as being a continuance of the state of pro-œstrus leading to continued growth of mucous membrane and also of the mammary gland. When the impregnation was no longer possible, with the discharge of the ovum, the secretion of this substance ceased, and the absence of the inhibitory stimulus caused break-down of the uterine mucous membrane as well as dissimilative activity of the mammary gland.

During sexual life, therefore, the ovaries are continually producing a substance which exerts an influence on both glands and uterus. With the occurrence of conception there is at once a great growth of what we may call germinal material. With the growth of the fertilised ovum the amount of hormone produced in the ovum must also increase in proportion. In the early stages of pregnancy the chief source of this hormone may perhaps be located in the chorionic villi, but with the growth of the body of the fœtus this latter must take a preponderating share in the preparation of the hormone. We have no reason to suppose that the fœtal elements of the placenta entirely lose this function of the germinal cells, but the negative results of injection of placenta in our experiments show that it is impossible to ascribe to the placenta, as is done by Halban, a preponderating part in the preparation of this hormone.

If the hormone is produced in the body of the fœtus, it might be objected that the formation should go on after birth, and therefore lead in the newborn animal to a continuance of the growth both of the mammary gland and of uterus. The profound changes in the environment of the new animal which occur at birth must, however, induce equally profound changes in its metabolism, and there is no difficulty in imagining that with the assumption of extra-uterine life the formation of this substance in the fœtus comes to an end.\*

The occurrence of growth in the mammary gland of a virgin rabbit and of secretion in the mammary gland of a multiparous rabbit from the injection of boiled extracts of fœtus, seems to indicate that the specific hormone, like adrenalin or secretin, is not destroyed by boiling. Further evidence,

\* In the ornithorhynchus pregnancy is associated with the growth of mammary glands, although the embryo in this animal is contained in an egg, and does not enter into any anatomical connection with the uterine wall. Halban points out, however, that the shell of the egg is porous, and that during its stay in the uterine cavity it increases in size, and the contained embryo grows, in consequence of the absorption of nutrient material from the fluid contained in the uterine cavity. If the embryo is able to absorb nutrient material from the uterine contents, it is equally able to give up to these contents diffusible substances, which may be taken up by the mucous membrane and carried by the circulation to the mammary glands. The condition in the ornithorhynchus cannot therefore be regarded as a disproof of the chemical theory which we have adopted throughout this investigation.

however, is required on this point, as also on the question whether the substance is specific to the animal, or whether the injection of extracts of the fetus of one animal would produce a growth of the mammary glands in another species. One experiment, in which we fed a kitten for three weeks on the fetuses of rabbits, was negative in its results. This might, however, have been due to the failure of the intestine to absorb the hormone without destruction, or to the failure of the immature glands to react to the minute stimulus which they received. So far as we know, secretin is not absorbed into the circulation when introduced into the stomach or intestine, and colossal doses of adrenalin have to be given by the mouth in order to produce any systemic effects.

The effect of the injections of foetal extracts on multiparous rabbits deserves some further mention. The multiparous rabbit differs from a virgin rabbit in possessing ready-formed alveoli, *i.e.*, secretory structures. On the theory which we have adopted, the circulation of the mammary hormone should diminish any secretion in these alveoli and should cause growth. In all our experiments at least 24 hours elapsed between each two injections. It is probable that the hormone was rapidly absorbed from the injection, and was therefore present in the blood of the animal only for a certain fraction, say a few hours, out of the 24. While it was circulating it should cause building up of the secreting cells. Directly, however, it ceased to circulate, the cells would enter into dissimilative activity resulting in secretion. By our injections, therefore, we are not able to imitate the continuous stimulus of pregnancy. We are rather producing each day a pregnancy of a few hours followed by a parturition. These factors should therefore result in the production of milk in any animals possessing the structures (*i.e.*, the alveoli), which are capable of secreting milk, and would therefore account for the secretion of milk observed by us in all the cases where multiparous rabbits were the object of our experiment.

#### CONCLUSIONS.

So far as our experiments go, they show that the growth of the mammary glands during pregnancy is due to the action of a specific chemical stimulus produced in the fertilised ovum. The amount of this substance increases with the growth of the fetus, and is therefore largest during the latter half of pregnancy. Lactation is due to the removal of this substance, which must therefore be regarded as exerting an inhibitory influence on the gland cells, hindering their secretory activity and furthering their growth. It is probable that the specific substance is diffusible, and will withstand the boiling temperature.

We cannot, however, claim that these conclusions of ours are firmly established. A final decision can only be given by a research carried on under more favourable conditions. One requires, in fact, a farm, where we could have at our disposal 500 rabbits, and could arrange for a plentiful supply each day of rabbits about the middle of pregnancy. Under these conditions it might be possible to determine both the seat and nature of the effective stimulus, as well as to test the influence of various reagents in splitting off the hormone from some possible precursor. Many of our experiments, carried out in a London laboratory, were brought to a premature conclusion by failure of material. If, however, the conception of the action of the mammary hormone, which was put forward by Hildebrandt and adopted by us, is correct, namely, that it is a substance which produces growth by inhibiting the normal activity of the gland cell, it should be possible to decide many questions affecting it by working on an animal, such as the goat, in lactation. Injection of the hormone should diminish or stop the secretion of milk while it was circulating in the blood, but should, as a secondary effect, produce an increased secretion as a reaction from the immediate assimilatory effect. The injection might, indeed, have to be prolonged for one or two days, since we know that in Man the onset of a renewed pregnancy during lactation stops the flow of milk only after some time (three or four weeks). At any rate, such experiments could be more rapidly carried out than those which have been the subject of this communication.

#### DESCRIPTION OF PLATE.

The drawings were made as follows:—The mammary glands were dissected out, pinned on corked rings, hardened in corrosive sublimate and formol, washed, and stained in very dilute hæmatoxylin. They were then dehydrated, cleared, and mounted as lantern slides in canada balsam between glass plates. (These specimens were shown by projection at the meeting of the Royal Society, on March 1, 1906.) An image of the specimens was thrown (without magnification) on to a piece of millboard, and the darkly stained glands were traced out in indian ink. The figures, therefore, reproduce the glands in natural size.

Fig. 1.—Gland from virgin rabbit.

- „ 2.—Mammary gland from primiparous rabbit, five days after impregnation.
- „ 3.—Mammary gland from primiparous rabbit, nine days after impregnation.
- „ 4.—Mammary gland from virgin rabbit which had received injections of extracts of fetuses, uterus, and placenta during five weeks (Exp. 10).
- „ 5.—Mammary gland of virgin rabbit, showing growth produced by injection of extracts of foetal viscera during a period of 17 days.
- „ 6.—Mammary gland of virgin rabbit, showing growth produced by injection of extracts of foetal bodies and placenta over 17 days.
- 7.—Mammary gland of virgin rabbit, showing slight growth induced by daily subcutaneous injection of rabbit's serum (from non-pregnant rabbits) during a period of three weeks.

*On the Origin and Life History of the Interstitial Cells of the Ovary in the Rabbit.*

By JANET E. LANE-CLAYPON.

(From the Physiological Laboratory, University College. Communicated by Professor E. H. Starling, F.R.S. Received June 16, 1905.)

[PLATE 1.]

The majority of the investigators of the subject consider that the cells of the germinal epithelium arise by differentiation of the peritoneum, and become embedded in the subjacent mesoblast, there being probably a dual process, namely, the downgrowth of the cells themselves and a simultaneous upgrowth of the subjacent mesoblast.

The fate of the cells thus embedded has given rise to much discussion. All observers agree in stating that they give rise to the ova, and most observers consider that they give rise also to the follicle cells; but de Foulis (8),\* Schrön (16), and Wendeler (20), believe these cells to be derived from the connective tissue.

Pflüger (15) and Waldeyer (18), although differing in regard to the development of the ovary, both consider that the germinal cells give rise to the cells of the follicular epithelium, there being most probably a previous division of the original cells.

Nagel (14) also agrees that the follicle cells are derived from the germinal epithelium. Balfour (4) believed that some of the cells of the egg-clusters became ova by differentiation, and he described besides a number of small cells, of which some formed the follicular epithelium and the others probably either served as foodstuff for the rest, or eventually themselves formed ova or follicle cells.

Bühler (7) describes the formation of the follicular epithelium by the

\* These numbers refer to the entries in the bibliography at end.

streaming inwards from the periphery of some of the cells of the germinal epithelium.

The changes connected with ovogenesis have been very fully described by v. Winiwarter (19). They may be briefly summarised as follows. The germinal cells of the second invagination are rather small and show a nucleus with some lumps of chromatin, being also rather granular. These he calls *protobroque* cells of the *a* variety. These divide, giving rise to other protobroque cells *a*, and also to a *b* variety. These last divide again giving rise to more cells of the *b* variety and to a new form of cell, *deutobroque*. The last are larger, and the nucleus more transparent. The deutobroque cells give rise to the ova by nuclear differentiation by means of the following stages. 1. The chromatin breaks up into fine filaments, which are distributed over the whole nuclear area; this is the *leptotenic* stage. 2. The filaments become gradually massed together until they show as a compact lump at one side of the nuclear area. This transformation is the *synaptenic* stage, which is succeeded by 3, the *pachytenic*. Here the filaments become again spread out, but they are much coarser than in the previous stages. The 4th stage, or *diplotenic*, is so called on account of the tendency of the chromatin strands to lie in pairs. In the final or *dictyate* condition the chromatin is distributed in a reticulum over the greater part of the nuclear area.

Balfour describes protoplasmic masses of young ova where the cells appear fused, and he suggests that one of these ova may grow at the expense of the rest. Van Beneden (5) describes multinucleated masses in the ovary of the adult bat, which he suggests may give rise to an ovum and its follicular epithelium.

The formation of follicles, which proceeds rapidly, gives rise in the ovary to two zones, an external or parenchymatous zone in which the follicles lie, and an interstitial vascular zone; these have been described with some modifications by various workers and for different animals. (His (11), Waldeyer, Born (6), Macleod (13), Van Beneden.)

The question of the post-natal formation of primordial ova has been the subject of many isolated observations. Pflüger believed he had evidence of the return of the ovary to the tubular formation at the rutting season, the object of the return being the formation of fresh ova. Waldeyer believed that all ova were formed in the young animal, and for this reason called all ova "primordial ova."

Schrön noticed an increase in the number of clear cells, presumably ova, near the periphery in cats and rabbits at the rutting season, and in women at the menstrual periods. Koster (12) describes prolongations of epithelium with formation of fresh ova and follicles in the ovaries of several recently

pregnant women; Wagener (17) records the thickening of the germinal epithelium near the attachment of the Fallopian tube in the pregnant bitch, a condition which he thinks denotes the formation of ova.

Amann (1) describes the presence of follicles in the ovaries of a woman of 63, where there was incipient cystadenoma, the follicles being in all stages of formation by means of invagination of the germinal epithelium. The interest of this observation lies in the age of the woman and in the apparent formation of fresh ova consequent on the stimulus caused by the incipient cystadenoma.

V. Winiwarter was not able to trace any of the stages of ovogenesis in any of the adult ovaries he examined, and considers this a necessary condition for the formation of ova. As far as the literature goes, we may consider the ovary to be formed by the embedding in the underlying mesoblast of the cells of the germinal epithelium, the embedding being brought about by a process of ingrowth of the cells and of upgrowth of the mesoblast. The cells thus embedded are oogonia, which give rise to ova by division, as also to the follicle cells, the future ova undergoing considerable nuclear transformation before reaching the condition of the fully-formed primordial ovum.

The post-natal formation of primordial ova has been recorded in certain cases, but there is not much evidence either in favour of or against it.

*Object of the Investigations.*—Certain features which I observed in the interstitial cells of the ovaries of rabbits at a late period of pregnancy led me to study the origin of these cells. This question would appear to have been neglected by previous workers on the ovary. The formation of an internal ovarian secretion (*cf.* Andrews (3)), which by analogy with the interstitial gland of the testis might be presumed to be derived from the interstitial cells (*cf.* Ancel and Bouin (2)), gives considerable interest to their origin.

This was studied by examining (1) the ovaries of rabbits from the twentieth day embryo up to those of the young rabbit about three weeks after birth; (2) the ovaries of pregnant rabbits at all stages.

*Methods.*—It is not easy to find a really good fixing agent for ovaries, especially adult ovaries. Hermann's, Flemming's (strong formula), Podwysoski's and Altmann's fluids, were all used. The last was found satisfactory for cytoplasm, but the sections obtained with the other fixatives were not good. The tissue was osmicated outside and insufficiently fixed inside. Finally,\* Gilson's fluid was used exclusively for all nuclear figures, and a

\* Gilson's fluid = abs. alc., 1 part ;  
glacial acetic, 1 part ;  
chloroform, 1 part ;  
the whole saturated with sublimate.

mixture of sublimate (saturated) 4 pints, formol 1 pint, and 1 per cent. glacial acetic for other purposes.

The sections fixed in Gilson's fluid were stained with iron hæmatoxylin, or hæmalum; those fixed in the other solution stain well either with iron hæmatoxylin, hæmalum and eosin, or toluidine blue and eosin.

*Changes in the Cells of the Germinal Epithelium in the Immature Rabbit.*

The origin of the germinal epithelium from the peritoneum by a process of differentiation has been so fully shown by several observers, that it will not be necessary to deal any further with the origin of the germinal cells. Also it has been shown that these cells become embedded in the underlying mesoblast; this state of affairs is seen in an embryo of the twentieth day.

The ovary is by this time a definite organ; it is intensely vascular, showing large blood spaces, especially in the parts lying immediately round the mesoblastic core. At this period the main mass of the germinal cells is situated peripherally, only a few isolated ones having penetrated into the core, which last is sending processes of connective tissue in between groups of germinal cells. Of these there are present a large number of protobroque and a few deutobroque; also a certain number of mitotic figures, but these are not numerous. (See Plate 1, fig. 1.)

From this time onward until after birth the changes in the ovary, as seen under the low power of the microscope, are not striking; there are more deutobroque cells, characterised by their transparent appearance, and there is an increase in the number of mitotic figures.

Studied under the high power of the microscope some of the deutobroque cells are seen to have entered upon the early stages of ovogenesis, and to have reached the leptotenic stage. There are large numbers of round cells showing a nuclear structure differing from either the protobroque or the deutobroque cells. The mitoses are chiefly found near the periphery, and the greater number of them seem to be taking place in the large cells. I do not altogether agree with v. Winiwarter on the question of mitosis in these cells. In the first place there appears to be very little distinction between the varieties of protobroque cell, *a* and *b*, and I shall not dwell upon it. The mitosis in the protobroque cells does not appear to be sufficient to account for the large number of deutobroque cells which are formed, and my observations are to the effect that by far the greater number of mitoses are taking place in the deutobroque cells themselves. Each class of cell divides, the protobroque less copiously than the deutobroque, giving rise to two cells of their own variety. There can be no doubt that the deutobroque cells are modified

germinal cells, but I hope to show that the process is one of differentiation and not of division.

The protobroque cell is the type of the original germinal cell; it is small, generally oblong or oval, and contains a large nucleus. The nucleus shows a number of chromatin masses of varying sizes, and the whole nuclear area gives a general impression of granulation (represented by shading in the figures (Plate 1, fig. 2 (*a*)). There are no chromatin filaments.

The deutobroque cell is very much larger, and the nucleus has for the most part a strikingly transparent aspect, the granular appearance noticed in the protobroque cell being confined to the periphery of the nuclear area. The chromatin is quite differently arranged; there are one or two irregular chromatin masses, and strands showing nodular enlargements where they intersect. See fig. 2 (*f*).

Sections of a young ovary very soon before or after birth show a large number of cells whose nuclei exhibit every phase of transition between these two varieties. These changes in the nucleus may be classed broadly into three divisions:—

1. The chromatin masses become fewer and larger.
2. There is considerable formation of chromatin strands.
3. The granular appearance gradually passes away from the centre of the nucleus towards the periphery, leaving the centre clear.

Some of these changes are shown in fig. 2 (*b*), (*c*), (*d*), (*e*). In the first stage the whole cell becomes rounder, as also the nucleus, and the chromatin has begun to aggregate, and there are traces of strands passing away from the masses. These features increase in intensity until there are only a few chromatin masses, but the strands are passing between them and intersect in parts. The granular appearance has begun to leave the centre, which is clear.

A further process on these lines brings the cell into the typical deutobroque condition. It would therefore seem that the change from the protobroque type is accomplished by means of transformations in the nuclear area, accompanied by a growth in size of the cell, and it is unnecessary to suppose, under these circumstances, that mitosis is also a method of formation. The protobroque and deutobroque cells are therefore all oogonia, either potential or actual, the transition from the one class to the other being probably accomplished by processes of nuclear differentiation.

At this period in the life of the ovary there is no appearance which could be characterised either as egg-tubules or egg-clusters; there are large collections of epithelial cells bounded centrally by the mesoblast, which presents the appearance of connective tissue. This tissue penetrates but slightly into the region lying peripherally to the main central core, but careful

inspection shows that there are a few fine processes pushing their way outwards and more or less enclosing large numbers of germinal cells. The latter are of all shapes and sizes, from the typical protobroque to the deutobroque type.

By the third day after birth the general configuration of the ovary has changed very considerably. There is still the central mesoblastic core, but the germinal cells have become more marked off than in the embryo, presenting the appearance of a definite zone of germinal epithelial cells. The cells are arranged, especially in the more central parts of the zone, in the form of solid rods or clusters, several cells thick, which press their rounded ends into the central mesoblast. Some of them might fairly easily be mistaken for tubules without a lumen, and there can I think be little doubt that these are what Pflüger took for tubules. Around the periphery the tubular formation is not so marked, the cells lying in irregular aggregations (fig. 3). As the mesoblast is centrally situated those parts of the germinal zone lying towards the centre get divided up earlier than the more peripheral parts, which retain the formation of an earlier stage. This lagging behind, as it were, of the periphery is quite characteristic of all the changes taking place in the young ovary; it applies to the formation of tubules and clusters, to the processes of ovogenesis, as pointed out by v. Winiwarter, as well as to the formation of interstitial cells, which will be dwelt upon later on.

From this time onwards up to about the twelfth day after birth the changes in the general configuration of the ovary are brought about by an amplification of the processes already described, namely, continued upgrowths of connective tissue, cutting off the tubules and clusters. The connective tissue likewise presses into the larger collections of germinal cells, thus cutting them off and dividing them again into smaller portions, so that as time goes on the clusters near the central parts consist of less cells, but are present in much greater number, while those parts more peripherally situated are in a somewhat earlier stage, the cluster formation being still fairly evident close to the periphery as late as the sixteenth day.\*

Before proceeding to the changes in the egg-clusters about the fifteenth

\* Too much stress, however, should not be laid upon the exact date of the young ovary in relation to its structural aspect. There seems to be an appreciable difference in the extent to which the ovary is developed in different animals about this age. v. Winiwarter does not describe any ovary between the tenth and the eighteenth day, because he does not consider the changes to be sufficiently striking to call for any description. Of two litters of rabbits I found slight differences in the ovaries of the same date, the sixteenth day, the changes being rather more advanced in one than in the other, and both were almost as advanced as v. Winiwarter's figure of the eighteenth day. The differences are probably determined by the varying nutrition of the animal, as also possibly by the kind of rabbit, some being far more advanced in outward aspect at this age than others.

day, the transformations which have been taking place in the deutobroque cells must be briefly touched upon, but they have been so fully described by v. Winiwarter that a lengthy exposition is quite unnecessary. I shall adopt his nomenclature throughout. It has already been stated that almost immediately after birth changes begin to take place in the deutobroque cells, which enter upon the leptotenic phase, the transformations beginning centrally. This is succeeded by the synaptenic, and by the third day there are already a very great number of this variety.

The leptotenic phase is characterised by the absence of visible nucleolus, and by the spreading out of the chromatin in the form of fine filaments over the whole nuclear area. This stage is evidently only a further step in the differentiation which has already taken place. It has been shown that the change from the protobroque to the deutobroque type is accomplished by the chromatin masses becoming gradually broken up into strands. In the leptotenic phase the process is merely carried further. Whereas in the deutobroque stage there are still one or two chromatin masses which have not become broken up into strands, in the leptotenic this is not the case, the whole chromatin being present in the form of filaments. The transition stage can be seen in an ovary of a few days after birth (fig. 6(1)). These filaments become gradually aggregated, passing to the synaptenic state. The leptotenic condition is a very fugitive one, whereas the synaptenic, owing presumably to the great variety of aspects through which the aggregation passes is very much more prolonged. The synaptenic is succeeded by the pachytenic, where the filaments are coarser, then by the diplotenic, and this in its turn by the dictyate condition, which is the typical nuclear appearance of the young ovum.

These changes pass gradually outwards, and by the tenth day even the cells quite at the periphery have passed through the earliest phases, whilst the central cells are reaching the final ones. By the fourteenth day there are a certain number of dictyate nuclei towards the centre. At this stage the central mesoblastic core is becoming obliterated, the egg-clusters of either side of the ovary very nearly meeting. The clusters are much smaller, having been split up by the ingrowing mesoblast. The number of dictyate nuclei now increases at a surprising rate, there being a great number by the fifteenth day, and a still greater number by the sixteenth day, by which time the clusters are almost indistinguishable, except round the periphery, their place having been taken by dictyate cells, some of which now show a surrounding follicular epithelium. There are also collections of small more or less rounded cells lying in between the young follicles, but not forming any part of the follicular epithelium. These are the interstitial cells of the ovary, and I propose now to trace their origin in detail.

*Origin of Interstitial Cells.*—By about the tenth day the ovogenetic processes in the central egg-clusters are at their height, and continue in this condition, passing through the various phases, until about the fourteenth day, when, as already stated, some have reached their final stage. Throughout the whole period, however, there are in almost all the clusters some three or four cells, or perhaps more, which remain in the ordinary deutobroque state, and do not undergo any of the ovogenetic phases, the number of these being greater in the peripheral clusters than in the central ones; by the fifteenth and sixteenth days, when the number of dictyate nuclei is increasing, there are few, if any, of the deutobroque cells to be found in the central parts, but instead, there is an increasing number of the small round cells already referred to. As the days pass on the number of the former decreases, and the number of smaller cells increases. Thus, there is throughout the ovary, but in different parts at slightly different periods, a reduction in the number of deutobroque cells, which have remained unchanged, and an increase in the number of small round cells.

Examination of sections of the fourteenth, fifteenth, and sixteenth days near the centre of the ovary, leaves no room for doubt that some of these cells form the follicular epithelium, gradually passing towards and arranging themselves around the young ova. At the fourteenth day the number of these cells to be found near the centre is not nearly sufficient to form the follicular epithelium for the large number of young ova, while near the periphery there are many more than would appear to be necessary for the requirements of this part. By the fifteenth day the number of these cells near the centre has increased very largely, still more so by the sixteenth day, by which time many of the young ova are surrounded with follicle cells, and there are also the collections of these cells already referred to. Their number has meanwhile diminished somewhat at the periphery. The appearance in the intermediate parts gives the key to the whole question. Here are seen large numbers of these cells streaming inwards from the periphery and making their way between the egg-clusters of the periphery towards the centre, where the cluster formation can now be scarcely recognised. Here they arrange themselves around the young ova, or pass into little groups by themselves. These groups are the first beginnings of the real interstitial tissue of the ovary, and mark the commencement of the adult aspect of the organ.

There are thus two main points to be emphasised at this period in the life of the ovary. First, the passage inwards of a large number of cells from the periphery, and secondly, the commencement of the adult formation by the formation of young follicles, and the appearance of interstitial cells.

This passage inwards of cells from the periphery was noticed by Bühler ; he realised that the number of small cells near the centre during the height of ovogenesis was not enough to provide a follicular covering for all the young ova which were there, and he describes the streaming inwards of the cells from the periphery, and their passage to the young ova, around which they arranged themselves, and formed the follicular epithelium.

Balfour noticed that in the later periods of ovogenesis there were present too many of the small cells, like the follicle cells, for them all to become arranged around the ova and give rise to the follicular epithelium. He was at a loss to account for the destiny of these supernumerary cells, and supposed that they must either eventually become ova or follicle cells, or be used up as food-stuffs for the other cells.

It seems to me, however, that these cells, supernumerary as far as the follicular epithelium is concerned, are in reality very important. They form the groups which represent the interstitial tissue of the fully-formed ovary, and thus, far from being unimportant, are absolutely essential for the performance of the functions of the ovary.

The question which now arises is, where do these cells come from, and what is their history of formation ? It has already been indicated that the number of unchanged deutobroque cells varies inversely with the number of these cells, since these last are greater in number in the region where there are most deutobroque cells present, namely, at the periphery, especially in the region of the poles, and the high power of the microscope reveals the fact that these cells are indeed metamorphosed deutobroque cells.

The ordinary deutobroque cell presents one or two irregular chromatin masses, from which pass out filaments of varying degrees of coarseness and fineness with nodules at their intersections. The centre of the nucleus is clear, whilst around the periphery is the granular appearance already described. See fig. 2 (*f*). In the ovary of about the eighteenth day the only regions where these cells are to be seen in any appreciable numbers are round the periphery and at the poles. They stand out even under the low power on account of their general transparent aspect as compared with the surrounding cells, and also in many cases on account of their rather larger size. There are also cells whose transparency is not so great, but which show up quite markedly in contrast to the rest and are rather smaller in size than the more transparent ones. These cells are transformation stages between the deutobroque and the ordinary interstitial type, and the process resembles very much in the inverse order that which has been already described for the deutobroque formation from the protobroque.

The first stage is the gradual massing of the chromatin into irregular masses

and the thinning of the chromatin strands, which become rather less in number, as do also the nodules (fig. 4(*e*)). At the same time the granular appearance extends gradually towards the centre, although it is not until quite a late stage that it reaches the centre itself (fig. 4 (*f*) and (*g*)). The size of the cell becomes gradually less, and the amount of protoplasm relatively greater.

The retraction, as it were, of the filaments and strands towards the chromatin masses is very much more marked in the cells of smaller size, where there is a tendency for the masses to pass towards the periphery, leaving the centre clear (fig. 4 (*d*) and (*e*)).

These changes continue until nearly all the chromatin is massed, the masses becoming rounder as the process goes on. There are always traces of strand formation left, in contrast to the protobroque nucleus, where it is markedly absent. Thus the small cell derived by differentiation from the deutobroque cell does not return to the characteristic protobroque type, but shows traces of its intervening deutobroque condition in the shape of strands of chromatin, and nodules on the strands. See fig. 4 (*g*).

The cells, once reduced in size, become true ovarian cells, and may either function as follicle cells or as interstitial cells (fig. 4 (*h*) and (*k*)).

Thus we find the following processes taking place in connection with the formation of the mature ovary. The cells of the germinal epithelium become embedded in the underlying mesoblast, and, once there, may either undergo differentiation, or apparently may remain in the protobroque condition. If the former be its fate, it must undergo nuclear transformation, together with growth in size, until it reaches the deutobroque stage. Arrived at this condition it probably divides, although possibly this is not an essential, and the two cells formed by this division are of the same type. There are now two courses open for the cells thus produced; they may undergo the nuclear transformations of ovogenesis, and become primordial ova, or they may rest for a time, and finally undergo regressive transformations, becoming either follicle cells or interstitial cells. Every cell of the germinal epithelium is probably a potential ovum, relatively very few remaining in the protobroque state, although some may still be seen at the periphery in ovaries of the eighteenth day. Incomparably the greater number pass to the deutobroque state, preparatory doubtless to the formation of ova. All cannot become ova, for the other forms of cell are necessary for the maintenance of the ovarian functions; possibly, therefore, only the most robust cells, and those which are most conveniently situated for obtaining nourishment undergo the ovogenetic changes. This would seem to be borne out by the fact that many more of the central cells, which are nearer their food supply, undergo ovogenesis, than

of the peripheral ones. The rest of the cells which are not able, for one cause or another, to undergo these changes, appear to remain quiescent for a while, until finally they regress and pass into a condition of subserviency to the needs of those which have become ova. Both follicle cells and interstitial cells are, however, still potential ova. They have passed through the initial stages, and only need enlargement and nuclear transformations in order to become ova, should the appropriate stimulus be given. This chance is not given to the follicle cells. As soon as the follicle begins to grow, they multiply rapidly, and probably provide, by their disintegration, the follicular secretion upon which the ovum feeds and grows. In the ripe follicle of the rabbit there is almost complete disintegration of the membrana granulosa, and the remains of the discus proligerus is presumably extruded with the ovum, perhaps serving it as food material prior to its fertilisation, and subsequent attachment to the uterine wall. The interstitial cells, however, have possibilities before them, being still capable of carrying out any function belonging to the true ovarian cell.

All the true ovarian tissue is derived from the germinal epithelium, this tissue forming in the adult rabbit by far the greatest part of the whole ovary. There is relatively little mesoblast, which subserves solely the function of support and of nutriment-carrier to the rest of the organ. We may, therefore, look upon the whole ovary as consisting of two classes of cells and of two only, namely, (1) those derived from the germinal epithelium and performing all the ovarian functions, and (2) those derived from the original mesoblast, which are supporting and vascular.

There remains only one feature to be dealt with in the immature ovary, one that has already been described by Balfour, namely, the protoplasmic masses formed by the aggregations of young ova. In the ovary of the sixteenth day the ova are all separate, but a day or two later this is not the case. There are now a large number of these masses of various sizes. They appear to consist of two, three, four, or even five young ova, to judge by the number of nuclei seen, but it is impossible to distinguish any trace of cell-boundary between them. Balfour suggests that these may either form as many ova as there are nuclei, or that one ovum may develop at the expense of the rest. This last point of view appears to be the more probable. It is evident that the massing takes place subsequent to the formation of the young ova, since it is not seen until after the appearance of the ova, and it would appear rather purposeless if they merely separated again a little later on. Moreover, in these masses one or two of the nuclei often look as if they were disappearing by gradual dissolution, and it is, therefore, probable that they will all ultimately serve as food-stuff for the one ovum

whose condition happens to have been best, and will, therefore, survive in the struggle for existence.

This cannibalism on the part of the young ovum is not surprising, if the life of an ovum be considered. It is really but the normal condition of the cell at all its stages of development; it grows and fattens at the expense of other cells. In the young ovary it is starting its first stage of growth and must devour other cells; later on, when it grows during the growth of the follicle, it lives upon the follicle cells, and later still, when, after fertilisation, the ovum in its extended sense refers to the young foetus, it lives on the material provided by the cells of the maternal organism.

This massing of cells and subsequent demolition of some of them for the benefit of one will be again dealt with in connection with the ovary of the pregnant rabbit.

#### *Changes in the Ovary during Pregnancy.*

The young ovary, after the period when it has reached a stage where the general aspect is that of an adult ovary, enters upon a period of slow growth, during which there is a continual formation of a considerable number of follicles, which having reached a state of partial maturity then begin to atrophy and finally disappear, leaving only a faint trace of their former existence in the shape of a scar.

Having reached sexual maturity, the ovary becomes subject to periodic influences, of the nature of which little, if anything, is known. According to Fraenckel (9), they are intimately connected with the hypertrophy of the mucous membrane of the uterus. The sum total of the influences at work results in the production of "heat," which occurs in the rabbit about once a fortnight, but the external changes in the vulva by which this is judged take place very gradually, so much so, that in the spring and summer time, when breeding is most prolific, the adult rabbit is scarcely ever out of one or other stage of "heat." It is fairly certain, therefore, that whatever changes may take place in the ovary during "heat," the condition recurs too frequently for these to be very marked. This does not refer in any way to the formation of the corpora lutea of "heat," which are, of course, very definite. It has recently been stated by Heape (10) that unless impregnation occurs the ripe follicles of "heat" do not burst, in which case, presumably, there can be no formation of corpora lutea. If this is the case it would seem that there can be no such thing as the corpus luteum of "heat," and the changes in the ovary during this period must be considered to consist merely of those taking place before sexual maturity, only rather more marked, namely, the formation of follicles, but after puberty these

reach the ripe state, since they will burst if impregnation occur, whereas this is probably not the case in the immature ovary. There would then, on this view of the case, be no ovulation except in the impregnated rabbit. It is quite possible that the additional stimulus of impregnation may hasten the bursting of the follicle, but it seems somewhat unlikely that without impregnation there should never be ovulation.

The changes resulting in the production of "heat" are obviously those preparatory to a possible pregnancy. Fertilisation appears to be in itself a stimulus, and sets up general hypertrophy of the entire genital apparatus, producing likewise an improved condition of the animal; as to the mechanism of the production of this hypertrophy, however, our knowledge may be said to be nil, and we are reduced to classifying the whole as the changes brought about by the stimulus of pregnancy.

*Naked Eye Changes.*—Fraenckel describes and figures very accurately the naked eye changes in the pregnant ovary of about the fifteenth day in his paper on the function of the corpus luteum.

These changes are very striking, and indicate in themselves some very definite alteration or increase in the function of the gland; apart from the formation of the corpora lutea, there is an immense increase in absolute size, the gradual occurrence of which will now be described.

The ovary of the non-pregnant rabbit is a small yellowish body, lying on either side against the posterior abdominal wall, a little below the kidney. It is usually about  $\frac{1}{2}$  inch in length and thin, being slightly wedge-shaped in transverse section and rather pointed longitudinally at either end; upon its surface may be seen clear round spots, showing the locality of the larger follicles, some of which, if they are nearly ripe, may even project slightly from the surface.

The bursting of the follicles and fertilisation lead to the formation of the corpora lutea, the so-called "true" corpora lutea of pregnancy, and the growth of these bodies during the early period are undoubtedly the most characteristic feature in the naked eye appearance of the ovary. If the pregnancy be one with a large number of foetuses, the ovary often looks gnarled, so large and numerous are the excrescences produced upon its surface by these bodies. If these, however, be cut off, and if the organ be carefully examined at about the fourteenth day, when the corpora lutea are at their maximum state of development,\* it will be readily seen that the ovary itself has increased in size, quite apart from the formation of the lutein tissue. The whole gland has a more swollen and rather less compact aspect; it is larger both in length and girth, and the wedge-shape of the

\* Cf. Fraenckel, *loc. cit.*

transverse section is less marked; there are also in many cases fewer follicles in an advanced condition than in the non-pregnant state. Just at this period the energies of the gland have apparently been directed rather to the formation of the lutein tissue than new follicles.

From the fourteenth to the eighteenth day the corpora lutea remain at their maximum, and then begin to diminish rapidly in size. Instead of being very vascular whitish bodies, projecting in many cases to the extent of three-quarters of their whole extent beyond the surface of the gland, they gradually diminish both in size and vascularity, until by about the twenty-second day of pregnancy they are merely elevations on the surface, showing the faintest possible trace of vascularity; this diminution continues steadily until, a little while before birth, the locality of these striking features of the fourteenth day of pregnancy is only seen by the presence of an opaque whitish circular area upon the surface of the ovary. The changes are so marked that it is possible after a little experience to diagnose very approximately the previous duration of the pregnancy from the appearance of the corpora lutea.

Whilst these external changes are taking place in the lutein tissue, the rest of the ovarian tissue has been also undergoing changes, which, if not so striking in appearance, are none the less evident. It has already been stated that the organ at the fourteenth day shows marked increase in size apart from the corpora lutea; whereas, shortly after this period, these bodies begin to diminish in size, the reverse takes place in the rest of the ovarian tissue; and whereas growth of the ovary as a whole has been slow up to the present, it now becomes rapid and continues until close upon the time of parturition.

By about the eighteenth or twentieth day all trace of wedge-shape in cross-section has completely gone, and the organ is nearly circular, the girth is much greater, and this increase extends right up to the poles. These changes become more and more marked, until at about the twenty-sixth day the organ is well over an inch in length, sometimes about  $1\frac{1}{2}$  inches, showing a proportionate increase in its other measurements, and having a shape very much resembling a spindle with blunted ends. The number of clear round spots has meanwhile been increasing rapidly, so that in the majority of cases the greater part of the surface is taken up either by them or by the round whitish patches, which mark the spots where the corpora lutea have been projecting above the surface. The formation of follicles appears to be somewhat inhibited during the rapid growth of the corpora lutea, but to be resumed with greater energy when these have reached their maximum development. At the time of parturition there are a large number of

follicles which have almost reached full maturity, and it is a well-known fact that rabbits can be readily fertilised immediately after parturition.

The gland, although soft, is not in any way brittle, and in spite of its great general enlargement retains on the whole the same shape, the most marked change being that from the wedge-shaped to the circular transverse section.

*Changes in Size of the Interstitial Cells.*—This great increase in size must be the result either of a large numerical increase, or of a very great increase in the size of the individual ovarian cells. The latter is at any rate the main, if not the only factor concerned, the change in size of the cell under the microscope being so marked as to attract attention even apart from any actual measurement.

The measurements were made with a micrometer eye-piece, gauged against a micrometer slide, this method being found quite sufficiently accurate for the purpose. It was not intended to record exactly the size of each individual cell, but rather by taking the measurements as accurately as possible of a large number, to find the average increase in size at different stages of pregnancy. In taking the measurements considerable selection was exercised in the cells measured; only those whose area in section was approximately circular, and where the nucleus was centrally situated being used, as it was hoped by these precautions to obtain measurements passing as nearly as possible through the centre of the cell. The measurements are given below of 10 cells from each date of pregnancy, but this does not by any means represent the number actually measured, but the same figures recur again and again, and the average works out to almost precisely that given.

Towards the end of pregnancy there is considerable difficulty in finding the right kind of cells to measure, nearly all of them being angular and irregular in outline, giving as a whole somewhat the appearance of a tessellated surface. The changes in general aspect of the sections, produced by the change in the size of the cells, will be returned to later on.

Interstitial Cells of a normal Rabbit.

Diameter at 14th day of pregnancy.

Diameter in mm.		mm.	
0.0162		0.0225	
0.0180		0.0216	
0.0171		0.0243	
0.0189		0.0207	
0.0180	Average = 0.0177 mm.	0.0252	Average = 0.0234 mm.
0.0153	= 17 $\mu$	0.0216	= 23.4 $\mu$
0.0180		0.0225	
0.0198		0.0270	
0.0171		0.0252	
0.0189		0.0243	
<hr/>		<hr/>	
0.1773		0.2349	

Diameter at 18th day.

Diameter at 20th day.

mm.		mm.	
0.0252		0.0342	
0.0279		0.0333	
0.0288		0.0306	
0.0279		0.0306	
0.0270	Average = 0.0272 mm.	0.0297	Average = 0.0319 mm.
0.0288	= 27.2 $\mu$	0.0315	= 31.9 $\mu$
0.0270		0.0324	
0.0252		0.0315	
0.0270		0.0324	
0.0279		0.0333	
<hr/>		<hr/>	
0.2727		0.3195	

Diameter at 22nd day.

Diameter at about 26th day.

mm.		mm.	
0.0324		0.0306	
0.0315		0.0288	
0.0324		0.0297	
0.0315		0.0342	
0.0315	Average = 0.0326 mm.	0.0270	Average = 0.0318 mm.
0.0306	= 32.6 $\mu$	0.0333	= 31.8 $\mu$
0.0360		0.0360	
0.0351		0.0351	
0.0342		0.0315	
0.0315		0.0324	
<hr/>		<hr/>	
0.3267		0.3186	

Diameter just before birth, at 28th or 29th day.		Diameter a few hours after parturition.	
mm.		mm.	
0·0288		0·0270	
0·0315		0·0279	
0·0270		0·0279	
0·0297		0·0270	
0·0288	Average = 0·0298 mm.	0·0270	Average = 0·027 mm.
0·0315	= 29·8 $\mu$	0·0261	= 27 $\mu$
0·0288		0·0252	
0·0306		0·0279	
0·0315		0·0270	
0·0306		0·0279	
<hr/>		<hr/>	
0·2988		0·2709	
Diameter after 3 days' lactation.		Diameter after 6 weeks' lactation.	
mm.		mm.	
0·0270		0·0180	
0·0252		0·0171	
0·0279		0·0162	
0·0270		0·0180	
0·0270	Average = 0·0268 mm.	0·0171	Average = 0·0171 mm.
0·0252	= 26·8 $\mu$	0·0153	= 17·1 $\mu$
0·0279		0·0180	
0·0261		0·0171	
0·0270		0·0162	
0·0279		0·0180	
<hr/>		<hr/>	
0·2682		0·1710	

Tabulating these results, one gets—

Approximate age of pregnancy.	Diameter of cells in $\mu$ .
0 (= normal) .....	17·0
14th day.....	23·4
18th „ .....	27·2
20th „ .....	31·9
22nd „ .....	32·6
27th „ .....	31·8
Shortly before birth .....	29·8
„ after „ .....	27·0
3 days „ „ .....	26·8
6 weeks „ „ .....	17·1

Taking the radius of the cells it is seen that the increase in its length in the cell during pregnancy is from 8·5 to 16·3 or very nearly double.

If the volume of the sphere be taken as  $\frac{4}{3} \pi \cdot r^3$  and the cell be taken as a sphere, the ratio of the non-pregnant cell to the cell at the maximum size attained during pregnancy becomes almost exactly 1:7, which would allow sufficient enlargement of the ovary to account fully for the increase in size. It is not to be supposed that all the cells enlarge to the same extent, but it may reasonably be supposed that they enlarge to about five times their normal size. This will account for the enlargement of the whole ovary, and there would seem therefore to be no necessity to seek any further cause of the enlargement of the ovary during pregnancy.

The only other possible cause which suggests itself at once is of course the division of cells, but although I have examined some hundreds of sections of pregnant ovaries, I have not found any trace of this happening. In giving the above figures I do not wish to suggest that the measurements are absolute. They are subject most probably to individual variations, depending possibly upon the number of fœtuses in each pregnancy, and on various other circumstances. The ovaries in question were, however, taken quite haphazard in regard to all external causes, which allows some scope for differences in the ovary, and the results are fairly definite. They show a great increase in the size of the ovarian interstitial cells during pregnancy, and that the main increase is reached by about the twenty-second day, and is sustained until just before birth, when there is a slight diminution in size.

In this connection there is one feature to be dealt with, namely, the shape of the cell. Up to about the twenty-fourth or twenty-fifth day it is not difficult to find approximately spherical cells to measure. After this period, however, the difficulty of doing so becomes very great, if not impossible. The cells are angular and seem crushed together, and I would suggest that possibly the cells may be really still undergoing slight increase in size, but that the capsule having almost reached its maximum stretching capacity does not admit of the desired expansion, and the cells instead of being spherical become more closely packed in order to find room for the additional bulk, filling in as it were the interstices rather than causing an increase in size in the spherical direction.

The rounded appearance is resumed very shortly after birth, and there is also a slight decrease in size. Why there should be a decrease before birth is a point upon which I feel it is impossible to offer any suggestion. The mechanism of the production of labour is a question upon which very little is definitely known; if, however, it be the function of the ovary to cause the adhesion of the fœtus to the uterine wall (Fraenckel), a function carried out

presumably by means of the interstitial cells, since these probably furnish the internal secretion of the organ, it seems not impossible that the diminution in size may be indirectly connected with the onset of labour.

*The Formation of "Primordial" Ova from the Interstitial Cells.*

In addition to the increase in size there are other changes taking place in some of the interstitial cells near the peripheral parts of the ovary, during the later third (approx.) of the period of gestation. It is a matter of common histological knowledge that over the surface of the ovary there is a layer of epithelial cells, roughly about two cells deep, although varying slightly in thickness at different places. Immediately below this is a layer of tissue in which are embedded the primordial ova in their early stages, when they have not yet acquired a follicular epithelium or when that epithelium is not very highly developed. There are in addition groups of small ovarian cells which will eventually, as occasion arises, form the follicle cells for the primordial ova. This whole layer together with the germinal epithelium varies very considerably in thickness in different animals, the variation having possibly some relation to the age of the animal under investigation (*cf.* v. Beneden).

In the non-pregnant animal and in the early periods of pregnancy, there is a fairly sharp boundary between these outer layers and the deeper lying interstitial cells. By about the twentieth day of pregnancy this state of affairs is seen to be gradually changing, and some of the interstitial cells are becoming surrounded by the connective tissue of the inner layer and thus getting cut off from their fellows below. Whether this is brought about by the passing outwards of the cells themselves or by the growth inwards of the connective tissue is very difficult to decide quite satisfactorily; but I think it is reasonable to suppose that both processes are involved. It has already been shown that there are two means whereby the germinal cells of the embryo become embedded in the subjacent mesoblast, namely, by an ingrowth of the germinal cells and by a simultaneous upgrowth of the mesoblast lying below. Here we have an analogous condition, but the positions are reversed; the germinal cells are now inside and the mesoblast outside.

This process, which is beginning to be evident about the twentieth day, continues throughout the rest of pregnancy, so that as the days go on more cells become cut off and press outwards, in many cases reaching almost to the periphery. The number of cells thus cut off varies appreciably in different animals, probably depending upon the age of the animal, but it is not excessive at any time; I have never found more than three or four rows

of cells cut off, and these rows do not form continuous layers round the ovary (*vide* fig. 5).

About the twenty-third or twenty-fourth day, and in ovaries of later dates of pregnancy, a somewhat striking feature about some of these cells is that they are no longer mononucleated; two nuclei are frequent, three quite common, whilst in some cases there may be as many as six. These nuclei are not massed together as in a giant cell, but lie separate in the cell protoplasm. The latter is very much greater in amount than in an ordinary interstitial cell, and is irregular in outline. The appearance of these multinucleated cells suggests that they have been derived from the fusion of the same number of interstitial cells as there are nuclei in the cell. It will be remembered that van Beneden pointed out this appearance in the bat's ovary, when he found in some cases as many as eleven nuclei, and he suggested that possibly one of them grew at the expense of the others, whom it used as food, or that one might become an ovum and the others the follicle cells.

Examination of a large number of these cell masses shows that in many cases there is undoubted atrophy of one or more of the nuclei going on. In some there is a clear space where a nucleus might have been expected, in others the nucleus stains very faintly or only in parts, whilst there is usually one nucleus which stains intensely, especially in the iron hæmatoxylin specimens, and in which the staining, even after extreme differentiation, is still so dark as to remove all possibility of tracing any nuclear structure. This points to some difference of metabolic condition, and the conclusion seems obvious that this nucleus is growing strong at the expense of the others; one is reminded of the protoplasmic masses described by Balfour in the young ovary and to which reference has already been made in this paper. Here we have a number of potential ova (for the fact has already been emphasized that all interstitial cells being derived from the germinal epithelium are potential ova) massed together, of which the nucleus of one of them grows at the expense of the others, which it uses as food material; in the young ovary the end-product is a primordial ovum. In the pregnant ovary the end-product is likewise a "primordial" ovum. The cells of these aggregations are all quite clearly ordinary interstitial cells, and the surviving cell is also an interstitial cell differing only in the intensity of its staining reaction.

It has I hope been conclusively shown, in the earlier part of this paper, that the interstitial cells have all been derived from the cells of the germinal epithelium, and have all passed through the deutobroque condition, and it has been pointed out by v. Winiwarter that if there is to be ovogenesis subsequent to the first great ovogenetic period, the cells which are to become

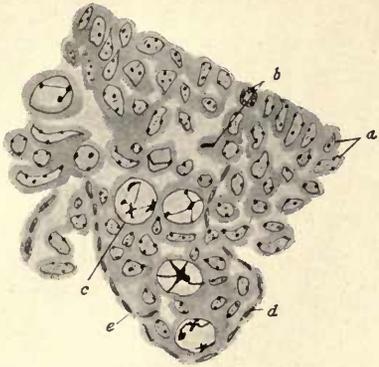


FIG. 1.

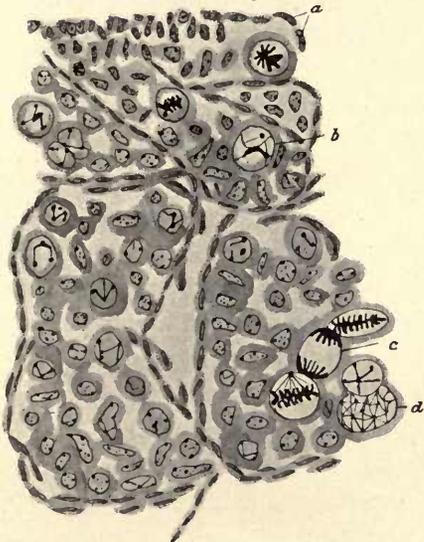


FIG. 3.

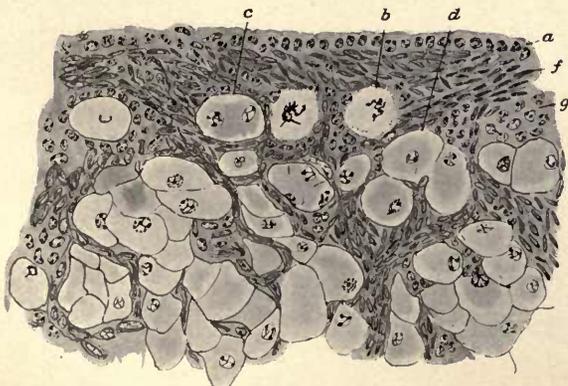


FIG. 5

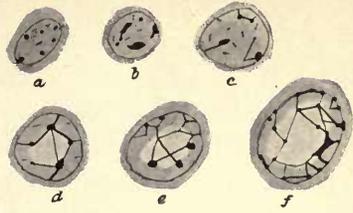


FIG. 2.

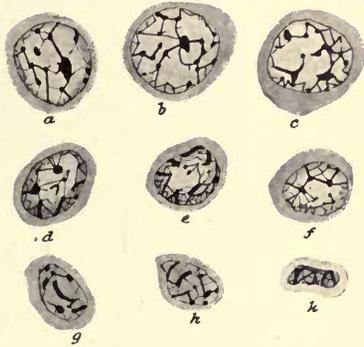


FIG. 4.

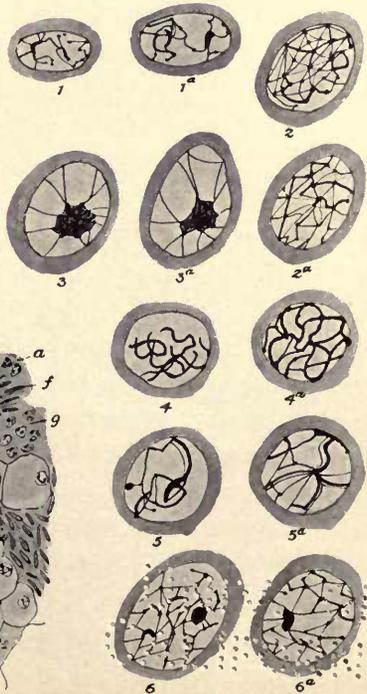


FIG. 6



ova must pass through the requisite nuclear changes. Also it is obvious, although this is not a point which he brings out, that there is a very great difference in size between the primordial ovum and the interstitial cell in a non-pregnant animal, and it is therefore necessary for the cell to enlarge at some period of the transformation. This requirement is fulfilled, as has already been shown, in the case of the pregnant ovary. The interstitial cell of the non-pregnant ovary has approximately a diameter of  $17 \mu$ , but increases up to  $29 \mu$  or even rather over  $30 \mu$  in the pregnant animal. The size of a primordial ovum before it begins to grow, preparatory to becoming a Graafian follicle, is very constant; I have taken measurements of a large number of ova both in the young ovary and in the pregnant as well as the non-pregnant animal, and the average diameter is  $27 \mu$ , the diameter reached by the interstitial cells about the eighteenth day of pregnancy.

It is about the twentieth day of pregnancy that the cutting off of the interstitial cells towards the periphery begins to be noticeable—that is to say, shortly after they have reached a diameter about equal to that of a primordial ovum. It is not, however, until a little later that the cells thus cut off begin to show any nuclear differentiation, in fact this is perhaps best seen in the ovary of a rabbit whose time of parturition has almost arrived. These changes are identical with those taking place in the deutobroque cells of the young ovary during the period of oogenesis. The only difference lies in the fact that whereas in the pregnant ovary the process is taking place only at the periphery, and in relatively very small numbers, in the young ovary there may be 20 or 30 nuclei undergoing changes in the same field. The fact of their presence at all in the pregnant ovary is, however, all proof that is necessary for the formation of ova. It is not for a moment to be supposed that any formation of fresh primordial ova after the first great period should take place to anything like the same extent. Probably the actual changes only occur over a period of a few days, commencing about the twenty-fifth day of pregnancy, or rather earlier, and extending probably to a little after parturition. In the young ovary the changes do not commence until after birth, and some of the cells have completed their changes by about the tenth or eleventh day, the process being probably considerably less lengthy than this for the individual cells, and taking still less time, if anything, in the pregnant rabbit, where there is obviously a state of stimulation during the whole period of pregnancy.

The first change passed through by the nucleus of an interstitial cell, which has passed to the periphery in order to become an ovum, is shown in fig. 6 (1). The nucleus shows chromatin filaments, in the middle of which are seen irregular lumps of chromatin. (In the diagrams the analogous stage of the

young ovary has been given side by side with that in the adult, and does not call for any special description.) This is a transition form from the interstitial nucleus to the leptotenic stage in the process of ovogenesis, and appears to be brought about by the breaking up of the nuclear chromatin into an immense number of filaments. The arrangement of the chromatin in the interstitial cells is, as a rule, discrete either in a rather loose reticulum or round the edges, usually the former.

The first change is therefore the formation of fine filaments. The *leptotenic* stage of v. Winiwarter is brought about by the enlargement of the nuclear area and the spreading out of the filaments over this increased space, thus producing a looser arrangement which consists of fine filaments with a rather nodular appearance where they intersect (fig. 6 (2)). This state would appear to be a very fugitive one (as observed likewise by v. Winiwarter), judging by the rarity of its occurrence. It is quickly passed through, and the nucleus enters upon the *synaptenic* condition (fig. 6 (3)). This stage occupies much longer than the last, and a relatively large number of nuclei are found in this condition, which has many modifications. The filaments at the leptotenic stage are spread out over the nuclear area, whilst at the final synaptenic the chromatin is massed into a lump at the side of the nucleus. All stages may be traced both in the adult pregnant ovary and the young ovary, but only the most characteristic phase is figured, namely, that where a very appreciable amount of massing has already proceeded, the mass being connected to the sides of the nucleus by a few very fine filaments.

The massing completed, there seems to be a rearrangement of the chromatin, and it becomes spread out again, but this time the filaments are thicker. This is the *pachytenic* stage (fig. 6 (4)). The number of nuclei found in this stage is less than in the synaptenic, but still there are a fair number in various conditions. The filaments are so markedly thicker and more bulky generally that it is impossible to confuse it in any way with the leptotenic phase. The chromatin does not fill the nucleus quite so much as in the young ovary, but I have found sections where this was more the case than in the one figured; moreover in some the chromatin seems to have a more continuous disposition than is here represented.

The transition stage between the pachytenic and dictyate or final stage is not, according to my observations, quite analogous to v. Winiwarter's, and I rather hesitate to call it diplotenic, as the duality of the filaments is not well marked (*see* fig. 6 (5)); the chromatin is still arranged in thick strands, and there is some trace of nucleoli, whilst at the same time there are a very few thinner nodulated strands, foreshadowing the condition called by v. Winiwarter *dictyate*, and which represents that of the young ovum.

The nucleolus in the dictyate condition (fig. 6 (6)) is very definite, and the chromatin is arranged more or less all over the nuclear area (which is now very large), and shows a number of small nodules both at what appear to be free ends and at the points of intersection. There can, in fact, be not much doubt that the changes taking place are identical with those seen in the young ovary, which lead to ovogenesis, and therefore it would appear that ovogenesis also takes place in the adult animal during pregnancy.

Previous observers on this subject appear to have all considered that formation of ova must be accomplished by means of fresh invaginations of germinal epithelium, and those who thought they saw invaginations concluded at once that there was therefore a formation of ova in later life, whilst those who failed to find them denied the possibility on this account. My observations show that fresh invaginations of the germinal epithelium are not a necessity, but that the "invagination" has taken place already in the embryo. The invaginated cells of the germinal epithelium give rise to all the cells of the true ovarian tissue, which are all capable of functioning in any true ovarian capacity—that is, they may become ova or follicle cells, or interstitial cells, and most probably also lutein cells, their destiny appearing to be a matter of chance. The interstitial cells, however, are still capable of becoming ova, and of undergoing the changes requisite for ovogenesis should the appropriate stimulus be given. This stimulus is supplied when the animal becomes pregnant, and the ovarian cells enlarge in size. Towards the end of the time of pregnancy some of them press towards the periphery and undergo the necessary changes, becoming true ova. Thus every pregnancy would seem to be a stimulus for the next, in the way of providing new ova, although even of the relatively small number found probably very few ever reach maturity.

*Conclusions.*—Summing up the conclusions reached in this paper we find—

1. That a large number of germinal cells become embedded in the subjacent mesoblast. Of these the great majority undergo transformations up to a certain stage. This stage having been reached, they may pass through the necessary processes of ovogenesis, or they may become modified to form either follicle cells or interstitial cells, this last process being the chief fate of the cells near the periphery, whilst ovogenesis is that of the more centrally situated ones.

2. The interstitial cells are thus potential ova, capable of becoming ova should the appropriate stimulus be given.

3. This stimulus is provided by pregnancy, during which period the interstitial cells undergo enlargement in size, exceeding that of a primordial ovum.

4. About the twenty-third day some of the interstitial cells become cut off near the periphery and pass through the nuclear transformations of ovogenesis, becoming true ova.

I wish to express my deep obligation to Professor Starling, under whose supervision this research has been carried out, and without whose never-failing assistance, interest, and sympathy at each step of the work it would have been impossible to carry out the investigations described above.

Also I desire to thank Mr. H. G. Plimmer for his kindness in giving me much valuable information in regard to the carrying out of the histological details.

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DESCRIPTION OF PLATE I.

FIG. 1.—Ovary of 20th day embryo (rabbit). Fixed in Gilson's fluid :—

- a* = protobroque cells.  
*b* = " (in mitosis).  
*c* = deutobroque cell.  
*d* = connective tissue cell.  
*e* = position of mesoblastic core.

FIG. 2.—Cells in ovaries of rabbits just before and after birth. Fixed in Gilson's fluid :—

- a* = protobroque cell.  
*b* }  
*c* } = transition forms between (*a*) and (*f*).  
*d* }  
*e* }  
*f* = deutobroque cell.

FIG. 3.—Ovary of three days old rabbit showing formation of egg-clusters. Fixed in sublimate solution :—

- a* = protobroque cell.  
*b* = deutobroque cell.  
*c* = " in mitosis.  
*d* = leptotenic stage in ovogenesis.

FIG. 4.—Cells in ovaries of young rabbits. Fixed in Gilson's fluid :—

- a* = deutobroque cell in ovary of three days old rabbit.  
*b* = " " about 18 days old rabbit.  
*c* }  
*d* } = transition stages from deutobroque to ovarian cell in ovary of about  
*e* } 18 days old rabbit.  
*f* }  
*g* }  
*h* = interstitial cell } from ovary of young rabbit (18 days).  
*k* = follicle cell }



THE SEPARATION OF PHOSPHORUS FROM CASEINOGEN BY THE ACTION OF ENZYMES AND ALKALI. BY R. H. ADERS PLIMMER AND W. M. BAYLISS. (Two Figures in Text.)

(From the *Physiological Laboratory, University College, London*.)

It was found by Bayliss<sup>(1)</sup> in his experiments on the kinetics of tryptic action, that the great increase of electrical conductivity in the initial stages of a caseinogen-trypsin and a gelatine-trypsin digestion could not be accounted for by the decrease of internal friction, but was in all probability due to a separation of electrolytes from the substances themselves. In the case of caseinogen, however, the increase of electrical conductivity would be partially derived from the separation of its phosphorus as inorganic phosphates. It was with the object of determining the rate at which this latter process took place under the action of trypsin, and comparing these results with those obtained by the electrical conductivity method, that the following experiments were commenced.

Before the commencement of our researches only two experiments had been carried out on the separation of phosphorus from caseinogen by the action of trypsin. In 1895, Sebelien<sup>(2)</sup> found that caseinogen, except for a negligible residue, was completely digested, but he made no investigations with regard to the nature of the phosphorus which had gone into solution. In 1898, Biffi<sup>(3)</sup>, a pupil of Salkowski, made much more detailed experiments; he also found that, except for a minute residue, in all probability derived from the trypsin employed, the whole of the phosphorus passed into solution; of this, only about 27 per cent. was precipitated by magnesia mixture, and consequently consisted of inorganic phosphoric acid, the remainder being contained in an organic form.

We have also determined at what rate the phosphorus of caseinogen is converted into a soluble form under the action of pepsin. A great many more investigations have been made upon this subject than upon

the above. Until 1893, when Salkowski<sup>(4)</sup> commenced his experiments, it was supposed that the whole of the phosphorus of caseinogen was contained in the insoluble paranuclein, which was considered by Liebermann to be a compound of albumin with metaphosphoric acid. Only Szontagh, as quoted by Salkowski and Hahn<sup>(5)</sup>, observed that the paranuclein was further digested, and that phosphoric acid was contained in the filtrate. From Salkowski's own observations and those in conjunction with Hahn, we now know that a great part of the phosphorus of caseinogen passes into solution, and that under the most favourable conditions, such as very fine division of the caseinogen and a great excess of pepsin, the solution may be complete. The presence of phosphoric acid as observed by Szontagh could not be confirmed by Salkowski, who stated that the whole of the phosphorus was contained in a soluble organic compound. About the same time v. Moraczewski<sup>(6)</sup> also investigated this subject. He found that the whole of the phosphorus never passed into solution, but that a portion was always contained in the insoluble paranuclein, which, on further digestion, lost albumin and became richer in phosphorus. In fact, the amount of this residue depended on the length of time of the digestion and on the dilution. His results were confirmed by Sebelien<sup>(7)</sup>. The greater the amount of ferment and the greater the dilution, then the greater is the amount of phosphorus which passes into solution. Both these investigators never observed the complete solution of the caseinogen, but always found that there was a small insoluble residue, so that with regard to this point they contradict Salkowski. This small residue has been since shown by Salkowski<sup>(6)</sup> to be so minute that it is negligible. The net result is, that, under the most favourable conditions of digestion, the whole of the phosphorus of caseinogen passes into solution. Our results do not altogether agree with those of Salkowski, but on the whole they are confirmatory.

So far as we know, no detailed experiments have been made upon the digestion of caseinogen by papain, this enzyme being supposed to have an action resembling that of pepsin, rather than that of trypsin. By studying its rate of action we have found that it is intermediate in its power of dissolving caseinogen between pepsin and trypsin. For comparison, we have also investigated the action of acid and alkali on caseinogen. One per cent. caustic soda was found to almost exactly resemble trypsin in its rate of separation of the phosphorus, but with regard to the products it was totally different.

*Method.* The method which we have employed for estimating the phosphoric acid at the different stages of the digestions was that recently described by A. Neumann<sup>(9)</sup>. It consists in oxidising the organic matter with a mixture of equal parts of nitric and sulphuric acids, precipitating the phosphoric acid as ammonium phospho-molybdate, dissolving this precipitate after washing free from acid in excess of semi-normal alkali and titrating with semi-normal acid. The difference multiplied by 1.268 gives the amount of  $P_2O_5$  in milligrammes.

Neumann stated that not more than 40 c.c. of the nitric acid mixture should be employed in the oxidation on account of the hindering effect of the sulphuric acid on the precipitation of the ammonium phospho-molybdate. In certain cases it was found necessary to use more than this amount so that a modification had to be made; 10 c.c. of conc. sulphuric acid were added directly and nitric acid, instead of the nitric acid mixture, was run in until the oxidation was complete. The solution was allowed to cool before fresh quantities of nitric acid, generally about 10 c.c., were added. For this amount of sulphuric acid, complete precipitation always occurred, when 30 c.c. of the 50 per cent. ammonium nitrate solution were added to the oxidised residue dissolved in 150 c.c. of water.

The greatest difficulty, however, occurred in the filtration of the precipitate of ammonium phospho-molybdate and the washing of it free from acid. This was found to be extremely slow, and, consequently, for the number of estimations which were required, a more speedy method had to be devised. We have obtained this by employing a special pattern of filtering tube, which consists of a glass tube about 8 cm. long and 2-2.5 cm. in diameter; at one end a perforated platinum plate is sealed into it and at this point the tube is drawn out like an ordinary filtering funnel. The apparatus resembles a Buchner filtering funnel but has the shape of a Soxhlet filtering tube. Either asbestos or filter paper may be placed on the plate: in these experiments filter paper was used exclusively. This apparatus was introduced to the notice of one of us several years ago and has been employed for the most various purposes. Unfortunately we are unable to find out who is the inventor of this filtering tube, so that we have described it again in some detail.

By this means the filtration and the washing of the ammonium phospho-molybdate can be completed in less than five minutes, and we have been able to carry out over 400 determinations of phosphoric acid during the course of the last eight months. With this rapid filtration

it is quite unnecessary to use ice-cold water for washing the precipitate, the whole of which may be collected on the filter. The precipitate is then dissolved by the alkali on the filter and the solution allowed to run into the original flask, into which the remainder, including the filter paper, is washed with water.

With regard to the accuracy of the method, as modified by us, we append the values which we have obtained with a standard solution of acid potassium phosphate (10 c.c. = 20 mgm.  $P_2O_5$ ).

(1) 10 c.c. = 20.03.	(4) 10 c.c. = 19.78.	(7) 10 c.c. = 19.65.	(10) 10 c.c. = 20.03.
(2) 10 c.c. = 20.03.	(5) 10 c.c. = 19.78.	(8) 10 c.c. = 19.65.	(11) 10 c.c. = 20.67.
(3) 10 c.c. = 19.91.	(6) 10 c.c. = 19.91.	(9) 10 c.c. = 19.65.	(12) 10 c.c. = 20.67.
Mean 10 c.c. = 19.98 mm. $P_2O_5$ .			

In some cases 10 c.c. nitric acid, in others 10 c.c. sulphuric acid were present. In all cases 30 c.c. of the ammonium nitrate solution were added, this being essential for the precipitation of the ammonium phospho-molybdate. It is advisable to avoid a great excess of ammonium molybdate since molybdic acid may then be precipitated. Under these conditions the method is extremely accurate.

#### THE SEPARATION OF PHOSPHORUS FROM CASEINOGEN.

*A. Separation by Trypsin.* Of the various methods which have been adopted for studying quantitatively the action of trypsin, we have employed the one put forward first by Hedin<sup>(9)</sup> in 1904, which was subsequently utilised by Cathcart<sup>(10)</sup> and then again by Hedin<sup>(9)</sup> himself. The method consisted in precipitating the digest with tannic acid, whereby any unchanged albumin and albumoses, but not peptones except imperfectly, are precipitated, and then estimating the phosphoric acid in a measured volume of the filtrate by Neumann's method, modified as above described. This phosphoric acid may be conveniently referred to as the "soluble  $P_2O_5$ ." The materials employed were Merck's caseinogen prepared by Hammarsten's method, and "Pancreatin absolut Rhenania" generally in such amount that the digest contained 0.2—0.3 per cent. of dry enzyme. Toluene was used as the antiseptic in these experiments and in those with pepsin, etc.

At various periods of digestion at 38° C. varying from ten minutes to several days 50 c.c. samples were removed with a pipette and dropped into an equal volume of tannic acid solution (Cathcart's recipe diluted with an equal volume of water). After allowing to stand for about

12 hours, in which time the precipitate had settled, a clear liquid was obtained on filtration; in a measured volume of this the phosphoric acid was estimated. The quantities so obtained were calculated out for the whole sample taken, so that the fraction of the soluble  $P_2O_5$  split off might be readily seen when compared with the total  $P_2O_5$  which was estimated in a separate sample, generally 10 c.c., not precipitated by tannic acid. The following experiments were carried out:—

(1) 450 c.c. 5% caseinogen in NaOH.  
50 c.c. 3% trypsin.

Time	$P_2O_5$ in mgm.
0	10.6
10 mins.	19.6
30 "	23.5
1 hour	25.9
2 hours	29.0
4 "	31.5
6 "	32.8 <i>i.e.</i> $\frac{5}{8}$ of total
24 "	36.8 <i>i.e.</i> $\frac{3}{4}$ of total
Total	48.8

(2) 450 c.c. 5% caseinogen in NaOH.  
50 c.c. filtered 3% trypsin.

Time	$P_2O_5$ in mgm.
5 mins.	16.1
15 "	16.9
30 "	19.6
1 hour	22.5
2 hours	26.3
6 "	30.4 <i>i.e.</i> $\frac{5}{8}$ of total
Total	47.4

(3) 800 c.c. 5% caseinogen in NaOH.  
80 c.c. 4% filtered trypsin.

Time	$P_2O_5$ in mgm.
0	5.1
10 mins.	10.3
30 "	18.6
1 hour	23.3
2 hours	28.7
4 "	34.2
7 "	36.1
10 "	36.1
21½ "	37.6
4 days	39.8 <i>i.e.</i> $\frac{5}{8}$ of total
Total	47.9

(4) 50 grs. caseinogen in 1000 c.c. water  
+ 62 c.c.  $\frac{N}{1}$  NaOH. 4 grs. dry trypsin  
added and well shaken.

Time	$P_2O_5$ in mgm.	
	(a) Tannic acid filtrate	(b) Trichloroacetic acid filtrate
0		
30 mins.	17.4	50.6
1 hour	30.9	45.1
2 hours	37.3	
4 "	39.1	
8 "	38.5	
12 "	40.1	
24 "	40.6	
2 days	43.1	
5 "	38.5	52.8
9 "	50.2	
14 "	49.0	52.8
Total	52.6	

In Experiments 1, 2, 3, only the soluble  $P_2O_5$  in the early stages of digestion was determined. From the data it will be seen that  $\frac{5}{8}$  of the total  $P_2O_5$  passes into solution in 6 hours; this value becomes  $\frac{3}{4}$  in 24 hours, and  $\frac{5}{8}$  after 6 days. The accompanying curve shows that this soluble  $P_2O_5$  is split off in a similar way to that of the soluble nitrogen

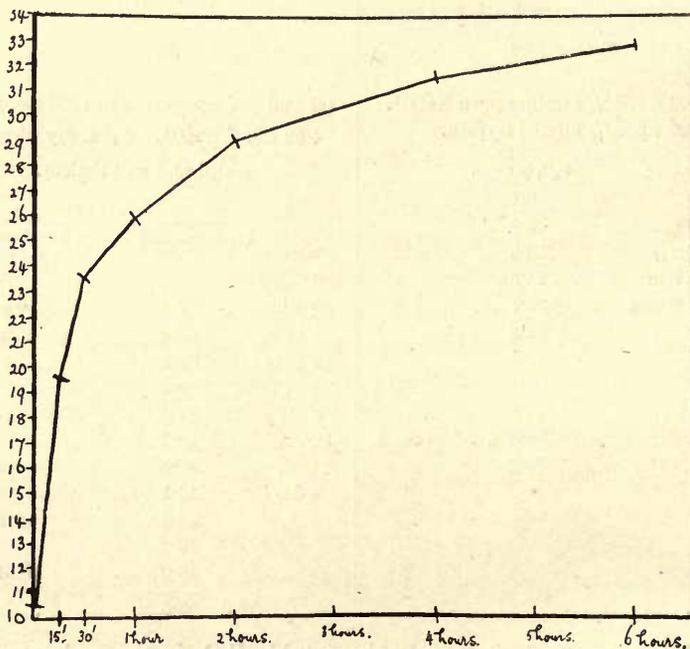
(5) 40 grs. caseinogen in 2000 c.c. water + 20 c.c.  $\frac{N}{1}$  NaOH. 130 c.c. 2% filtered trypsin.

Time	P <sub>2</sub> O <sub>5</sub> in mgm.		
	(a) Tannic acid filtrate	(b) Tri-chloroacetic acid filtrate	(c) Neutral tannic acid filtrate
0	4.2	5.7	
3½ hours	16.2	19.3	
20 "	17.7	19.1	
27 "	18.4	19.3	16.1
2 days	18.4	17.9	17.9
3 "	19.1	17.7	
5 "	19.4	19.1	
Total	20.3		

(6) 50 grs. caseinogen suspended in 1000 c.c. water. 4 grs. dry trypsin added and well shaken.

Time	P <sub>2</sub> O <sub>5</sub> in mgm.
0	7.6
8 hours	22.1
24 "	33.0
32 "	37.8
48 "	41.6
55 "	43.6
3 days	45.1
5 "	46.7
6 "	46.2
7 "	46.7
9 "	47.7
Total	52.6

as shown by Hedin<sup>(9)</sup> and corresponds in its early stages to the electrical conductivity curve as obtained by one of us<sup>(1)</sup>.



In two Experiments, 3 and 4, the electrical conductivity was determined and at the same time the soluble P<sub>2</sub>O<sub>5</sub> was estimated. The curves were found to be nearly parallel up to the period of 7 to 8 hours, the conductivity curve then continued to rise more steeply than the

curve for soluble  $P_2O_5$ . The presence of soluble  $P_2O_5$  cannot therefore account for the continued increase of electrical conductivity, though the preliminary rapid rise of electrical conductivity might be partially explained by the presence of phosphates as electrolytes<sup>1</sup>.

In Experiments 4 and 5 the determinations of soluble  $P_2O_5$  were carried on for several days, not only in the tannic acid filtrate, but also in the filtrate obtained after adding an equal volume of 5% trichloroacetic acid. Tannic acid was found to be a better precipitant than trichloroacetic acid, which, from the data, did not completely precipitate albumoses which would presumably be present after 30 minutes' digestion. In Experiment 5 tannic acid in neutral solution was tried as precipitant in two samples; under these conditions its power of precipitating was not very different to that in acid solution, so that its use was not continued. The amount of alkali in this experiment was only just sufficient to dissolve the caseinogen (Laqueur and Sackur<sup>(12)</sup> give 0.8 c.c.  $\frac{N}{1}$  alkali for 1 gm. caseinogen); in the previous experiments it was in such amount as to redden phenolphthalein.

Experiment 6 was carried out in a solution containing no alkali, so that the acidity was that of the caseinogen itself; as expected, the rate of separation of soluble  $P_2O_5$  was much slower. This is partly due to small lumps of caseinogen and partly to the retarding effect of acids upon the action of trypsin.

It will be observed that, in every experiment, the whole of the phosphorus of caseinogen did not pass into solution, a small quantity being always precipitated by the tannic acid. An indigestible residue containing phosphorus, which was precipitated on acidifying tryptic digests of caseinogen, had been observed by both Sebelien<sup>(2)</sup> and Biffi<sup>(3)</sup>, who came to the conclusion that it had no relation to the caseinogen, but was derived from the trypsin, since its amount was greater when the quantity of ferment was increased.

Upon this point we have carried out a series of experiments, since estimations of the phosphorus in trypsin did not give an amount sufficient to account for it.

20 c.c. filtered 3% trypsin solution gave 17.5 mgm.  $P_2O_5$ .

20 c.c. filtered 3% trypsin solution after precipitating by tannic acid gave 12.6 mgm.  $P_2O_5$ .

*i.e.* 0.6 gm. trypsin contains 5 mgm. residual phosphorus, and a 50 c.c. sample, when the digest contains 0.3% ferment, contains 1.2 mgm.

<sup>1</sup> This point will be dealt with in greater detail in a future paper by one of the present authors.

Firstly, autodigestion experiments of the trypsin, similar to those above described, were carried out with the following results:—

(1) 2 grs. trypsin dissolved in water.  
Filtrate 105 c.c. allowed to digest.

Time	Soluble $P_2O_5$ in mgm.
0	19.9
19 hours	21.6
2 days	23.1
3 "	20.7
6 "	19.8
	Total 26.0

(2) 5 grs. trypsin dissolved in 250 c.c. water + 5 c.c.  $\frac{N}{1}$  NaOH (*i.e.*  $< 1\%$  alkali).

Time	Soluble $P_2O_5$ in mgm.
0	7.6
1 hour	11.2
2 hours	10.6
3 "	10.8
4 "	11.4
20 "	11.4
28 "	11.1
44 "	11.6
	Total 17.1
Total filtered from insoluble residue	16.8

There is, thus, a considerable residue containing phosphorus which does not pass into the form of soluble  $P_2O_5$ , even after several days' digestion, so that the results of Sebelien and Biffi are confirmed. It will also be observed that the soluble  $P_2O_5$  is already contained in the trypsin solution as such, since on digestion this quantity is scarcely increased.

The residue, however, in the trypsin-caseinogen digest is not altogether derived from the trypsin, since experiments on the digestion by trypsin of paranuclein (the insoluble residue remaining when caseinogen is digested by pepsin) showed that its phosphorus, which amounted to 5.5 per cent.  $P_2O_5$ , was not completely converted into a soluble form. Two experiments were made:—

(1) 215 c.c. of 0.5% solution of paranuclein in NaOH just alkaline to phenolphthalein.  
20 c.c. of 3% filtered trypsin.

Time	Soluble $P_2O_5$ in mgm.
0	6.2
30 mins.	13.3
2 hours	16.1
6 "	16.5
	Total 17.3

(2) 500 c.c. paranuclein solution neutral to litmus. 50 c.c. of 2% filtered trypsin.

Time	Soluble $P_2O_5$ in mgm.
0	4.1
1½ hours	10.0
4 "	18.4
6 "	21.0
22 "	26.0
30 "	25.7
2 days	28.1
3 "	27.1
5 "	28.1
	Total 32.3

In the second experiment the total  $P_2O_5$  was estimated before adding the trypsin. There was an insoluble residue containing 4 mgm.  $P_2O_5$ , which could not therefore be derived from the trypsin; the amount obtainable in a 50 c.c. sample was less than 1 mgm., since the 50 c.c. of the 2 per cent. solutions of trypsin only contained 8 mgm.  $P_2O_5$ .

Again, in our experiments on the digestion of casein by 1 per cent. caustic soda, a similar insoluble residue containing phosphorus was left, so that the residue of a caseinogen-trypsin digestion is derived partly from the trypsin and partly from the caseinogen.

Trypsin, in the manner usually prepared, contains nucleo-proteid or its decomposition products. Nucleo-proteid is digested with difficulty by trypsin, and leaves a residue containing phosphorus. We have investigated the action of trypsin on yeast-nuclein (Merck) with regard to the rate of separation of soluble  $P_2O_5$  and soluble nitrogen in order to determine to what extent it was dissolved by trypsin:—

10 gm. yeast-nuclein dissolved in 40 c.c.  $\frac{N}{2}$  NaOH for solution and 500 c.c. of water: 1 gm. trypsin added.

Time	Soluble $P_2O_5$ in mgm.	Soluble N in grs.	Time	Soluble $P_2O_5$ in mgm.	Soluble N in grs.
0	19.5	0.0392	3 days	33.2	0.1155
1 hour	23.1	0.0553	6 "	35.2	0.1162
3 hours	24.8	0.0700	10 "	38.3	0.1218
8 "	26.4	0.0868	15 "	41.1	0.1078
24 "	29.4	0.1008	21 "	40.1	0.1022
2 days	32.5	0.1078	Total	49.5	0.1666

The yeast-nuclein was therefore digested by trypsin, but not completely, a residue containing one-fifth of the total  $P_2O_5$  and about three-fourths of the total nitrogen being left in the precipitate by tannic acid. After 10 days the amount of soluble nitrogen decreased, but we have not determined whether any synthetical action had occurred.

From the small quantity of indigestible residue left by caseinogen when it is digested by trypsin or by alkali, it seems extremely probable that this is present as impurity and is of the nature of nucleo-proteid. Traces of nucleo-proteid might be expected in milk, as a result of the disintegration of the secreting cells of the mammary glands.

The conclusions which can be drawn from these experiments are, (1) that the whole of the phosphorus of caseinogen is converted into a soluble form (*i.e.* is not precipitated by tannic acid) by the action of trypsin; (2) that the small insoluble residue is derived from nucleo-proteid which is contained partly in the caseinogen as impurity and partly in the trypsin.

Investigations have also been made on the chemical nature of the soluble  $P_2O_5$ ; these will be described more conveniently later.

*B. Separation by Pepsin.* A great deal of work has already been done upon the behaviour of the phosphorus of caseinogen, when this substance is submitted to the action of pepsin. Moraczewski<sup>(6)</sup>, Sebelien<sup>(2)</sup>, and Salkowski<sup>(4), (5), (7)</sup>, have each shown that except for a small residue the whole of the phosphorus passes into solution, but they made no determinations with regard to the rate of its solution. We have therefore made investigations upon this point, and have employed the same method as above described with trypsin, using tannic acid as precipitant and estimating the  $P_2O_5$  in the filtrate. In the earlier experiments the caseinogen was simply suspended in 0.4 per cent. hydrochloric acid solution, but, in the later Experiments, 3, 4, 5, the caseinogen was previously dissolved in the amount of alkali recommended by Salkowski<sup>(13)</sup>. In this way lumps of caseinogen were avoided, as it is reprecipitated, on acidifying in a fine state of division, so that, according to Salkowski, it can pass completely into solution under the most favourable conditions. Using this method we found that a greater quantity of the phosphorus went into solution, and also that its solution was more rapid. The following are the data which were obtained:—

(1) 20 grs. caseinogen ; 0.1 gr. pepsin. 1000 c.c. 0.4 per cent. HCl.				(2) 20 grs. caseinogen ; 0.1 gr. pepsin. 1000 c.c. 0.4 per cent. HCl.			
Time	Soluble $P_2O_5$ in mgm.	Time	Soluble $P_2O_5$ in mgm.	Time	Soluble $P_2O_5$ in mgm.	Time	Soluble $P_2O_5$ in mgm.
0	6.3	7 hours	5.9	0	5.1	3 days	10.1
30 mins.	5.4	22 "	8.1	17 hours	7.3	5 "	10.8
1 hour	6.0	51 "	10.9	24 "	7.3	10 "	13.3
2 hours	5.9	5 days	12.5	41 "	8.7	17 "	19.8
4 "	6.0			2 days	9.8	28 "	21.1
		Total	34.2			Total	34.2

The splitting-off of the phosphorus was in all cases very slow, but its rate was quite regular and comparable to that obtained by other observers on enzyme action. The whole of the phosphorus never passed into the soluble form. Even after 149 days' digestion the quantity dissolved was only about  $\frac{3}{4}$  of the total phosphorus (Experiment 4); in Experiment 5 the quantity was about  $\frac{7}{10}$  or rather less. This was probably due to the difference in the amount of acid employed, the larger quantity in Experiment 4 having possibly a slight effect in the prolonged digestion. The ratio of soluble nitrogen to total nitrogen was also determined in

this experiment and found to be  $\frac{11}{14}$  after 146 days, so that not only phosphorus but also nitrogen was contained in the insoluble residue.

(3) 20 grs. caseinogen dissolved by 10 c.c. $\frac{N}{I}$ NaOH + 100 c.c. H <sub>2</sub> O. 1000 c.c. 0.4 % HCl added. 0.2 gr. pepsin.		(4) 40 grs. caseinogen dissolved in 1500 c.c. H <sub>2</sub> O + 20 c.c. $\frac{N}{I}$ NaOH. 500 c.c. $\frac{N}{I}$ H <sub>2</sub> SO <sub>4</sub> added. 0.4 grs. pepsin. Acidity = 1 %.		(5) 20 grs. caseinogen dissolved in 200 c.c. H <sub>2</sub> O + 10 c.c. $\frac{N}{I}$ NaOH. 10 c.c. $\frac{N}{I}$ H <sub>2</sub> SO <sub>4</sub> + 800 c.c. 0.4 % HCl. 0.4 grs. pepsin.	
Time	Soluble P <sub>2</sub> O <sub>5</sub> in mgm.	Time	Soluble P <sub>2</sub> O <sub>5</sub> in mgm.	Time	Soluble P <sub>2</sub> O <sub>5</sub> in mgm.
0	13.4	0	4.2	0	2.0
1½ hours	13.7	6 hours	4.4	1 day	2.0
6 "	14.0	1 day	5.9	2 days	3.2
2 days	18.9	2 days	6.2	4 "	4.6
3 "	20.2	3 "	7.1	6 "	4.2
4 "	21.3	4 "	7.4	8 "	5.1
5 "	21.6	5 "	7.6	11 "	6.1
6 "	20.6	7 "	8.4	15 "	5.6
8 "	20.6	9 "	10.0	19 "	6.8
10 "	21.0	11 "	9.8	27 "	7.1
17 "	21.6	15 "	10.0	33 "	7.6
31 "	24.0	44 "	12.5	41 "	9.0
Total	32.3	58 "	13.2	55 "	9.1
		72 "	13.7	69 "	9.1
		95 "	13.4	92 "	12.4
		128 "	13.4	125 "	12.9
		149 "	15.7	146 "	13.2
		Total	20.3	Total	20.3

In Experiment 4 the quantity of acid was 1 per cent. H<sub>2</sub>SO<sub>4</sub>. This strength was chosen, since Langstein<sup>(14), (15)</sup>, who found that amino-acids were produced from albumins on prolonged peptic digestion, employed this quantity in his experiments and subsequently stated that 1 per cent. H<sub>2</sub>SO<sub>4</sub> produced no amino-acids from albumin after one year's action at 37° C. We have examined the effect of 1 per cent. H<sub>2</sub>SO<sub>4</sub> and our result will be mentioned later.

The fact that so much as two-thirds of the total phosphorus passed into the soluble form does not agree with the result obtained by Salkowski<sup>(16)</sup>, who stated that the whole of phosphorus of caseinogen was converted into paranucleic acid by the action of pepsin, and that this compound is precipitated by tannic acid. The prolonged digestion, which was carried out by us, is sufficient to account for the difference in our results, since paranucleic acid would most probably undergo further change and be converted into a peptone containing phosphorus, which is not thrown down by tannic acid. This explanation appears to be the

most satisfactory, since, as is shown below, the soluble  $P_2O_5$  consists only to a very small extent of inorganic phosphoric acid.

### THE ACTION OF PAPAIN ON CASEINOGEN.

Of recent years a great many investigations have been made upon the digestion of various albumins by papain. The most recent observers, Chittenden, Mendel, Harlay, all seem to agree that leucine, tryptophane, and tyrosine, the typical products of a tryptic digest, are not formed, but the various statements with regard to the medium in which papain exerts its greatest effect are very contradictory. Mendel and Underhill<sup>(17)</sup>, in 1901, found that papain acted both in acid and alkaline media, the most favourable being 1 per cent.  $NaHCO_3$  or 0.5 per cent.  $Na_2CO_3$ , but as to the actual extent and the rate of action of papain no experiments have as yet been made.

Our experiments have been carried out in precisely the same way as described above: we have determined not only the soluble  $P_2O_5$  but also the soluble nitrogen, so that a better idea of the actual change which occurred might be obtained. The results are best seen from the experimental data:—

50 grs. caseinogen dissolved in 2070 c.c. water + 30 c.c.  $\frac{N}{1}$  NaOH and divided into three portions:

Time	(1) 700 c.c. + 2 grs. papain (Merck).		(2) 700 c.c. + 15 c.c. $\frac{N}{1}$ $H_2SO_4$ = 0.1 % $H_2SO_4$ + 2 grs. papain.		(3) 700 c.c. + 3.5 grs. $Na_2CO_3$ = 0.5 % $Na_2CO_3$ + 2 grs. papain.	
	Soluble $P_2O_5$ in mgm.	Soluble N. in grs.	Soluble $P_2O_5$ in mgm.	Soluble N. in grs.	Soluble $P_2O_5$ in mgm.	Soluble N. in grs.
0	7.6	0.0560	2.5	0.0280	1.3	0.0261
1 hour	13.2	0.1064	10.1	0.0812	2.3	0.0336
3 hours	15.2	0.1155	14.2	0.1099	2.0	0.0343
21 "	17.5	0.1379	18.5	0.1463	2.8	0.0378
24 "	17.5	0.1281	19.3	0.1365	3.0	0.0434
2 days	18.5	0.1414	19.8	0.1561	3.3	0.0434
3 "	18.5	0.1400	20.5	0.1498	4.1	0.0357
5 "	19.5	0.1575	20.8	0.1540	5.1	0.0483
7 "	20.8	0.1631	—	—	5.1	0.0490
16 "	21.3	0.1764	—	—	6.8	0.0588
28 "	22.1	0.1736	—	—	8.9	0.0623
41 "	—	0.1645	—	—	10.6	0.0567
55 "	—	0.1624	—	—	11.7	0.0539
70 "	—	0.1575	—	—	12.4	0.0588
Total	21.6	0.2100	21.6	0.2128	21.6	0.1960

(4) 15 grs. caseinogen in 500 c.c. water + 10 c.c. $\frac{N}{1}$ NaOH. 30 c.c. $\frac{N}{1}$ H <sub>2</sub> SO <sub>4</sub> + 2 grs. papain = 0.2% H <sub>2</sub> SO <sub>4</sub> .			(5) 20 grs. caseinogen in 500 c.c. water + 15 c.c. $\frac{N}{1}$ NaOH. 65 c.c. $\frac{N}{1}$ H <sub>2</sub> SO <sub>4</sub> + 2 grs. papain = 0.5% H <sub>2</sub> SO <sub>4</sub> .			(6) 20 grs. caseinogen in 500 c.c. water + 15 c.c. $\frac{N}{1}$ NaOH. 1 gr. Na <sub>2</sub> CO <sub>3</sub> + 2 grs. papain = 0.2% Na <sub>2</sub> CO <sub>3</sub> .		
Time	Soluble P <sub>2</sub> O <sub>5</sub> in mgm.	Soluble N. in grs.	Time	Soluble P <sub>2</sub> O <sub>5</sub> in mgm.	Soluble N. in grs.	Time	Soluble P <sub>2</sub> O <sub>5</sub> in mgm.	Soluble N. in grs.
0	4.8	0.0383	0	0	0.0336	0	3.3	0.0315
$\frac{1}{2}$ hour	6.6	0.0665	1 day	3.5	0.0357	1 day	8.1	0.0637
1 "	8.4	0.0819	2 days	3.8	0.0364	2 days	11.4	0.0784
3 hours	11.9	0.1008	3 "	2.5	0.0343	3 "	13.7	0.0896
8 "	16.0	0.1281	5 "	1.0	0.0231	5 "	17.5	0.0966
24 "	20.0	0.1477	7 "	0.8	0.0231	7 "	19.5	0.0934
2 days	21.8	0.1701	13 "	3.3	0.0259	13 "	22.1	0.1064
3 "	24.3	0.1869	The samples in this experiment could not be taken with a pipette since the caseinogen was precipitated in lumps on adding the acid which could not be taken up in this way. They were therefore taken in a measuring cylinder and hence the large variations in the results.			17 "	25.1	0.1078
6 "	26.4	0.1939				22 "	26.1	0.1176
10 "	28.7	0.2128				29 "	26.6	0.1232
15 "	29.4	0.1979				42 "	32.3	—
Total	29.2	0.2772	Total	39.9	0.2982			

Papain, thus, exerts its greatest action on caseinogen in a neutral or faintly acid medium, and resembles trypsin more than pepsin with regard to the amounts of P<sub>2</sub>O<sub>5</sub> and N converted into a soluble state. In alkaline media, even in 0.2 per cent. Na<sub>2</sub>CO<sub>3</sub> solution, the action was very slow, and resembled pepsin, but was more rapid. The results do not agree with those of the previous observers, but can be easily explained by the difference in the method employed. Their methods were to obtain acid or alkali albuminate on neutralisation, or to observe the solution of fibrin under the action of papain, methods which give no true estimate of the amount of action.

The net result of our experiments is that papain acts best in media which are neutral, or very slightly acid or alkaline; its power of hydrolysing albumins is intermediate between that of pepsin and trypsin; it resembles the latter rather than the former in its rate of dissolving caseinogen and converting it into substances which are not precipitated by tannic acid.

#### DIGESTION OF OVOVITELLIN BY TRYPSIN AND BY PEPSIN.

The digestion of ovovitellin by enzymes has up to the present time been very little investigated. Beyond the facts that ovovitellin is a nucleo-albumin, like caseinogen, and that it leaves a residue of paranuclein on peptic digestion, no observations as to the actual extent of its digestion by ferments have been made. We have therefore studied

this in a similar manner to that of caseinogen with the following results:—

Ovovitellin from 15 egg-yolks in 1500 c.c. water + 7.5 grs.  $\text{Na}_2\text{CO}_3$ . 2 grs. Rhenania trypsin.

Time	Soluble $\text{P}_2\text{O}_5$ in mgm.	Soluble N. in grs.
0	8.1	0.0182
1 hour	8.6	0.0322
2 hours	9.9	0.0381
3 "	10.6	0.0455
4 "	11.7	0.0511
5 "	12.2	0.0567
6½ "	11.9	0.0588
24 "	14.4	0.1057
30 "	15.7	0.1113
2 days	16.5	0.1197
3 "	18.3	0.1288
6 "	20.5	0.1484
8 "	22.1	0.1547
12 "	22.3	0.1652
15 "	21.8	0.1645
21 "	23.3	0.1666
29 "	24.6	0.1722
36 "	24.6	0.1694
Total	43.1	0.1820

Ovovitellin from 10 egg-yolks in 700 c.c. 0.4 per cent. HCl. 0.6 gr. pepsin + 0.5 gr. pepsin on 8th day.

Time	Soluble $\text{P}_2\text{O}_5$ in mgm.	Soluble N. in grs.
0	10.1	0.0280
1 day	10.4	0.0784
2 days	11.4	0.0931
3 "	11.7	0.0980
4 "	13.2	0.1064
5 "	10.9	0.1113
8 "	14.2	0.1281
9 "	15.5	0.1344
10 "	13.9	0.1337
11 "	16.2	0.1435
12 "	17.0	0.1498
14 "	17.0	0.1547
16 "	18.0	0.1631
18 "	18.8	0.1666
21 "	19.5	0.1715
24 "	20.5	0.1792
Total	72.9	0.3262

The ovovitellin employed was obtained from egg-yolks by repeatedly extracting them with ether until the residue was quite colourless. This residue was then taken and digested by the enzyme.

The digestion of ovovitellin by trypsin was very slow in comparison with that of caseinogen. In 24 hours only one-third of the total phosphorus and about one-half of the nitrogen were converted into a soluble form. Even in 36 days little more than half the total phosphorus had undergone conversion, though practically the whole of the nitrogen had passed into the soluble form.

The digestion by pepsin was extremely slow. In 24 days less than one-third of the total phosphorus had been changed into a soluble form and at the same time the amount of soluble nitrogen was rather more than one-half. There is, in fact, a great resemblance to the caseinogen-pepsin digestion.

As is well known, it is extremely difficult to prepare ovovitellin free from lecithin. When it is obtained in a state of purity, free or nearly free from lecithin, it no longer has the same properties, such as solubility

in saline solutions, which it had before purification. It is supposed therefore to be combined with lecithin and to be a lecith-albumin. Since we made no attempt to purify the ovovitellin it may be assumed that the phosphorus, still remaining in an insoluble form, may be contained to a large extent in the lecithin portion of the molecule. (Lecithin, being an ester, would not be acted on by trypsin unless lipase were also present. The presence of lipase is, however, very improbable since aqueous extracts of dried trypsin preparations do not possess any lipolytic power. Lipase is generally only obtainable from fresh preparations of pancreas<sup>1</sup>.) If this should be the case, then half of the phosphorus of ovovitellin is combined in the form of lecithin. Lecithin, again, contains only a very small amount of nitrogen. In our experiment, the whole of the nitrogen, except about one-ninth, was converted into a soluble form. This result bears out our suggestion that the lecithin portion of the ovovitellin contains about one-half of the total phosphorus.

#### DIGESTION OF CASEINOGEN BY ACID AND BY ALKALI.

It was shown by Langstein<sup>(14)</sup> that, when albumins were digested by pepsin in 1 per cent. sulphuric acid solution at 37° C. for one year, they were converted to a small extent into amino-acids. His results were criticised by Salaskin and Kath. Kowalevsky<sup>(18)</sup>, who suggested that the separation of these simple products were due to the action of the 1 per cent. sulphuric acid for the prolonged period of one year. Langstein<sup>(15)</sup> replied to this criticism and quoted an unpublished experiment by Neuberg on the digestion of gelatine by 1 per cent. acid which confirmed his result. We have made an experiment upon this point and have found that at any rate no soluble  $P_2O_5$  was separated in the course of 4 days by 1 per cent.  $H_2SO_4$ , the figures being 3.4 mgm.  $P_2O_5$  at zero time and 3.5 mgm.  $P_2O_5$  after 4 days' digestion. As soon as pepsin was added separation of soluble  $P_2O_5$  took place, so that we can in so far confirm Langstein's result. The experiment was not continued, since it was of greater interest to us to determine what strength of acid produced soluble  $P_2O_5$ . By employing about 15 per cent. hydrochloric acid (equal volumes of HCl sp. gr. 1.16 and water) we observed a somewhat rapid rate of separation of soluble  $P_2O_5$  from caseinogen, comparable to that of pepsin.

<sup>1</sup> H. Engel has quite recently found that Rhenania pancreatin "absolut" when extracted with glycerine yields an active lipase.

50 grs. caseinogen suspended in 500 c.c. water + 500 c.c. HCl. Sp. gr. 1.16.

Time	Soluble P <sub>2</sub> O <sub>5</sub> in mgm.	Time	Soluble P <sub>2</sub> O <sub>5</sub> in mgm.
0	6.8	3 days	25.1
21 hours	17.3	5 "	27.8
1 day	19.2	10 "	32.7
2 days	23.2	28 "	44.0
		Total 44.7	

We have not made any further experiments upon the action of acids on caseinogen, since this subject has been investigated in great detail by Goldschmidt<sup>(10)</sup> from another point of view, and we found that the results obtained by the action of 1 per cent. alkali were of far greater interest.

Having found that 1 per cent. sulphuric acid produced no separation of soluble P<sub>2</sub>O<sub>5</sub> from caseinogen in 4 days, we investigated the action of alkali of the same strength. A great difference was observed. Soluble P<sub>2</sub>O<sub>5</sub> was separated at a rate almost identical with that obtained in the trypsin digestions. The whole of the phosphorus was split off in 24 hours, as the following data show:—

(1) 40 grs. caseinogen in 1500 c.c. water + 20 c.c. $\frac{N}{1}$ NaOH.		(2) 10 grs. caseinogen in 375 c.c. water + 5 c.c. $\frac{N}{1}$ NaOH.		(3) 80 grs. caseinogen in 1500 c.c. H <sub>2</sub> O + 50 c.c. $\frac{N}{1}$ NaOH.	
	500 c.c. $\frac{N}{1}$ NaOH.		125 c.c. $\frac{N}{1}$ NaOH.		500 c.c. $\frac{N}{1}$ NaOH.
Time	Soluble P <sub>2</sub> O <sub>5</sub> in mgm.	Time	Soluble P <sub>2</sub> O <sub>5</sub> in mgm.	Time	Soluble P <sub>2</sub> O <sub>5</sub> in mgm.
5 mins.	3.1	0	1.7	0	4.6
1½ hours	7.4	1 day	18.3	2 hours	14.2
5 "	12.9	2 days	18.5	5 "	24.8
25 "	18.6	4 "	19.0	8 "	29.4
2 days	19.0	6 "	19.5	24 "	36.5
5 "	18.7	7 "	18.8	28 "	37.0
8 "	19.1	8 "	18.5	32 "	36.5
12 "	20.3	9 "	20.0	48 "	36.5
Total	21.6	12 "	18.3	Total	39.3
		Total	20.9		

In every case the caseinogen was previously dissolved in a small quantity of alkali. It might be supposed that even this small quantity would cause a separation of soluble P<sub>2</sub>O<sub>5</sub>, but two experiments to determine this showed that no splitting-off of phosphorus occurred. In 24 hours in one experiment there was increase of 0.6 mgm. soluble P<sub>2</sub>O<sub>5</sub>, in another experiment there was no increase of soluble P<sub>2</sub>O<sub>5</sub> after 21 hours. Thus, very dilute alkali sufficient only to dissolve the caseinogen caused no splitting-off of soluble P<sub>2</sub>O<sub>5</sub>. The results show

clearly that, except for quite a small residue, the whole of the phosphorus of caseinogen goes into solution in 24 hours. The residue in the trypsin-caseinogen digestion is thus partly derived from the caseinogen.

In two other experiments we also determined the rate at which soluble nitrogen was produced by 1 per cent. caustic soda, at the same time making determinations of the soluble  $P_2O_5$ . The results are best seen from the data and the accompanying curve (Exp. 5):—

(4) 20 grs. caseinogen in 750 c.c.  $H_2O$   
+ 10 c.c.  $\frac{N}{I}$  NaOH. 250 c.c.  $\frac{N}{I}$  NaOH.

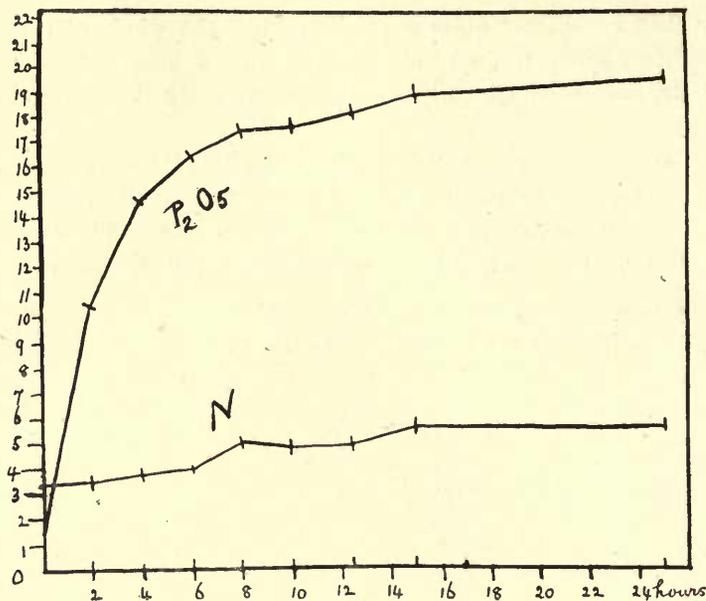
(5) 20 grs. caseinogen in 750 c.c.  $H_2O$   
+ 10 c.c.  $\frac{N}{I}$  NaOH. 250 c.c.  $\frac{N}{I}$  NaOH.

Time	Soluble $P_2O_5$ in mgm.	Soluble N. in grs.
0	1.5	0.0147
1 hour	6.6	0.0168
2 hours	8.1	0.0161
3 "	11.8	0.0175
4 "	11.7	0.0189
5 "	13.2	0.0217
6 "	13.7	0.0203
7 "	15.2	0.0231
8 "	15.5	0.0217
24 "	17.7	0.0238
28 "	17.7	0.0224
32 "	17.3	0.0231
48 "	17.5	0.0231
30 days	17.3	0.0357
Total	19.6	0.1316

Time	Soluble $P_2O_5$ in mgm.	Soluble N. in grs.
0	1.5	$0.0168 \div 5 = 3.36$
2 hours	10.6	$0.0175 = 3.5$
4 "	14.7	$0.0189 = 3.8$
6 "	16.5	$0.0203 = 4.0$
8 "	17.5	$0.0252 = 5.0$
10 "	17.7	$0.0245 = 4.9$
12½ "	18.3	$0.0245 = 4.9$
15 "	19.0	$0.0280 = 5.6$
25 "	19.5	$0.0280 = 5.6$
Total	21.1	$0.1449 = 28.98$

Contrary to expectation the soluble nitrogen scarcely increased in 24 hours whereas the soluble  $P_2O_5$  in this time reached a maximum. It seems as if the 1 per cent. alkali had the special effect of only splitting off soluble  $P_2O_5$  from caseinogen.

During the action of the 1 per cent. caustic soda it was noticed that the digest became distinctly opalescent. On exact neutralisation with the calculated quantity of sulphuric acid there was no change, but on slightly acidifying (not sufficient to turn congo red paper blue) a voluminous precipitate was thrown down and the solution became almost free from opalescence. We have made a few preliminary experiments upon this precipitate, and have obtained a small quantity as a white dry powder by redissolving in alkali and reprecipitating and then drying with alcohol and ether. Its composition is under investigation. The acid solution, which remained, contained albumoses precipitable by half saturation and complete saturation with ammonium sulphate.



#### THE SOLUBLE P<sub>2</sub>O<sub>5</sub>.

It was found by Biffi<sup>(3)</sup> that 27 per cent. of the total phosphorus of caseinogen was converted into inorganic phosphoric acid by the action of trypsin, the remainder being combined in an organic form not precipitable by magnesia mixture. It might be concluded from our results that the whole of the phosphorus was changed into inorganic phosphoric acid. The precipitation of albumoses and peptones by tannic acid, however, is known to be by no means complete, at any rate in acid solution such as we have employed, and a further investigation of the nature of this soluble P<sub>2</sub>O<sub>5</sub> was necessary. For this purpose we employed the method of precipitation of phosphates as ammonium magnesium phosphate without previously oxidising the organic matter.

(1) *The soluble P<sub>2</sub>O<sub>5</sub> obtained by the action of 1 per cent. caustic soda.* The opalescent solution obtained by the action of 1 per cent. alkali on caseinogen was first slightly acidified, in order to remove the substance thrown down by acid, as above mentioned, in order that the solution should contain less albuminous matter, which might hinder the precipitation of the ammonium magnesium phosphate. The acid solution so obtained was then employed directly. It was made alkaline

with ammonia in the usual way and excess of magnesia mixture was added. This gave immediately a small white precipitate, which caused the values to be erroneous as the figures show:—

50 c.c. oxidised and precipitated by Neumann's method	gave 27.01 mgm. $P_2O_5$ .	
50 c.c. precipitated by magnesia mixture	gave 36.13 mgm.	} mean = 35.18 mgm. $P_2O_5$ .
50 " " " " " "	34.23 " "	

This error, however, was entirely obviated by employing the magnesium citrate method instead of magnesia mixture for precipitating the phosphoric acid. No precipitation occurred on its immediate addition, but the ammonium magnesium phosphate was thrown down on stirring vigorously:—

50 c.c. precipitated by magnesium citrate	gave 25.74 mgm.	} mean = 26.42 mgm. $P_2O_5$ .
50 c.c. " " " " " "	27.13 " "	
50 c.c. " " " " " "	27.05 " "	
50 c.c. " " " " " "	25.74 " "	

The phosphoric acid is thus completely precipitated by this method, the small difference of 0.59 mm. being due to experimental error and not to incomplete precipitation in presence of albuminous matter. We have proved this by making further determinations in the presence of a known volume of standard acid potassium phosphate solution (10 c.c. = 20 mm.  $P_2O_5$ ) as follows:—

0 c.c. + 10 c.c. $KH_2PO_4$ solution precipitated by magnesium citrate	gave 46.40 mgm.	} mean = 46.19 mgm. $P_2O_5$ .
0 c.c. + 10 c.c. $KH_2PO_4$ " " " " " "	46.15 " "	
0 c.c. + 10 c.c. $KH_2PO_4$ " " " " " "	46.02 " "	

Hence 50 c.c. gave 26.19 mgm.  $P_2O_5$ .

Thus, the soluble  $P_2O_5$  split off from caseinogen by the action of 1 per cent. caustic soda consists entirely of inorganic phosphoric acid. The small quantity of less than one milligramme which is not precipitated is negligible. It could be demonstrated by oxidising the filtrate from the ammonium magnesium phosphate by Neumann's method and precipitating as ammonium phospho-molybdate. In all probability it is derived from the small quantity of nucleoproteid present in the caseinogen as above mentioned.

(2) *The soluble  $P_2O_5$  obtained by the action of trypsin.* The solution remaining in Experiment (5) was employed after the digestion had been continued for nearly two months. It contained a small precipitate from which it was filtered and the filtrate was examined in the following way:—

50 c.c. precipitated by tannic acid and oxidised by Neumann's method &c. gave 20.79 mgm.  $P_2O_5$ .

50 c.c. contained (*i.e.* 10 c.c. oxidised directly by Neumann's method, &c.) 20.92 mgm.  $P_2O_5$

*i.e.* the total phosphorus was present in a soluble form.

50 c.c. precipitated by magnesium citrate gave 6.59 mgm. }  
50 c.c. " " " " " 7.64 " } mean = 7.11 mgm.  $P_2O_5$ .

The inorganic phosphoric acid was completely precipitated, as was shown by the following experiments where 10 c.c. standard acid potassium phosphate were also added :

50 c.c. + 10 c.c.  $KH_2PO_4$  solution precipitated by magnesium citrate gave 27.64 mgm. } mean = 27.45 mgm.  
50 c.c. + 10 c.c.  $KH_2PO_4$  " " " " " 27.26 mgm. }  $P_2O_5$

*i.e.* 50 c.c. gave 7.45 mgm.  $P_2O_5$ .

Thus, by the action of trypsin on caseinogen, the whole of its phosphorus is not split off as in organic phosphoric acid but only 35 per cent. This value is 8 per cent. higher than that obtained by Biffi, but the discrepancy in the values can be accounted for by the length of time of the digestion, which, in our experiment, was considerable.

Since 1 per cent. alkali separated the whole of the phosphorus of caseinogen as inorganic phosphoric acid and trypsin only 35 per cent., the question arose, Will 1 per cent. caustic soda convert the remaining 65 per cent. of the soluble  $P_2O_5$ , *i.e.* the phosphorus contained in organic combination of a trypsin-caseinogen digestion, into inorganic phosphoric acid? To determine this, to 200 c.c. of the above trypsin digest were added 100 c.c. water and 100 c.c.  $\frac{N}{1}$  NaOH solution. These quantities bring the percentage of alkali to 1 per cent. A precipitate was obtained on adding the caustic soda; this was filtered off and the clear filtrate was kept at 38° C. for 40 hours and then examined for inorganic phosphoric acid :—

50 c.c. precipitated by magnesium citrate gave 4.95 mgm. } mean = 5.26 mgm.  
50 c.c. " " " " " 5.58 " }  $P_2O_5$   
50 c.c. + 10 c.c.  $KH_2PO_4$  solution precipitated by mag- }  
nesium citrate gave 24.34 mgm. } mean =  
50 c.c. + 10 c.c.  $KH_2PO_4$  solution precipitated by mag- } 24.91 mgm. } mean = 5.03 mgm.  
nesium citrate gave 25.48 mgm. }  $P_2O_5$

*i.e.* 50 c.c. gave 4.91 mgm.  $P_2O_5$ .

The original solution was diluted by an equal volume of water and alkali;  $2 \times 5.03 = 10.06$  mgm.  $P_2O_5$  are therefore precipitated after the action of 1 per cent. caustic soda, an increase of  $10.06 - 7.45 = 3.61$  mgm. This corresponds to 50 per cent. of the total soluble  $P_2O_5$ .

A similar result had been arrived at by Biffi<sup>(9)</sup>, who, on boiling a trypsin digestion of caseinogen with barium carbonate, noticed an increase of inorganic phosphoric acid from 27 per cent. to 56 per cent. He also obtained inorganic phosphoric acid on boiling his trypsin digestion with dilute caustic soda after previously removing the inorganic phosphoric acid already present, but he made no estimation of its amount.

There is, therefore, a very marked difference in the action of 1 per cent. caustic soda on caseinogen directly and on caseinogen previously digested by trypsin. In the former case, the total phosphorus is split off as inorganic phosphoric acid, in the latter only 50 per cent. of the total phosphorus is separated in this form. We are not yet able to explain this difference, but it seems that the manner of the combination of the phosphorus in caseinogen undergoes some change when it is acted upon by trypsin; if combined in the same way in the products produced by trypsin as in the caseinogen itself it would be split off by 1 per cent. caustic soda in 24 hours as inorganic phosphoric acid. The isolation of this body containing phosphorus which is produced by the action of trypsin and is resistant to 1 per cent. alkali is at present under investigation, but we may state that it is completely precipitated by ferric alum in the same way as paranucleic acid obtained by the action of pepsin on caseinogen (Salkowski<sup>(10)</sup>).

(3) *The soluble P<sub>2</sub>O<sub>5</sub> obtained by the action of pepsin.* The clear solutions filtered from the paranuclein of Experiments (4) and (5) were employed and the following results were obtained:—

50 c.c. precipitated by magnesium citrate gave 1·77 mgm.	} mean = 2·10 mgm. P <sub>2</sub> O <sub>5</sub>
50 c.c. + 10 c.c. KH <sub>2</sub> PO <sub>4</sub> solution precipitated by magnesium citrate gave 22·44 mgm.	
i.e. 50 c.c. gave 2·44 mgm.	
50 c.c. precipitated by magnesium citrate gave 0·63 mgm.	} mean = 0·96 mgm. P <sub>2</sub> O <sub>5</sub>
50 c.c. + 10 c.c. KH <sub>2</sub> PO <sub>4</sub> solution precipitated by magnesium citrate gave 21·3 mgm.	
i.e. 50 c.c. gave 0·96 mgm.	

Thus, by the action of pepsin on caseinogen only a minute quantity of inorganic phosphoric acid is formed. Salkowski in his experiments obtained no inorganic phosphoric acid. Hence the soluble P<sub>2</sub>O<sub>5</sub> obtained by the action of pepsin on caseinogen is combined in an organic form.

## SUMMARY.

1. The whole of the phosphorus of caseinogen, except for a very small residue, is converted into a soluble form by the action of trypsin in 24 hours.

2. The curve for its rate of separation is exactly parallel to the curve of the electrical conductivity during the first 7—8 hours; its rate of separation after this time is less rapid.

3. The small insoluble residue is partly derived from the trypsin and partly from the caseinogen and consists most probably of the products of decomposition of nucleo-proteid.

4. The "soluble  $P_2O_5$ " consists of inorganic phosphoric acid—35 per cent.—and organic phosphorus, *i.e.* phosphorus combined in an organic form, 65 per cent.

5. The phosphorus of caseinogen is very slowly converted into a soluble form by the action of pepsin. The quantity, thus changed is only 70 per cent. in 149 days, and consists, except for a negligible quantity of inorganic phosphoric acid, of organic phosphorus.

6. Papain, in its action on caseinogen, is intermediate in power between pepsin and trypsin. In its rate of splitting-off of "soluble  $P_2O_5$ " and "soluble nitrogen" it resembles trypsin the more closely, but it is much slower.

It acts best in a neutral or faintly acid medium; its action is slower in a slightly alkaline medium and is almost inhibited by 0.5 per cent. sulphuric acid and 0.5 per cent. sodium carbonate.

7. Ovovitellin, containing lecithin, is very slowly digested by trypsin in comparison with caseinogen. Only one-half of its phosphorus is converted into a soluble form in 36 days. A similar quantity is probably contained in the lecithin portion of the molecule.

8. One per cent. caustic soda converts the whole of the phosphorus of caseinogen into "soluble  $P_2O_5$ " in 24 hours. It resembles trypsin very closely in its rate of action.

9. The "soluble  $P_2O_5$ " produced by 1 per cent. caustic soda in 24 hours consists entirely of inorganic phosphoric acid. In the same time the quantity of "soluble nitrogen" scarcely increases.

10. The organic phosphorus produced by the action of trypsin on caseinogen is not completely converted into inorganic phosphoric acid by the action of 1 per cent. of caustic soda. The total quantity of inorganic phosphoric acid produced by trypsin and subsequently by 1 per cent. caustic soda is 50 per cent. of the total phosphorus of caseinogen.

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ON METHODS SUPPOSED TO LOCALIZE PHOSPHORUS  
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*Introduction.* The various tests for phosphorus which have been proposed depend on the use of a solution of ammonium molybdate in nitric acid. It has been assumed that the nitric acid would liberate the phosphorus from its organic combinations as orthophosphate, and that this would then be precipitated as ammonium phosphomolybdate. To distinguish the yellow precipitate from the yellow colour produced by the action of the nitric acid on the proteid, agents have been employed to reduce the phosphomolybdate to one of the coloured oxides of molybdenum. The dark coloration obtained in this way was considered to indicate the presence of phosphorus.

Lilienfeld and Monti<sup>(1)</sup>, who were the first to try to localize the compounds of phosphorus in cells, after first treating the tissues with the nitromolybdate reagent then treated them with a solution of pyrogallol, which turned parts of the tissue yellow, brown or black. These colours were believed to indicate the presence of phosphorus. Raçiborski<sup>(2)</sup>, in a criticism of Lilienfeld's and Monti's work, pointed out that pyrogallol, both in the test tube and in tissues, reduces ammonium phosphomolybdate to the blue oxide of molybdenum while it blackens ammonium molybdate. He therefore regarded Lilienfeld and Monti's results (yellow, brown or black) as due to the reduction of ammonium molybdate which had not been washed from the tissues. He also pointed out that the nitromolybdate reagent will not react with nuclein compounds even after 48 hours, while on the other hand these nuclein compounds have a great power of retaining ammonium molybdate and are therefore blackened on subsequent treatment with pyrogallol. The same affinity of nucleins for ammonium molybdate was independently pointed out by Gilson<sup>(3)</sup>.

Pollacci<sup>(4)</sup> using zinc chloride as the reducing agent found colours varying from dark blue to grey which he considered to indicate the presence of phosphorus. Heine<sup>(5)</sup>, however, found that phosphorus-free as well as phosphorus-holding bodies would, after treatment with the nitromolybdate reagent, turn blue with zinc or stannous chloride.

Macallum<sup>(6)</sup>, believing in the principle of the Lilienfeld and Monti reaction, used phenyl-hydrazine as the reducing reagent since, in the presence of acids, this will reduce the phosphomolybdate but leave ammonium molybdate unaffected. This is a great improvement on zinc or stannous chloride, as it is impossible to wash the sections free from adsorbed ammonium molybdate. The reaction with phenyl-hydrazine has been used more or less extensively as a micro-chemical test for phosphorus,—among others by the writer<sup>(7)</sup>. It has, however, recently been pointed out by Bensley<sup>(8)</sup> that this reaction of Macallum, when applied to tissues, often gives misleading results and shows a green colour in the tissue where nuclein compounds do not exist. On investigation Bensley discovered that the blue colour produced by the phenylhydrazine was due to the reduction of adsorbed molybdic acid and had nothing to do with phosphomolybdate, as his results show there could be no phosphomolybdate present in the sections. The reaction with phenylhydrazine, if Bensley be correct, is therefore no test for phosphorus.

*Observations.* I thought it desirable to determine in the first place whether the yellowing of the tissues, which is produced by the nitromolybdate reagent, might not be taken as sufficient evidence of the presence of phosphorus.

In the nitromolybdate reagent there are two factors tending to cause a yellowing of the tissue, viz. the nitric acid producing the xanthoproteic reaction, and the formation of ammonium phosphomolybdate from any inorganic orthophosphate which may be present. On investigation it was found that, if great excess of acid be avoided, a solution of ammonium molybdate in hydrochloric acid is as good a precipitant of orthophosphate as a solution in nitric acid. The reagent I used had the following proportions:

Ammonium molybdate 10% solution	80 c.c.
Hydrochloric acid sp. gr. 1.16	12 c.c.
Ammonium chloride	20 grs.
Potassium persulphate saturated solution	10 c.c.

The molybdate is poured slowly into the acid with continued shaking, then the chloride added, and when this has dissolved the

persulphate also added. The mixture should be allowed to stand for some time in order to allow any phosphate, which may be present in the reagents, to be precipitated.

The above reagent is as sensitive as the nitromolybdate reagent and will give an undoubted reaction with as little as .005 mgm. of sodium phosphate. The addition of an oxidizing agent (persulphate, hydrogen peroxide, chromic acid) is only necessary when the reagent is to be applied to tissues, but without such oxidizing agent the tissues will turn blue owing to the reduction of the molybdic acid. (Bensley observed the same for a solution of molybdic acid in hydrochloric acid.)

Sections of alcohol hardened material may be left in this reagent for days at 37°, and if they have been properly freed from inorganic phosphate no yellowing of the tissue is seen. Sections of testis (frog) were chiefly used in this work but sections of bone marrow, liver, pancreas and cerebellum were also tried. The nuclei of the fresh blood corpuscles of the newt do not turn yellow in this reagent even after 4 or 5 days. The same absence of effect is noticed if instead of sections small pieces of tissue are taken. If these have been freed from inorganic phosphate they do not show any yellow coloration, but if they contain inorganic phosphate they give an immediate reaction. Even lecithin compounds do not give a reaction (turn yellow) either with the nitro- or the hydrochloric molybdate reagent. If, however, small masses of lecithin are put in either of these reagents one finds after a few days a crop of phosphomolybdate crystals on the sides of the flask. This shows there is no localizing action, and that even from such compounds as lecithin the first effect of the reagent is to liberate some soluble compound of phosphorus which, however, is not acted upon by the reagent until it is further hydrolysed. Lecithin may be boiled with acids on a water bath and, unless the hydrolysis be continued for 4 or 5 hours, no inorganic orthophosphate is formed.

The reason for the absence of results with these micro-chemical reagents has been found by a study of the hydrolysis of tissues with acids at various temperatures. This work has been done in conjunction with Dr R. H. Aders Plimmer who has kindly allowed me to make use of these results. The method followed was that used by Bayliss and Plimmer<sup>(9)</sup> in their work on the liberation of phosphorus from caseinogen. The acid was added to the ground tissue (ox testis), thoroughly mixed, and then a sample (50 c.c.) withdrawn in a pipette and dropped into 50 c.c. of tannic acid reagent (Cathcart's<sup>(10)</sup> formula + an equal volume of water) and allowed to stand till the next day

when it was filtered. A definite volume of the filtrate was taken and the phosphorus determined after oxidation by the Neumann method as modified by Plimmer. The phosphorus in this tannic acid filtrate we term soluble phosphorus. The amount of inorganic phosphate in the digest was determined by first filtering and then throwing down the phosphate in a definite volume of the filtrate with ammonia and magnesium citrate. That the organic substances present did not inhibit the precipitation of the ammonium magnesium phosphate was shown by the result of control experiments in which a definite amount of inorganic phosphate was added to a sample before precipitation. After the precipitate had been dried, burnt and weighed, and the amount deducted for the quantity of phosphate added, the remainder was found to correspond with the amount obtained from a sample of the same fluid to which no inorganic phosphate had been added. The action of acid in liberating the phosphorus from tissue will be seen in the following tables.

TABLE I. *Ox testis, fresh.* 510 c.c.  $\frac{2N}{1}$  HNO<sub>3</sub> added to 170 grs. of tissue. Kept at 38°.

		Mgms. P <sub>2</sub> O <sub>5</sub> in 50 c.c. of digest as 'soluble' phosphorus
Sample 0	taken as soon as possible	contained 43.8
" 1	taken after 19 hours	contained 59.2
" 2	" 24 "	62.7
" 3	" 43 "	64.1
" 4	" 48 "	64.6
" 5	" 67 "	65.2
" 6	" 91 "	64.8
Total phosphorus in 50 c.c.		= 72.3

In the above table two facts are to be noticed, first, the ease with which most of the phosphate present is converted into 'soluble' phosphorus, and second, that there is a residue of phosphorus which does not pass into the soluble state. The large amount of phosphorus present in sample 0 is partly due to the difficulty in obtaining the first sample. The inorganic phosphate was not determined in this case at the commencement, but 2 days after sample 6 was taken it was found to be only 18 mgms. of P<sub>2</sub>O<sub>5</sub> in 50 c.c. Thus although the acid liberates  $\frac{5}{8}$  of the phosphorus as 'soluble' phosphorus yet only  $\frac{1}{4}$  of it was present as inorganic phosphate. Subsequent experiments show that this inorganic phosphate is not derived from nucleins but from inorganic salts originally present in the tissue.

Table II shows that hydrochloric acid liberates the phosphorus in a similar manner to nitric acid. The amount of nitrogen in the filtrate was also determined.

TABLE II. *Ox testis, fresh.* 880 c.c. HCl (contained 130 c.c. HCl, sp. gr. 1.16) added to 340 grs. of testis. Kept at 38°.

	Mgms. $P_2O_5$ in 50 c.c. of digest as 'soluble' phosphorus	Grs. of nitrogen in 50 c.c. of digest (after filtration)
Sample 0 at commencement contained	19.3	0.0189
„ 1 after 16 hours contained	45.4	0.0364
„ 2 „ 24 „	50.5	0.0434
„ 3 „ 2 days contained	55.5	0.0470
„ 4 „ 3 „	59.0	0.0595
„ 5 „ 8 „	60.8	0.0896
„ 6 „ 12 „	62.6	0.1057
„ 7 „ 19 „	62.9	0.1123
	Total in 50 c.c. = 68.5	Total = 0.2156

Inorganic phosphate at time of sample 5 = 18.3 mgms.  $P_2O_5$ .

Experiments were also made with other strengths of nitric and hydrochloric acid and with sulphuric acid, but as the results show the same general features the figures are not given.

Much more interesting from our present standpoint were the experiments carried out on testis which had been coagulated with alcohol and then extracted with hot alcohol and ether until all the phosphorus compounds soluble in these fluids had been removed. This material still contained considerable quantities of inorganic phosphate, but experiments with it showed that while, under the influence of acids, the 'soluble' phosphorus increased in the usual manner, the inorganic phosphate remained constant. As these experiments were complicated by this inorganic phosphate, which can readily be removed by extraction with water, I will proceed to the experiments with the tissue from

TABLE III. *Ox testis, free from lecithin-like compounds, and also from inorganic phosphates, i.e. testis containing presumably only nucleic compounds of phosphorus.* To 100 grs. of moist material 500 c.c.

$\frac{2N}{1}$   $HNO_3$  added.

	Mgms. $P_2O_5$ in 50 c.c. of digest as 'soluble' phosphorus	Inorganic phosphate
Sample taken at commencement contained	2.8	Absent
„ after 24 hours contained	14.2	„
„ „ 48 „	16.2	„
„ „ 168 „	17.5	„
	Total phosphorus in 50 c.c. = 24.0	

which this inorganic phosphate had also been removed. Table III shows that from such material no phosphorus is liberated as inorganic phosphate, i.e. capable of being precipitated with ammonia and magnesium citrate, by digestion with acids for seven days at 38°.

The above table shows that, from the nuclein bodies, acids liberate the phosphorus not as inorganic phosphate, but as some soluble organic compound in which the phosphorus is still masked. This soluble phosphorus compound does not react with either the nitric or the hydrochloric molybdate reagent. Testis prepared as above may be hydrolysed on the water bath with hydrochloric or sulphuric acid until it gives no precipitate with tannic acid and yet none of the 3 reagents, i.e. ammonia and magnesium citrate, the hydrochloric molybdate or the nitromolybdate, will show any inorganic phosphate.

The above figures show the reason for the failure of the nitro- or the hydrochloric molybdate reagent when applied to tissues. They show *that acids readily remove most of the phosphorus from nuclein compounds in some soluble form but not as inorganic phosphate*. The whole principle of the Lilienfeld-Monti-Macallum reaction is therefore wrong and all deductions as to the distribution of phosphorus compounds (other than inorganic orthophosphates) which have been deduced from the use of their reagents are valueless.

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One is therefore justified in some degree in distinguishing with Schulz<sup>1</sup> between the 'essential' and 'accidental' ash-constituents of proteins. The discussion as to the state of combination of these constituents in the protein molecule concerns in fact only the former, that part namely which it appears difficult or impossible to remove without destroying the character of the protein itself.

Now of recent years a considerable amount of research has been undertaken with regard to the nature of the phenomena known by the name of 'absorption' or 'adsorption'.<sup>2</sup> Too much space would be taken up in giving a complete list of this work, and I must content myself with mentioning the names of Van Bemmelen, Biltz, Schmidt, Walker and Appleyard, and Craw.

The main result of these investigations, so far as concerns us for the present purpose, is the discovery of what we may call the 'Law of Adsorption.' Suppose we have a series of solutions of such a dye as Congo Red, for example, in progressively diminishing concentration, and in each of these we place the same amount of filter-paper, we find that a part of the dye is taken up by the paper *and in relatively larger proportion the more dilute the solution.* To take some examples from the paper by G. C. Schmidt.<sup>3</sup>

Picric acid and cellulose :

Concentration of picric acid	Amount adsorbed
0.889 ...	0.111
0.340 ...	0.095

Eosin and silk :

Concentration of eosin	Amount adsorbed
0.666 ...	0.84
0.376 ...	0.75

Iodine and carbon :

Concentration of iodine	Amount adsorbed
0.161 ...	3.275
0.087 ...	2.958

1. *Loc. cit.*, p. 11.

2. Notwithstanding the suggestion of Zsigmondy (*Zur Erkenntniss der Kolloide*, Jena, 1905, p. 61, footnote) to make use of the name 'absorption' in honor of Van Bemmelen, the chief worker on the question who uses this form, I think that, in English at all events, 'adsorption' is less likely to lead to confusion, and 'absorption' can be still used for phenomena like the solution of gases in water.

3. *Zeitsch. f. Physik. Chemie.*, XV, p. 60. 1894.

These numbers show that there is some kind of 'affinity' between the bodies adsorbed and those which take them up. Put in other words the law states that there is not a proportionality between the concentration of the solution or partial pressure of the solute and the amount adsorbed. Ostwald<sup>1</sup> indeed points out that there is no hard and fast distinction to be drawn between chemical affinity and adsorption which latter he sometimes speaks of as 'mechanical affinity.' He calls attention to the complete series of transitions between the two phenomena, and, in referring to Van Bemmelen's researches, he remarks that they frequently leave it a matter of uncertainty as to whether the facts brought forward should be regarded as of a chemical or physical nature. In any case all the evidence shows that electrolytes when adsorbed are non-ionized and no longer take part in the electrical conductivity of the solution. This circumstance renders the determination of conductivities a rapid and convenient method of investigating certain aspects of the problem as will be seen later.

In the course of a series of experiments on the changes of conductivity in protein solutions brought about by the action of enzymes I have had occasion to take into consideration the possibility of the splitting-off of inorganic electrolytes under these conditions. The results of some preliminary experiments suggested to me that the most satisfactory explanation of the nature of the 'combination' between protein and ash was that the constituents of the latter are present in adsorbed form rather than in true chemical union.

Before passing on to the description of the experiments made to throw light on this problem, I may, in order to make clear the point of view, give the results of a preliminary experiment on an undoubted case of adsorption, viz., congo-red and filter-paper.

*Experiment:* Seven flasks were taken, each containing 50 c.c. of a solution of congo-red in 10 per cent. alcohol. These solutions were of a regularly diminishing concentration from 0.014 to 0.002 per cent. Congo-red forming a colloidal solution is very readily precipitated from watery solution by traces of electrolytes, so that an alcoholic

1. *Lehrbuch d. Allgem. Chemie.*, 2te. Aufl. Bd. I, p. 1084, et seqq.

solution is more easily worked with. For the same reasons, the filter-paper used was Schleicher and Schüll's analytical paper, and of this a disc 12.5 cm. diameter was added to each of the above flasks. After 24 hours standing the depth of colour in each solution was determined by Gallenkamp-Heele's colorimeter, the results being as follows:—

Concentration of solution	Proportion of dye in solution	Proportion of dye in paper
0.014	40 %	60 %
0.012	20 %	80 %
0.010	9.3 %	90.7 %
0.008	4 %	96 %
0.006	1.3 %	98.7 %
0.004	trace	practically all
0.002	"	"

The figures show at once that the amount of dye taken out of solution by the paper is not directly proportional to the concentration of the solution; nor, on the other hand, is it independent of this concentration as would be the case if a true chemical compound were formed. In fact, from a 0.014 per cent. solution  $\frac{40}{100} \times \frac{0.014}{2} = 0.0028$  gram is taken up by a certain amount of paper, whereas from a 0.008 per cent. solution only  $\frac{4}{100} \times \frac{0.014}{2} = 0.00028$  gram, or one-tenth of the former, is taken up by the same amount of paper, although there was still a quantity of free congo-red in the solution. The peculiarity of these phenomena, which I have spoken of above as 'the law of adsorption,' is well shown by the series of numbers in the third column.

Now, if we put these data into the form of a curve as is done in Fig. 1, where the ordinates represent the percentage of dye left in solution and the abscissae the original concentration of the solution, we see that the curve forms part of a hyperbola, and only approaches the axis (*i.e.*, zero concentration) asymptotically.<sup>1</sup> In other words, however diluted the original solution may be, there will practically be always a certain amount of the dye left unadsorbed by the paper. It follows

1. This hyperbolic form of the curve is pointed out by Ostwald, *Allgemein. Chem.*, Bd. I, p. 1,096.

Walker and Appleyard (*Jour. Chem. Soc.*, 69, 1896), p. 1334, also find a logarithmic formula for the adsorption of picric acid by silk.

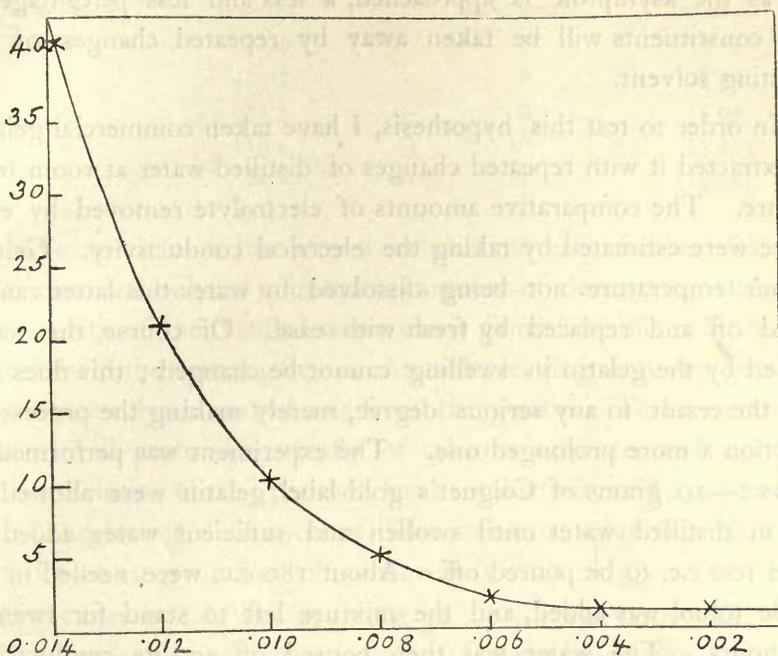


FIG. 1

also that if we attempt to wash out the dye by means of 10 per cent. alcohol it will require, theoretically, an infinite number of changes of the solvent to effect complete removal of the dye. I found, in fact, that if the piece of paper which was stained deep-red in the 0.002 per cent. solution was allowed to soak in 10 per cent. alcohol, a trace of dye only was removed each time a fresh supply of alcohol was added; the alcohol became very faintly pink, the colour only to be detected in a deep layer, while the paper did not perceptibly lose in depth of colour after several extractions.

## II. WASHING-OUT OF ELECTROLYTES

It will be seen, on a little consideration, that the great difficulty of removing the last small part of the ash-constituents of proteins may be readily explained on the hypothesis that the case is one of adsorption. The hyperbolic form of the characteristic curve shows

that, as the asymptote is approached, a less and less percentage of these constituents will be taken away by repeated changes of the extracting solvent.

In order to test this hypothesis, I have taken commercial gelatin and extracted it with repeated changes of distilled water at room temperature. The comparative amounts of electrolyte removed by each change were estimated by taking the electrical conductivity. Gelatin at room temperature not being dissolved by water this latter can be poured off and replaced by fresh with ease. Of course, the water imbibed by the gelatin in swelling cannot be changed; this does not affect the result to any serious degree, merely making the process of extraction a more prolonged one. The experiment was performed as follows:—10 grams of Coignet's gold-label gelatin were allowed to soak in distilled water until swollen and sufficient water added to enable 100 c.c. to be poured off. About 180 c.c. were needed in all. A little toluol was added, and the mixture left to stand for twenty-four hours. The water was then poured off and its conductivity determined. A fresh 100 c.c. of water were then added, and the process repeated every twenty-four hours. After the first three or four extractions the conductivity of the extract was so low that I found, with the apparatus used, that more accurate readings were obtained by concentrating the fluid in a platinum capsule over the water-bath to a definite volume (12 c.c.) before measuring its conductivity. Even when their concentration is thus increased, the electrolytes may be looked upon as practically completely dissociated, so that no appreciable error can be put down to this circumstance. It might be objected that impurities in the distilled water would also be concentrated, but I found on testing this possibility that the conductivity of the distilled water used, which had originally a conductivity of 5 gemmhos (= reciprocal megohms) did not increase by concentrating 90 c.c. down to 12 c.c. Presumably, this is to be accounted for by the fact that the slight conductivity was almost entirely caused by carbon dioxide, which would be driven off on heating. The numbers obtained were as follows:—

No. of extract	Conductivity in gemmhos	No. of extract	Conductivity in gemmhos	
1	1094	...	7	23.25
2	475.5	...	8	18.25
3	232.3	...	9	15.94
4	120.4	...	10	15.45
5	62.8	...	11	15.40
6	29.41			

We see at once that after a certain time each further change of water contracts only an infinitesimal amount of electrolytes. Since the conductivity of the distilled water was 5 gemmhos, this must be subtracted from the values given in the table, leaving only 10 gemmhos for the electrolytes washed out from the gelatin. These electrolytes consist in all probability chiefly of calcium sulphate, and a calcium sulphate solution of 0.25 per cent. has a conductivity of 2039 gemmhos, so that, neglecting differences of dissociation, a solution with a conductivity of 10 gemmhos will contain only  $0.25 \times \frac{10}{2039} = 0.0013$  per cent. It may be said that at this stage the ash was nearly all washed out. In order to test this possibility, I determined the ash of the gelatin. The incineration was performed in a platinum capsule, care being taken not to let the temperature get above dull redness, and not to prolong the heating after all charred portions had lost their blackness. The original gelatin contained 0.55 per cent. of ash, and after extracting with water eleven times it still contained 0.036 per cent. Therefore, when the rate of extraction had already become almost negligible, there was still left in the gelatin a considerable amount of ash.

On the hypothesis that the electrolytes are merely mixed with the gelatin and washed out by diffusion, the rate of diminution of conductivity of the successive extracts would have been much greater. This can be seen by calculating on the basis of the amount washed out by the first and second extractions, what would have been, *e.g.*, the conductivity at the seventh extraction. The proportion removed at each change would, obviously, have been the same. If the above calculation be made it will be found that the conductivity of extract No. 7 would have been 7.4 gemmhos instead of 23.25 gemmhos as found.

Fig. 2 gives the curve of this experiment showing the same hyperbolic form as the adsorption curve of congo-red, Fig. 1.

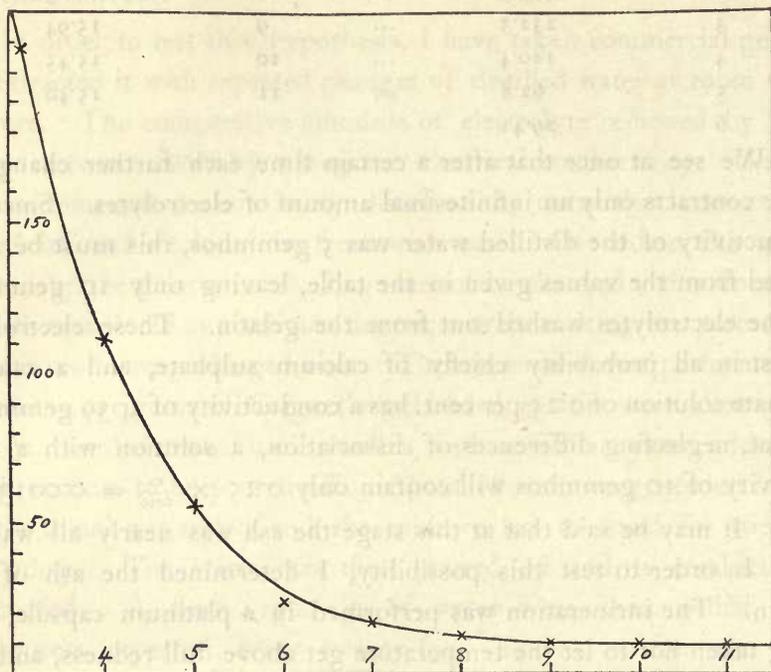


FIG. 2

Since the last values are the most interesting, and if the whole had been plotted out, the final course of the curve would have been on a much smaller scale than in the figure, the first two values are omitted, the curve commencing at the third. The ordinates represent specific conductivities of the extract in gemmhos, the abscissae are at equal intervals. The value No. 6 is obviously an experimental error. In drawing the curve the conductivity of the distilled water used is taken as the axis of abscissae. The position of the other asymptote is not shown since only part of the hyperbola has been drawn.

Before leaving this experiment I may mention that an attempt was made to re-dissolve the ash and determine its conductivity. A quantity of the gelatin after the last extraction was dried in a toluol oven and weighed 0.75 grams. This was incinerated and the ash redissolved in the quantity of water corresponding to that in which the

gelatin had soaked, viz., 13.5 c.c. It did not, however, entirely go into solution; yet notwithstanding this, the conductivity was 29 gemmhos as compared with the 10 gemmhos of the water in which the gelatin had been soaked. The signification of this fact is that it confirms the deduction drawn from the previous experiment that electrolytes are held by the gelatin in such a manner that they can only be separated by an enormous number of changes of water.

If gelatin which has been washed free from the greater part of its electrolytes be placed in a dilute potassium chloride solution, it takes up a certain quantity of this electrolyte, which can be washed out by repeated changes of water, and the curve of the conductivities of the series of extracts is of exactly the same form as that of Fig. 2.

The fact mentioned in the last paragraph makes it of interest to see whether direct evidence can be obtained of the adsorption of electrolytes by gelatin. This can be done in the following way:—

*Experiment*: 30 c.c. of  $\frac{N}{10}$  KCl were found to possess a conductivity of 16,600 gemmhos at 39.7° C. At room temperature 2.3 grams of 'washed' gelatin were placed therein and allowed to remain for 24 hours. The conductivity of the solution was now found to be diminished to 14,590 gemmhos at 39.7°. This means that at least 12 per cent. of the potassium chloride had been taken out of solution.

The fact can also be shown in other ways though in not so obvious a manner. If we take distilled water and add successively equal amounts of KCl, each increment produces a slightly less increase of conductivity than the previous one. The reason is that as the solution becomes more concentrated the KCl becomes less dissociated; to a less degree also increased ionic friction will contribute to the result. If, on the other hand, we add to the water first some 5 per cent. or so of gelatin and afterwards the successive doses of KCl the result is different. In a particular experiment 10 grams of gelatin were placed in 200 c.c. of distilled water, the gelatin having been previously washed fairly free from electrolytes. The conductivity of the water was raised to 26.9 gemmhos. When 2 c.c.  $\frac{N}{10}$  KCl were added and allowed to stand for 24 hours the conductivity was increased by 200 gemmhos,

Another 2 c.c. were added, and after 24 hours the conductivity was found increased by 208 gemmhos. That is, as I interpret it, a part of the first dose of  $KCl$  was taken up by the gelatin. It is impossible to compare directly these figures with those obtained on a similar experiment with distilled water without gelatin, since the proportion of water taken up in the swelling of the gelatin is unknown.

Again, if gelatin has the power of taking up electrolytes, it is to be expected that commercial gelatin and 'washed' gelatin would show a difference in their power of taking up more, since commercial gelatin has already a larger percentage. The following experiment was made to find out if this is so :—

*Experiment :* 12 grams (air-dry) of commercial and 'washed' gelatin were soaked for 20 hours in distilled water. The conductivity of the water was then found to be :—

Commercial gelatin :	553 gemmhos.
Washed „ :	77 gemmhos.

Two solutions (very dilute) of calcium sulphate and phosphate were made equal in conductivity to each of these extracts by adding, drop by drop, a saturated solution of these two substances in distilled water. Of these solutions a volume was taken equal to that of the water in two flasks in contact with the two kinds of gelatin, this was found to be (by weighing) 176 grams. Then to each of the four flasks 15 c.c. of the above-mentioned saturated solution of calcium sulphate and phosphate were added, and the increase of conductivity so produced was determined. If a part of the added electrolytes were taken up by the gelatin there should be a greater rise of conductivity in the cases of the watery solutions than in those where gelatin was present. The results actually obtained are rather difficult to interpret. To take first the 'washed' gelatin and the watery solution equal to it in conductivity. The rise of conductivity in the latter by the addition of 15 c.c. of the calcium sulphate solution was 163 gemmhos at  $10^{\circ}$ . Where the gelatin was present the first effect was a rise of 238 gemmhos, owing, no doubt, to the electrolytes not at once diffusing into the water contained in the swollen gelatin, this steadily diminished, and in about forty-five minutes had become 128 gemmhos. But then

the conductivity commenced to go up again, finally becoming 160 gemmhos. If, therefore, we take this last as the true value, the amount of electrolyte taken up by gelatin was very small, a difference of 3 gemmhos only between the two cases. If, on the other hand, we take the lowest value to which the conductivity fell, the difference is considerable, viz., 75 gemmhos.

In the case of the commercial gelatin and its equivalent calcium sulphate solution, the rise produced by the addition of 15 c.c. of saturated calcium sulphate and phosphate was 68 gemmhos in the watery solution and 56 gemmhos in that where the gelatin was present, a difference of 12 gemmhos. The addition of the electrolyte at first caused a rise of 106 gemmhos where the gelatin was present, it fell to 72 in one hour, but the final value was not reached for several hours, as opposed to the first case, where it was reached in two-and-a-half hours.

This experiment affords a certain amount of evidence that electrolytes are taken up by gelatin, but does not distinguish between adsorption and chemical combination. As we shall see later, the effect of heat shows it to be, in all probability, a case of the former.

### III. THE KINETICS OF ADSORPTION

#### 1. *Velocity of Reaction.*

Very little information is to be obtained as to the rate at which bodies are taken up by adsorbing substances. A few experiments that I have made on this question may therefore be of interest.

*Experiment:* 50 c.c. of 20 per cent. alcohol in test-tube lined with filter-paper. In thermostat at 39°. When warmed, 1 c.c. warmed 0.5 per cent. congo-red added, stirred, conductivity taken at intervals by pipette electrodes. The curve (Fig. 3) shows the time course of the diminution of conductivity. It will be noted that the curve is hyperbolic and that it arrives nearly at the asymptote in 10 minutes. At this temperature the velocity is considerable. The first part of the curve in all probability escaped observation, owing to the great initial velocity of the reaction as shown by the shape of the curve.

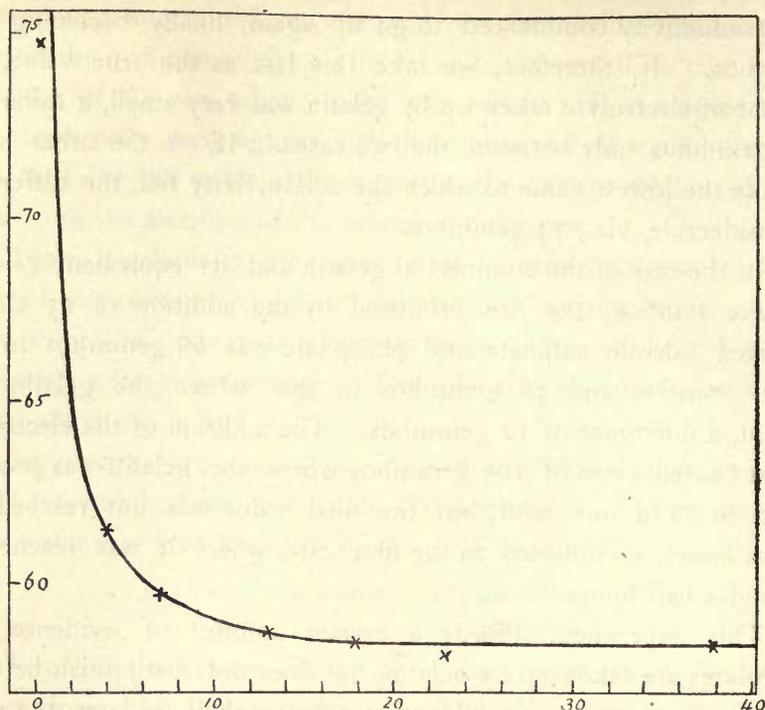


FIG. 3

In another experiment, in which the proportion of paper to dye was greater than in the previous one, equilibrium was reached in six minutes. So that the rate of change is proportional in the first place to the concentration of the adsorbing body. I have not enough data, however, to warrant any further discussion of this point. The reaction is one taking place in a heterogeneous system and its mathematical expression a complex one. It is probable that diffusion plays a considerable part as indicated by the results of the next experiment, which was made to obtain some idea of the temperature coefficient of the reaction-velocity. It was found that, for some reason or other—perhaps some action of the platinized electrodes on the dye—at low temperatures the electrical conductivity method did not give satisfactory results, so that the colorimetric method, though more laborious and less sensitive, was made use of.

*Experiment:* 50 c.c. of 0.005 per cent. congo-red in 5 per cent. alcohol in each of two flasks, a circle of filter-paper added to each, and

one kept at  $50^{\circ}\text{C}$ , the other at  $10^{\circ}\text{C}$ . After a certain time the solution was poured off and a new piece of paper and fresh dye solution, previously warmed, or cooled respectively, added and a different time of action allowed. The flasks were thoroughly shaken at frequent intervals to allow access of the solution to the whole surface of the paper. This shaking caused the separation of bits of paper which rendered it necessary to allow the solution to deposit before making colorimeter observations; neglect of the precaution caused the loss of several of the earlier observations. Filtration is naturally inadmissible. The results were as follows, the colorimeter readings expressing the relative percentage of dye left in solution, the original solution = 100.

Duration of action	Colorimeter readings	
	$50^{\circ}$	$10^{\circ}$
5 minutes	95	circ. 98
8 "	90	...
20 "	85	...
30 "	...	90
40 "	80	...
100 "	77	70
200 "	...	60
5 hours	75	...
7 "	...	55
14 "	75	48
24 "	...	45

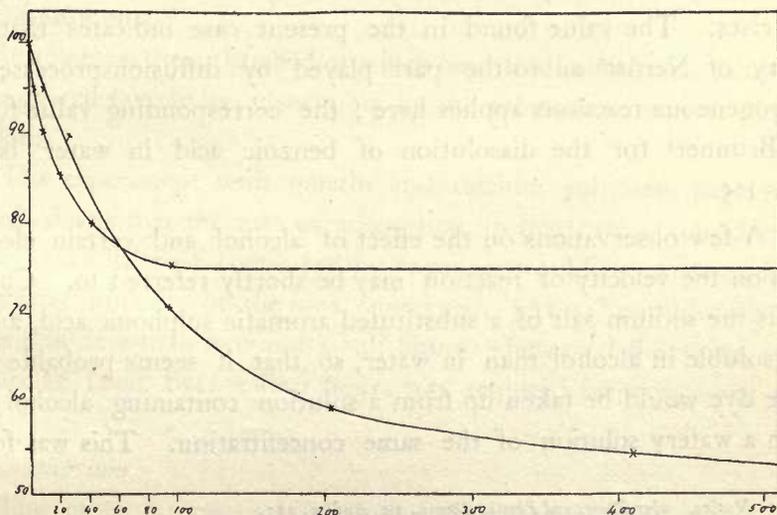


FIG. 4

The curves (Fig. 4) reproduce these two series up to seven hours. What we notice is that at the higher temperature the velocity is greater until the equilibrium position at this temperature is approached. The question of equilibrium will be discussed in the next section. In estimating the value of the temperature coefficient of the velocity, some uncertainty exists, since it will differ according to what stage of the reaction we take. If we take as criterion the time taken to reduce the concentration of the solution to a given percentage, say 90 per cent. at  $10^{\circ}$ , this time is about 2.5 times that at  $50^{\circ}$ , if we take 80 per cent. it is about 1.3 times. In either case, however, it is extraordinarily low; taking the higher value at  $10^{\circ}$  it takes twenty-five minutes to become 90 per cent., and at  $50^{\circ}$  it takes ten minutes, a difference of fifteen minutes for  $40^{\circ}$ ; so that, assuming uniformity of increase for the total interval, the time taken at  $40^{\circ}$  would be only  $\frac{15}{4} = 3.6$  minutes more than at  $50^{\circ}$ , or ten minutes and 13.6 minutes respectively. This gives a temperature coefficient of the extremely low value of  $\frac{13.6}{10} = 1.36$ . In the table given by Van t' Hoff<sup>1</sup> there are only two values below this, viz., the dissociation of  $\text{PH}_3$  and  $\text{AsH}_3$  probably depending on the high temperatures at which the observations were taken, since, as Van t' Hoff points out, the velocity-ratios for  $10^{\circ}$  usually diminish as the temperature rises. The value found in the present case indicates that the theory of Nernst<sup>2</sup> as to the part played by diffusion-processes in heterogeneous reactions applies here; the corresponding value found by Brunner<sup>3</sup> for the dissolution of benzoic acid in water is, in fact, 1.5.

A few observations on the effect of alcohol and certain electrolytes on the velocity of reaction may be shortly referred to. Congo-red is the sodium salt of a substituted aromatic sulphonic acid, and is less soluble in alcohol than in water, so that it seems probable that more dye would be taken up from a solution containing alcohol than from a watery solution of the same concentration. This was found

1. Vorles. über Theor. und Physik. Chemie., 2te Aufl. p. 225.
2. Zeitsch. f. Physik. Chemie., XLVII, 1904, p. 52.
3. Zeitsch. f. Physik. Chemie., XLVII, 1904, p. 62.

to be the case. Two solutions containing 0.005 per cent. of congo-red were taken, one in 50 per cent. alcohol, the other in water. From the former a circle of filter paper adsorbed at 14° in seventy minutes 45 per cent. of its contents, and from the watery solution only 30 per cent.

The action of a neutral electrolyte, sodium chloride, was similar, but more pronounced. From a solution containing 0.0042 per cent. dye and 2 per cent. NaCl, 75 per cent. was taken up in fifty minutes, and from a solution of the same concentration in dye, but without NaCl, only some 25 per cent. was taken up at the same time.

The action of strong acids is, of course, to set free the sulphonic acid from its compound, the free acid while more soluble in alcohol is less so in water, so that again we have a favouring effect on adsorption as follows:—Two flasks of the usual 0.005 per cent. solution of dye with each one circle of paper to 50 c.c. solution were prepared, one remained neutral, the other was acidified by the addition of one drop of 5 per cent. HCl. Though the solution became blue no precipitation of the colour-acid occurred in this weak concentration. After two hours at 13° to 14°, 35 per cent. was taken up from the acid solution and 12 per cent. from the neutral one. Before taking the colorimeter reading the acid solution was neutralized by a drop of ammonia.

It appears, then, that bodies which tend to diminish the solubility of congo-red favour its adsorption by the substances immersed in its solution.

The experiment with gelatin and calcium sulphate, previously related, shows that the rate of adsorption in that case is considerably greater than that of congo-red by paper, as would be expected from the greater mobility of the ions concerned. At 11° equilibrium was attained in less than two-and-a-half hours, whereas at the same temperature at least twenty-four hours was required for congo-red and paper.

## 2. *Equilibrium*

The most interesting fact under this head is the influence of temperature. As the curve (Fig. 4) shows, at the higher temperature,

less of the dye is contained in the paper and more in the solution. The curve (Fig. 5) shows the results of a few observations at various temperatures, ordinates being percentages in paper and abscissae temperatures. It will be noted that a straight line is formed. The values at the lower temperature are not quite so accurate as at the higher,

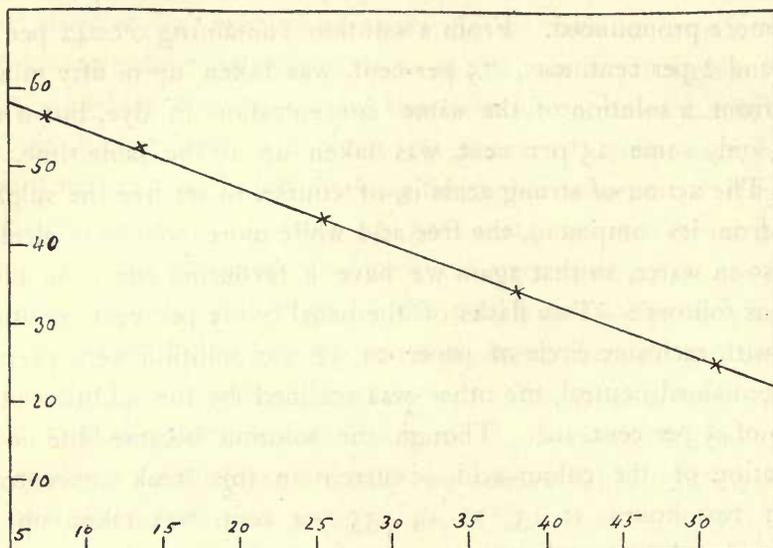


FIG. 5

since the former were obtained by allowing the flasks to stand in the laboratory, the temperature of which was not constant within  $2^{\circ}$  or  $3^{\circ}$  during the time necessary for equilibrium to establish itself. The upper values are accurate, being taken in a thermostat.

Attention has already been directed to the length of time necessary at low temperatures for the establishment of equilibrium in these dilute solutions. Ostwald also notes this fact.<sup>1</sup>

The dissociation of adsorption compounds by raising the temperature can also be shown in the case of gelatin and inorganic electrolytes, though complicated in this instance, by the passage from the state of hydrogel to that of hydrosol. There is, however, as I find, no sudden change in passing from the one state to the other as regards conductivity. If one warms a gelatin hydrogel from  $15^{\circ}$  to  $40^{\circ}$  determining

1. *Loc. cit.*, p. 1093.

conductivity at frequent intervals, the curve, which is in fact a straight line, shows no kink of any kind at the point where the hydrogel liquefies to a hydrosol.

The experiment previously described on the relations of gelatin to calcium sulphate was continued as follows:—The two flasks containing gelatin and calcium sulphate were warmed to  $53^{\circ}$ , the rise of conductivity in the case of the washed gelatin was 397.2 gemmhos, in the commercial gelatin 1317 gemmhos. Now the point of interest is, whether this rise is greater than would be the case if no separation of additional electrolyte had taken place under the influence of heat. To decide this, solution of calcium sulphate was prepared, having at  $10^{\circ}$  the same conductivity as the solutions in contact with the two kinds of gelatin. These were then warmed to the same temperature as the gelatin. The rise of conductivity in the more dilute amounted to 376 gemmhos, and in that of the stronger to 1446 gemmhos. There is therefore an increase in electrolytes in the case of the washed gelatin corresponding to a difference in conductivity of 21 gemmhos. It is to be remembered, moreover, that gelatin in the state of hydrosol has a slight effect similar to that of other non-electrolytes in diminishing the conductivity of solutions in which it is present. This is, no doubt, due to an increase of internal friction in some way or other, and amounts, according to determination I have made, to 3.4 per cent. diminution of conductivity for each 1 per cent. of the gelatin present. So that each of the values for the gelatin solutions should be increased by, approximately,  $3.4 \times 4.9$  per cent., the gelatin solution being 4.9 per cent. in concentration. This increases the two values to 463 and 1536 gemmhos. The differences in favour of the gelatin come out now to be 87 gemmhos for the washed, and 90 gemmhos for the commercial sample. The closeness of these two numbers serves to strengthen the view that the electrolytes causing the increase were in some way separated off from the gelatin under the influence of heat.

It has been shown above that the conductivity of a solution of the ash from a given sample of gelatin is greater than that of the solution with which the gelatine is in equilibrium. I thought it interesting, therefore, to compare the ash of the particular 'washed'

gelatine of the above experiment, as regards its conductivity, when re-dissolved to original volume, with the conductivity of the gelatin solution at  $10^{\circ}$  and at  $53^{\circ}$ . The ash from 100 c.c. of the gelatin solution weighed 0.0262 gram, it was re-dissolved in 96.6 c.c. of distilled water (to allow for the volume of the gelatin), the whole of it not going into solution. Now the conductivity of the gelatin solution was found at  $6.2^{\circ}\text{C}$ . to be 141 gemmhos, that of the re-dissolved ash was 270 gemmhos. It is obvious that this is more than sufficient to account for the additional electrolytes split off on heating. It also confirms the view already expressed that inorganic electrolytes are held in some kind of combination by gelatin. Since they are also separated to a certain degree by warming, the combination in question is in all probability of the nature of adsorption.

The various substances which increase the velocity of adsorption also affect the final equilibrium position but in the opposite direction to that in which it is affected by heat. The action of neutral salts is the most interesting and of the greatest practical and theoretical importance. Sodium chloride has a very striking effect, even in very low concentration, as the following experiment shows :—

Solution of congo-red 0.004 per cent. containing various percentages of sodium chloride. After a circle of paper had lain in each for 2 hours the following colorimeter readings were obtained :

Percentage of NaCl	Percentage of dye left in solution	Percentage of NaCl	Percentage of dye left in solution
0	85	0.02	33
0.0005	77	0.1	25
0.001	71	0.2	16
0.005	50		

The effect of sodium chloride, then, appears to be proportional to its concentration but not in direct linear proportion. The fact that in so weak a concentration as 0.0005 per cent. an obvious effect is produced indicates that the action of inorganic electrolytes is rather to be brought into relation with the well-known effect of these bodies on typical colloids, rather than with the precipitating action of alcohol, which requires a much higher relative concentration in order to produce a comparable effect. In investigating this action of electrolytes

the phenomena met with, although of considerable interest, showed themselves to be of a very complex nature, so that it will be better to devote a special section to the discussion of them later on.

At first sight the action of heat in dissociating the adsorption compound, as above described, appears at variance with the common practice of histologists in staining their preparations. I find that the beneficial effect of heat in this case is probably due to the fact that a piece of paper, for example, dyed with congo-red and exposed to a temperature of  $100^{\circ}\text{C}$ . loses the power to a large extent of giving up the dye to water, in other words it is fixed more firmly than if merely stained at room temperature.

According to the well-known laws of Van't Hoff, since the compound of cellulose and congo-red is dissociated more and more as the temperature rises, the formation of the compound should be accompanied by evolution of heat. I have attempted in various ways to detect such a production of heat but have been unable to do so. The two methods used were : (1) Immersion of a Beckmann thermometer in the centre of a series of co-axial cylinders of filter paper in a large beaker filled with  $\text{NaCl}$  solution to facilitate adsorption. A test-tube of 1 per cent. solution of congo-red was also immersed in the solution, and when the whole was at the same temperature, the dye was mixed with the saline solution. No temperature change was to be observed, although by aid of a lens, the thermometer could be read to  $\frac{1}{1000}$ th of a degree ; (2) A disc of paper moist with  $\text{NaCl}$  solution was allowed to rest on the face of an ordinary bismuth-antimony thermopile contained in a vacuum-jacketed vessel and connected to a low-resistance d'Arsonval galvanometer. The vessel also contained a small tube of concentrated congo-red solution, closed at the bottom by a glass rod with indiarubber tube around its lower end. When the galvanometer was steady the glass rod was raised and the dye allowed to flow over the paper. No deflection indicating the production of heat was observed. It is to be noted, however, that at the temperature of these experiments, the process would not be very rapid, and it is possible that a slight heat production would be conducted away as fast as it was formed.

### 3. *Reversibility*

The question as to how far the process is a reversible one is of some theoretical interest. It has been already mentioned that if the temperature is raised up to  $100^{\circ}\text{C}$ ., a part of the dye is fixed in the paper, so that under certain conditions, the reversibility is not complete. At ordinary temperature it appears complete. If a circle of paper be dyed with congo-red, rinsed with water and then placed in water along with another similar circle of paper, it will be found that after some time the two pieces of paper will be of the same depth of colour. At the same time, as already pointed out at the beginning of this paper, to completely extract all the dye from a piece of paper needs an enormous number of repeated changes of water.

As regards temperature reversibility is apparently complete.

*Experiment:* Two flasks containing congo-red and filter-paper were placed, one at  $50^{\circ}$ , the other at  $11^{\circ}$ ; next day both flasks were placed in a thermostat at  $25^{\circ}$ , when equilibrium was attained after about 30 hours, the colorimeter readings were:

From  $50^{\circ}$  - 53 %  
 „  $11^{\circ}$  - 57 %

Again, if sufficient time be allowed for equilibrium to be reached, a preparation may be alternately warmed and cooled, and nearly the same readings obtained at the same temperature. It appears, however, that after several days contact the dye becomes partly fixed. For example, in one case the reading at  $38^{\circ}$  was 66 per cent., it was then cooled to  $13^{\circ}$  with a reading of 48 per cent.; on again warming to  $38^{\circ}$ , 50 per cent. only was reached, and on cooling again to  $13^{\circ}$ , after two days the value became 25 per cent.

Gelatin, also, shows fairly complete heat reversibility as regards its adsorbed electrolytes. At  $11^{\circ}\text{C}$ . a particular hydrogel had a specific conductivity of 162 gemmhos, after being heated to  $53^{\circ}$  and again cooled to  $11^{\circ}\text{C}$ . its conductivity was 157.8 gemmhos. Another preparation containing more electrolytes had an initial conductivity of 609 gemmhos, after heating to  $55.5^{\circ}$  and cooling again to  $11^{\circ}$ , it became 542 gemmhos, or less than its original conductivity. Perhaps in the first measurement equilibrium was not completely attained.

When adsorption has taken place under the action of electrolytes a very considerable degree of fixation occurs, so that the dye is not given up again to water, or only to a very slight degree.

*Experiment:* Two similar pieces of paper dyed to same depth of colour, one in solution of  $\text{NaCl}$  0.02 per cent., the other in distilled water. Of course the latter solution was more concentrated as regards the dye. These pieces were rinsed in water, pressed between blotting-paper, and then placed in equal volumes of distilled water along with another piece of paper each. After 24 hours the one which had been dyed in distilled water had given up a considerable amount of pigment to the water, and the two pieces of paper were very nearly equal in depth of tint. The paper dyed in the presence of  $\text{NaCl}$  gave up no colour to be detected by the eye, although the second piece of paper was faintly pink, showing a slight extraction from the dyed piece.

#### IV. THE ACTION OF ELECTROLYTES

When we consider that a solution containing only 0.0005 per cent. of  $\text{NaCl}$  has a distinct effect in augmenting adsorption of congo-red by paper, viz., 23 per cent. taken up as against 16 per cent. from distilled water, it is plain that the phenomenon is not of the nature of a 'salting-out,' comparable to the precipitation say of egg-albumen by ammonium sulphate. The precipitation of such solutions as those of the colloidal metals and hydroxides is rather suggested. Now, there is considerable evidence that a large number of the anilin dyes exist in watery solutions in a colloidal form. Congo-red, having a molecular weight of nearly 700, would be expected to be one of these. Its solutions, in fact, do not diffuse through Schleicher and Schüll's parchment-paper thimbles, although they do so, very slowly, through ordinary parchment-paper. According to Michaelis<sup>1</sup> under the ultra-microscope they are heterogeneous, being resolvable into sub-microscopic particles. In the electric field the dye migrates to the anode, so that the particles are negatively charged. It is, however, somewhat difficult to make oneself certain of this fact on account of

1. *Deutsche Med. Wochenschrift*, 1904, No. 42.

the electrolytic decomposition. Colloids do behave as electrolytes, as is well-known<sup>1</sup>, and I have made one rough determination, by Whetham's boundary method, of the velocity of the coloured ion in congo-red solution. At 13° the boundary, which was not very sharp, moved 11 mm. in one hour under a potential fall of 3 volts per cm. Hardy<sup>2</sup> finds for globulin about 7 mm. in the same time, and for methylene-blue considerably more, about 40 mm. The electrical conductivity of congo-red solutions is comparable with that of inorganic electrolytes, a  $\frac{M}{100}$  solution at 40° having a specific conductivity of 5600 gemmhos. I do not lay any stress on the absolute value of this measurement since the preparation was not specially purified, and possibly contained a small amount of inorganic salt.

On the whole, then, we may regard congo-red as being a negatively charged colloid. As such it would be specially sensitive to di- and tri-valent kations, and this is in fact the case.

All the experiments to be described in this section, except when otherwise stated, were made in the same way, viz., to 50 c.c. of the solution of electrolyte 10 c.c. of  $\frac{M}{1000}$  dye solution and one piece 12.5 cm. diam. of Schleicher and Schüll's extracted filter paper added. After about 24 hours the amount of dye taken up by the paper was estimated by taking the colorimetric value of the dye left in solution.

It is easily seen at once that calcium salts are much more active in promoting adsorption than those of the monovalent alkali metals, e.g., the amount taken up from a solution containing  $\frac{M}{500}$   $\text{CaSO}_4$  was 85 per cent., from a  $\frac{M}{200}$   $\text{KCl}$  67 per cent.

Considering this fact the effect of tap-water is not surprising, since New River water contains an equivalent in  $\text{Ca}^{++}$  ions of about equal amount to the content therein of  $\frac{M}{300}$   $\text{CaSO}_4$ . From tap-water 85 per cent. was adsorbed in one experiment, and from distilled water only 27 per cent.<sup>3</sup> Moreover, these facts show that in order to obtain any reliable data, special care must be taken as to the purity of the paper used. As an illustration, I give the following experiment :

1. Hardy, *Journal of Physiology*, XXXIII, 1905, p. 292.

2. *Loc. cit.*, p. 291 and p. 289.

3. The absolute amounts in different experiments can only be compared by taking their *ratio* to the amount taken up from distilled water in each case, since the temperature was not the same in all.

Equal weights of the following samples of filter-paper were placed in 50 c.c. distilled water with 10 c.c.  $\frac{M}{1000}$  dye as usual. The amounts taken up by each are given :

1.	Dreverhof's ordinary, No. 333	...	...	95 %
2.	„ „ washed in distilled water			59 %
3.	Dreverhof's extracted paper	...	...	46 %
4.	„ „ washed	...	...	46 %
5.	Schleicher and Schull's extracted paper	...		46 %

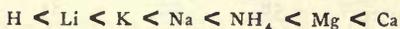
I may mention here that Dreverhof's extracted paper turns blue when a drop of dilute congo-red is placed on it, this does not happen with Schleicher and Schüll's, nor with Swedish paper. As the above experiment shows this slight acidity does not seem to affect its adsorbing power, and as will be seen later  $H^+$  ions have comparatively little favouring effect.

The result of this experiment seems to suggest the possibility, that if one could obtain a complete absence of electrolytes no adsorption would take place.

As regards trivalent kations I have only tested aluminium sulphate, but as even in a concentration of  $\frac{M}{1000}$  precipitation took place the result was valueless.

It is scarcely necessary, perhaps, to remark that if the dye is precipitated by the electrolyte no adsorption by the paper takes place, the large particles merely float about in the liquid. What is needed is the local concentration of electrolyte on the surfaces of the adsorbing solids.<sup>1</sup>

Although the greater favouring power of divalent as compared with monovalent kations is easy to demonstrate the order in each of these classes is difficult to decide since the action of each member is so nearly the same as that of the others. From a number of experiments the following seems to be the order :



1. Notwithstanding the fact that actual precipitation must not take place in these experiments the addition of electrolytes to congo-red, for example, causes an increase in the size of the colloidal particles even when no actual precipitation occurs ; so that the solution is on its way to precipitation even when this does not actually occur. The specimen of congo-red used in all my experiments showed the Tyndall phenomena very faintly in solution in distilled water ; but when NaCl was added the beam of light became much more distinct and the light reflected from it at right angles was polarized. When  $CaCl_2$  was added in sufficient amount to slowly precipitate the dye (about  $\frac{M}{100}$ ) the polarization ceased as the particles became larger than the mean wave-length of light.

This is very nearly the same as that found by Pauli<sup>1</sup> in the case of precipitation of proteins.

It is a matter of some interest to see whether in this case of adsorption the anions have a retarding action like that shown by Pauli in the above-mentioned experiments. That there is some action of this kind can I think be shown by consideration of the comparative action of chloride and sulphate of the same metal, say potassium. The difficulty lies in the fact of the great preponderance of the opposite action of the kation, probably on account of the negative charge of the congo-red. If we take equimolecular solutions of  $KCl$  and  $K_2SO_4$  we find their effect almost exactly the same within limits of experimental error. Now there are, in the dilute solutions employed, very nearly twice the number of kations in the  $K_2SO_4$  than in that of the  $KCl$ , necessarily, therefore, the  $SO_4$  ion has a greater retarding power than the  $Cl$  ion. In determining the order of the anions in this respect the same difficulty presents itself, as in the case of the opposite effect of the kations, but in a more marked degree. The approximate order as regards power of inhibition is the following:—



The results of these experiments will be best shown in a table analogous to those given by Pauli. The numbers give the percentage amount of dye adsorbed at room temperature under the influence of the body formed by the combination of kation and anion shown vertically above and horizontally at the left-hand side of each number, and in  $\frac{m}{200}$  concentration.

	CONGO RED						
	H	Li	K	Na	$NH_4$	Mg	Ca
OH	26	58	...	68	$79 \left(\frac{m}{12}\right)$	...	89
CNS	...	...	...	...	64	86	...
Acetate	...	...	58	70	81	...	...
Cl	...	...	70	75	72	...	91
F	...	...	...	75	...	...	...
Oxalate	12	...	80	...	65	...	...
$SO_4$	4	71	74	78	78	91	> 100
$PO_4$	...	...	...	...	74	...	...

1. Hofmeister's Beiträge, III, p. 225; V, p. 27; VI, p. 233; and VII, p. 531.

There are some irregularities especially as regards  $\text{NH}_4$  salts. The value put against  $\text{CaSO}_4$  means that the concentration had to be taken at  $\frac{m}{500}$  to avoid precipitation, and at this strength the value was 84 per cent.

The action of  $\text{OH}^-$  and  $\text{H}^+$  ions needs a little further explanation since it seems to vary according to concentration in both cases. It is somewhat remarkable that in concentrations of  $\frac{m}{100}$   $\text{NaOH}$  and  $\text{NaCl}$  have almost exactly the same amount of favouring action; in  $\frac{m}{1000}$  on the contrary, while  $\text{NaCl}$  has a distinct favouring action, viz., 58 per cent. adsorbed from  $\text{NaCl}$  as against 30 per cent. from water,  $\text{NaOH}$  has a very slight inhibiting action, viz., 25 per cent. against 26 per cent. from water.

In one experiment I took three solutions of equal content in  $\text{OH}^-$  ions, viz. :—

$\frac{m}{1000}$ $\text{NaOH}$	...	31 %
$\frac{m}{12}$ $\text{NH}_4\text{OH}$	...	79 %
$\frac{m}{100}$ $\text{Na}_2\text{CO}_3$	...	89 %

The amount adsorbed in presence of these is shown opposite each one.

I could not detect any influence of anilin-water on the process.

Sulphuric acid  $\frac{m}{2000}$  has a favouring action, 33 per cent. against 21 per cent. from water, while in  $\frac{m}{200}$  it has the opposite effect as the table shows. I am unable to suggest any explanation of these facts.

In connection with the well-known mutual precipitation of oppositely charged colloids<sup>2</sup> the behaviour of congo-red as a negative colloid is of interest. Colloidal platinum prepared by Bredig's method does not precipitate, but has a chemical action, turning the dye brown. This platinum sol is negatively charged so that precipitation would not be expected. On the other hand, according to Pauli<sup>1</sup> and other investigators, the precipitating action of salts of the heavy metals on egg-white is due to the presence in their solutions of metallic hydroxide in the colloidal form and presumably positively charged.

1. See Picton and Linder, *Journ. of Chem. Soc.*, 1892, page 148, etc., and W. Biltz, *Ber. d. Deutsch. Chem. Ges.*, 1904, Bd. XXXVII, page 1111.

2. Hofmeister's *Beiträge*, VI, page 257, 1905.

This being so, the powerful action of such salts on adsorption is not to be wondered at. In fact, I find  $\frac{m}{5000} \text{Zn}^{+2}$  to have an equal effect to that of  $\frac{m}{200} \text{KCl}$ .

This action of zinc sulphate gives the opportunity for testing by experiment whether in this case, as in Picton and Linder's cases of precipitation of colloids by electrolytes, the precipitating ion is carried down with the colloid to become attached to the paper. Since the 'precipitating' agent in the adsorption of a negative colloid is the positively-charged kation, and the most convenient method of detecting a disappearance of an ion, is the use of a concentration battery in the manner of Nernst, it is plain that we cannot conveniently determine the question where salts of alkali metals are concerned. A concentration battery in  $\text{Zn}^{++}$  ions is, on the contrary, easily arranged. In the present case I took two vessels, each containing an amalgamated zinc electrode immersed in  $\frac{m}{500} \text{ZnSO}_4$  solution and connected together by an inverted U-tube filled with the  $\text{ZnSO}_4$  solution. Such a battery being symmetrical has no E.M.F. A piece of filter paper was now placed in one of the vessels, the E.M.F. began to rise and attained a value nearly equal to what it would be by the Nernst formula, if all the  $\text{Zn}^{+}$  ions had disappeared from this vessel, viz., 0.0257 volt. This effect was, in all probability, due to adsorption by the paper. On now adding 5 c.c. of 1 per cent. congo-red in  $\frac{m}{500} \text{ZnSO}_4$  the E.M.F. went down again, due to the addition of more  $\text{Zn}^{+}$  ions. Presently, as the dye became adsorbed, the E.M.F. commenced to rise again and when plotted on squared paper showed the usual form of the adsorption-curve. As far as it goes, then, the experiment shows a diminution in concentration of  $\text{Zn}^{++}$  ions. But there are two circumstances which deprive it of much value. In the first place I found that next day the E.M.F. of the battery had risen to 0.094 volt, a value above that possible by removal of all the  $\text{Zn}^{++}$  ions; there must have been, therefore, some secondary process set up by the congo-red. In the second place the possibility must be taken into account, that when congo-red and zinc-sulphate are mixed, a zinc-salt of the congo-red may be formed by double decomposition which might not be completely dissociated, this

salt might be the actual body adsorbed by the paper. Considering the great dilution of the solution, however, I do not think the latter objection is very serious.

Another property of colloids is that of being protected from precipitation of electrolytes when a small amount of a stable colloid, such as gelatin, is present. As pointed out by Zsigmondy,<sup>1</sup> the fact was known to Faraday, although its meaning was, of course, not understood at the time. The following experiment shows that gelatin also protects congo-red from the action of an electrolyte :—

- A. 50 c.c. H<sub>2</sub>O + 1 c.c. 0.5 % dye  
 B. 50 c.c. 0.05 % gelatin + 1 c.c. 0.5 % dye  
 C. 50 c.c. 0.038 % NaCl + " "  
 D. 50 c.c. 0.038 % NaCl containing 0.05 % gelatin + 1 c.c. 0.5 % dye

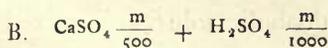
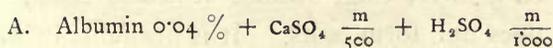
Adsorbed in three hours :

A.	30 %	...	C.	74 %
B.	31 %	...	D.	34 %

It will be seen that NaCl is practically without effect in presence of gelatin.

Egg-albumin has a similar effect, but less marked, probably on account of its content in electrolytes. I did not subject it to dialysis.

Gelatin is stated by Victor Henri<sup>2</sup> to be a negative colloid ; serum-albumin has recently been shown by Pauli<sup>3</sup> to be positive in acid solution, and negative in alkaline solution. It was of interest therefore, to see whether any difference in the protective action of egg-albumin was to be noted in the two instances. The result showed that electro-positive albumin *increased* the action of CaSO<sub>4</sub>, while electro-negative albumin *diminished* it :—



+ dye + paper as usual

Adsorbed :

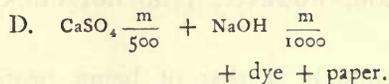
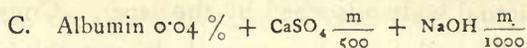
A. > 80 % (partially pptd.)

B. 36 %

1. *Loc. cit.*, p. 65, footnote.

2. *Rev. Gen. des Sciences*, 1905, p. 641.

3. Hofmeister's *Beitrage*, VII, p. 535 and p. 536.



Adsorbed :

C. 75 %

D. 98 %

It is difficult to compare with these the action of the neutral albumin since acid and alkali have themselves an influence. But it appears that the sign of the charge on the stable colloid determines the nature of its action, although the particular albumin in this experiment had also a protective action in neutral reaction. I am unable to state whether it was naturally electronegative or otherwise.

Other electro-negative dyes, such as anilin-blue and nigrosin, under the action of electrolytes, give similar results to congo-red. The colloidal condition of the solution is, however, apparently a *sine qua non*. I could not detect any influence of electrolytes on picric acid, although it is difficult to observe a small difference, because of the comparatively feeble colouring power of this dye. I compared the depth of tint of the two solutions, one with electrolyte, the other without, in both of which the same amount of paper had been immersed, by placing them in two cells of equal thickness in front of a piece of plantinotype paper and exposed to light. No difference could be detected in the depth of tint on development of the paper, although, as is well known, this paper shows very small differences of tone.

Scharlach R. is interesting, because it is a so-called 'indifferent' dye, that is it does not form salts. It is insoluble in water; but as Michaelis<sup>1</sup> points out, when an alcoholic solution is mixed with five to six times its volume of water, it is not precipitated, but becomes a colloidal solution. In this state it behaves towards electrolytes like a negative colloid, and, in fact, in the electric field, as I find, it wanders to the anode.

Rosolic acid shows similar properties.

1. *Deutsche. Med. Wochensch.*, 1904, No. 42.

In order to save space, I have put together in a table at the end of this paper a number of facts relating to various dyes in common use which have a bearing on the question under discussion.

The only other point I will refer to as regards the negative dyes is that eosin, although its colloidal properties are doubtful (see table), shows a much greater sensitiveness to the anion than the dyes hitherto mentioned, alkali in this case causes the taking up of *less* colour by paper than is taken up from water. We found in the previous cases that the action of the  $\text{OH}^-$  ion was overpowered by the opposite action of the kation ( $\text{Na}^+$ ).

We turn now to the electro-positive dyes like toluidin-blue. It would be expected that in this instance the anions would have a favouring action, the kations the reverse. This is, indeed, the case, but like the negative dyes the effect of the kation is predominant, so that the result of adding any electrolytes, except alkalies, is to *lessen* adsorption. In the absence of electrolytes, as a rule, more dye is taken up by the paper than from the solution of an electro-negative dye. This would appear to be at variance with the general use of congo-red to dye cotton, but it is to be remembered that in practice there is always sufficient electrolyte present to reverse the relative behaviour of negative and positive dyes as shown when dissolved in pure distilled water.

For details as to various positive dyes see the table.

Methylene blue precipitates the electro-negative colloidal platinum.

I have not been able to detect any marked protective action of stable colloids in the case of electro-positive dyes.

In the circumstance that both the + and - dyes are more sensitive to kations than to anions they behave like albumin solutions to salts of the heavy metals as shown by Pauli.<sup>1</sup>

On reference to the table at the end of this paper it will be noticed that in all the cases investigated where the dye is in the form of a salt with an inorganic base or acid, as the case may be, the sign of the charge of the colloid is determined by the organic constituent of

1. Hofmeister's *Beiträge*, VI, pp. 233 to 249.

the salt. In this respect they differ from the globulin of Hardy and the serum-albumin of Pauli, which in acid or alkaline solutions assume the sign of the charge of the  $H^+$  or  $OH^-$  ions respectively. They are in fact more like certain complex ions; such as the ferrocyan ion,  $Fe(CN)_6$ , which, although containing an iron atom is, nevertheless, electro-negative.

I have been unable, for want of time, to test the behaviour of more than a few typical dyes of the two classes, but it appears that there is considerable difference of degree in the sensitiveness to electrolytes. This property indeed seems to depend on the degree of colloidalness of their solution and the amount of their electric charge. Each individual dye requires separate investigation.

As to the explanation of the action of electrolytes, I think the clue is given by the following considerations. V. Henri and Larguier des Bancel's,<sup>1</sup> in their work on colloids, observed that gelatin, as hydrogel, when immersed in a solution of anilin-blue or congo-red in distilled water took up little or none of the pigment, and give as the reason for this that both the bodies are electro-negative colloids and therefore mutually repel one another. If, on the contrary, a solution of a bivalent metal, such as barium nitrate, is added the gelatin becomes deeply stained. The negative charge of the anilin-blue is neutralized by the positive barium ions so that it can now freely attach itself to the gelatin. This case then resolves itself into one of the mutual precipitation of colloids, and the only doubtful point about the explanation is whether the negatively-charged particles of the gelatin hydrosol may be regarded as retaining their charge when in the form of gelatine. There seems no *à priori* reason why this should not be so, and there is also experimental evidence in favour of it. Picton and Linder<sup>2</sup> in referring to the adsorption affinity of the hydrogels of ferric hydroxide and arsenious sulphide for anilin dyes state that these hydrogels 'retain the same selective affinity for the dyes which will coagulate them' as they possessed as hydrosols, viz., 'the hydrogel of ferric hydroxide for anilin-blue, that of arsenious

1. C.R. Soc. de Biologie, LIX, p. 132, 1905.

2. Journ. Chem. Soc., 88, 1905, p. 1934.

sulphide for methyl-violet. We regard this fact as evidence that the granular aggregates in these hydrogels still retain some charge. In other words, the difference of potential existing between the molecular aggregates and the field in the hydrosol state is not destroyed by coagulants, but only reduced to a point at which the forces of cohesion are just able to overpower the forces of repulsion brought into play by such difference of potential.'

It will readily be seen that if paper assumes a negative charge when immersed in water the phenomena I have described above fall into line with the behaviour of electrically charged colloids. We notice that of a negative colloid like congo-red the less is taken up by paper the more perfect the freedom of the water from electrolytes, there is mutual repulsion until the dye is discharged by a kation. On the other hand, the opposite holds as regards the positive dyes, there is attraction of the negative paper for the positive dye. The kation in this case probably acts by discharging the paper, being attracted thereto by its opposite charge.

Prof. Donnan, to whom I propounded the question, refers me to the work of Coehn<sup>1</sup>, who found that when various non-conducting bodies are immersed in fluids of different dielectric-constants they assume a positive or negative charge according as their own dielectric-constants are higher or lower than the fluid with which they are in contact. For instance, glass (5.6) is negative in water (80) or alcohol (26), whereas in turpentine (2.2) it is positive. Now, paper according to Thwing<sup>2</sup> has a dielectric-constant of 2.82, and would therefore be electrically negative to water.

Moreover, Quincke<sup>3</sup>, in the course of his investigations on electrical endosmosis found that, as a rule, all non-conducting bodies in water took on a negative charge. Among the substances tested by him were cotton-wool and silk; particles of these in water exposed to an electric field wandered to the anode and were therefore negatively charged.

1. *Wied. Ann.*, 64, page 217, 1898.
2. *Zeitsch. f. Physik. Chemie.*, XIV, page 292, 1894.
3. *Pogg. Ann.* 113, page 583, 1861.

The conclusion is, I think, justified that paper takes up very little electro-negative dye because it is itself negatively charged, and that when the dye is discharged by the addition of an electro positive colloid or a kation, there is no longer the same mutual repulsion between the dye and paper.

It would be of interest to test the behaviour of paper in turpentine, in which it would be electro-positive, if one could obtain an electro-negative colloidal dye in solution in turpentine.

This interpretation is confirmed by the results of experiments on silk :—

*Experiment*: From a watery solution of congo-red a piece of paper took up 26 per cent., a piece of silk (well washed), of the same weight took up only 5 per cent. From a  $\frac{m}{200}$   $MgSO_4$  solution, paper took up 91 per cent. and silk 98 per cent. From toluidin-blue in water, paper took up 85 per cent. Silk rapidly took up the whole, so that I added another double amount of dye solution, the colorimeter reading thus showed that 67 per cent. of the whole, that is  $67 \times 3 = 201$  as compared with 85 in the case of paper was taken up. The action of neutral-salts on the adsorption of positive dyes by silk was of the opposite sign to their effect on paper. For example :—

Paper : From water	...	...	85 % adsorbed
„ $\frac{m}{200}$ $MgSO_4$	...	...	51 % „
Silk : From water (concentration of dye three times that in case of paper)			67 % adsorbed
„ $\frac{m}{200}$ $MgSO_4$	...	...	91 % „

The results may be explained by the consideration that silk, owing to its lower dielectric constant than that of paper, would no doubt have a higher negative charge, which would make it less accessible to negatively-charged dyes, but more so to positively-charged dyes. I am unable to state why the action of electrolytes on the positive dye is opposite in the two cases of silk and paper, unless that for some reason silk is relatively more sensitive to the adjuvant action of the anion. If this were so, however, one would expect a more marked

inhibitory effect of the anion in the case of electro-negative dyes and silk, whereas we see that the action of  $MgSO_4$  is rather greater in the case of silk than in that of paper.

The troublesome stain which appears on the surface of glass containing electro-positive dyes is no doubt due to the negative charge of the glass. This film is so adherent that water will not remove it, and I have found it necessary, when working with these dyes, to rinse all flasks used with concentrated nitric acid, in order to obtain concordant results.

Another fact receives its explanation from the negative charge of paper, viz., that from alcohol more negative dye is taken up than from water, in one experiment :

From 50 % alcohol - 65 % taken up  
 „ water - 53 % „

Since alcohol has a lower dielectric-constant than water it would naturally be expected, since the negative charge of paper is due to the difference between its dielectric-constant and that of the fluid in which it is immersed, that the negative charge would be greater the greater this difference is, and therefore in alcohol the charge would be less than in water and the attachment of a negative dye less difficult. On the other hand, *less* + dye is taken up in the presence of alcohol, thus :

From 50 % alcohol - 5 % taken up  
 „ water - 80 % „

Finally, I may refer to the action of acid in preventing the favouring action of electrolytes on adsorption of a negative dye, for example :

CONGO-RED				
From water	...	...	...	27 % adsorbed
„ $\frac{m}{200}$ NaCl	...	...	...	75 % „
„ $\frac{m}{200}$ NaCl + $\frac{m}{2000}$ $H_2SO_4$	...	...	...	31 % „

This result is probably to be explained by the fact that in the presence of sulphuric acid the colour-acid is set free from congo-red. This colour-acid is, no doubt, more strongly electro-negative

than the salt, so that the amount of kation which would suffice to discharge the salt would be insufficient to discharge the acid, it would therefore be comparatively ineffective in promoting adsorption.

In view of the theory set out in the preceding paragraphs it will be of interest to give two curves showing the form of the relation between the concentration of the kation and its effect on congo-red. The ordinates in Fig. 6 represent the percentages of congo-red taken up from a  $\frac{m}{6000}$  solution containing electrolyte of the concentration given by the abscissae. The upper curve is that of  $\text{CaSO}_4$ , the lower curve that of  $\text{KCl}$ .

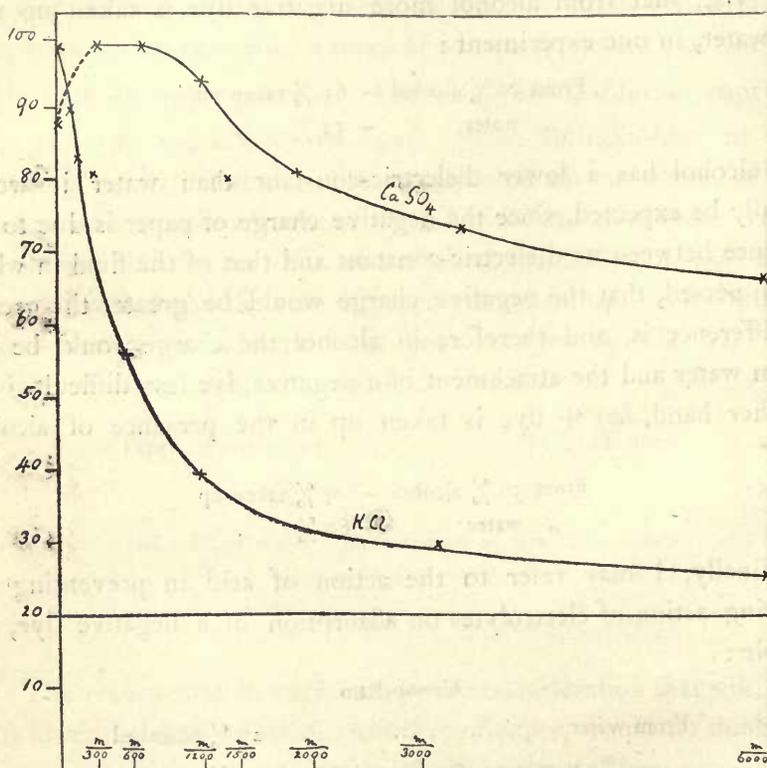


FIG. 6

The commencement of the  $\text{CaSO}_4$  curve is irregular, owing to partial precipitation of the dye having taken place. The inclination to an S-shape at the beginning of the  $\text{KCl}$  curve is, probably, also an indication of incipient precipitation. The extreme sensitiveness of

congo-red to electrolytes is also shown by the curves; even at the low concentration of  $\frac{m}{6000}$  of the electrolyte they do not fall to the level of the amount taken up from the particular sample of water used, which amount is represented by the horizontal line with an ordinate value of 20 per cent. It is noticeable, on the contrary, that the curves tend to become asymptotes. I am not prepared to give any interpretation of the form of these curves.

## V. CONDITIONS OF DISSOCIATION OF ADSORPTION COMPOUNDS

Recent research has brought to light the important part played in vital phenomena by the mutual relations of colloids to electrolytes and to other colloids. Since these relations are apparently those of adsorption, it is of some importance to know what are the conditions under which this association is broken down.

### 1. *Temperature*

We have already seen that dissociation is favoured by rise of temperature, and that, on the other hand, if suddenly exposed to the temperature of boiling water, a dyed piece of paper becomes to a certain degree fixed in its condition, so that the dye can only with difficulty be extracted by water.

### 2. *Precipitation*

It was noticed by Cramer and Swale Vincent<sup>1</sup> that the inorganic constituents of various organic bodies are set free when these bodies are acted upon by precipitants of bases. I have observed the same fact in the case of the precipitation of gelatin by tannin.

*Experiment:* A 15 per cent. solution of gelatin had a specific conductivity of 1570 gemmhos at 38.5. 20 c.c. of this solution were mixed with 90 c.c. water and 10 c.c. of 10 per cent. tannin solution, filtered, and 100 c.c. filtrate concentrated to 20 c.c. The conductivity of this was now 2750 gemmhos. This 20 c.c. would represent  $\frac{100}{120}$  of the first, so that the conductivity should be multiplied by  $\frac{120}{100} = 3300$  gemmhos, that is, the conductivity was doubled by the action of

<sup>1</sup> *Journ. of Physiology*, XXX, p. 150, 1903.

tannin. The amount of tannin added was intentionally less than that required for complete precipitation, so that the filtrate contained a little unaltered gelatin, but no tannin.

### 3. *Action of Chloroform*

Moore and Roaf<sup>1</sup> have shown that electrolytes are set free in blood by the action of chloroform.

### 4. *Excitation, Injury, and Death*

Macdonald<sup>2</sup> found that, as a result of injury, potassium salts are set free in the axis-cylinders of nerve fibres. Under certain conditions these salts may be taken up again by the colloids, and Macdonald suggests a theory of excitation and inhibition on this basis.

Hoeber<sup>3</sup> finds that the excitability, the capacity of staining, and the colloidal consistency of nerve vary concurrently.

Wakelin Barratt<sup>4</sup> finds that chlorine ions are set free when unicellular organisms are injured or killed.

Brailsford-Robertson<sup>5</sup>, as the result of his work on the phenomena of the heart-beat, comes to the conclusion that these phenomena are such as would be expected if there were a periodic driving out by anions of kations from combination with proteid.

Howell,<sup>6</sup> having found that the vagus cannot exert its action on the heart-muscle in the absence of potassium ions, brings forward the hypothesis that the inhibition produced by the vagus is due to splitting off potassium from combination in the muscle.

In order to see whether there is any evidence of a sudden separation of electrolytes at the moment of death, I have made the following experiment:

The skins of two frogs were immersed in isotonic cane-sugar solution. The electrical resistance of the solution was measured, after soaking for some time, and found to be at 11° C., 6407 ohms. The solution containing the skins was then slowly warmed and the resistance determined at frequent intervals up to 56° C. The

1. *Proc. Roy. Soc.*, 73, p. 382, 1904, and 77 B, p. 98, 1906.

2. *Thompson-Yates L.b. Reports*, Vol. 4, p. 213, 1902.

3. *Centralb. f. Physiologie.*, XIX, page 390, 1905.

4. *Zeitsch. f. Allgem. Physiologie*, V, page 33, 1905.

5. *Pflüger's Archiv.* 110, page 623, 1905.

6. *American Journ. of Physiology*, XV, page 291, 1905.

values when plotted on squared paper formed a regular curve, somewhat concave towards the axis of abscissae. There was no indication of any kink in the curve except for one determination at  $16^{\circ}$ , which was obviously an experimental error. That electrolytes were given off is shown by the fact that the diminution of resistance was considerably greater than would have been the case if the diminution depended only on the effect of the rise of temperature on electrolytes present at the beginning. The temperature-coefficient was, indeed, 4.14 per cent. per degree, instead of, at the most, 2.75 per cent., according to the determinations of Arrhenius. The separation of electrolytes also showed itself to be irreversible, since cooling down to  $11^{\circ}$  C., the resistance only increased to 4128 w. The shape of the curve shows that the rate of separation was greater at the lower than at the higher temperatures, but, as already remarked, there was no point on the curve to indicate that death occurred. It is obvious that the results may also be accounted for by gradual destruction of the impermeability of the living cell for most inorganic salts, but, if so, this process must also be a gradual one.

To compare with this experiment I made a similar one with egg-white, raising the temperature to  $95^{\circ}$  C. In this case the result was opposite to the above, viz., there was evidence of taking up of electrolytes. The physical state of the coagulated egg-white is, however, so different from that of the fresh substance that it is difficult to interpret the meaning of the observation.

##### 5. *Coagulation of blood.*

It was found by E. G. Martin<sup>1</sup> that  $Ca^{++}$  ions have a marked stimulating influence in causing freshly isolated strips of the tortoise ventricle to commence beating spontaneously. This effect is shown by serum, but not by uncoagulated plasma. It appears, thus, that  $Ca^{+}$  ions are set free during the process of clotting, so that there should be an increase of electrical conductivity in the process. Robert T. Frank<sup>2</sup> was unable to detect any change of this kind. I have, on the contrary, succeeded in showing that there is such a change, not in

1. *Amer. Journ. of Physiology*, XI, p. 117, 1904.

2. *Amer. Journ. of Physiology*, XIV, p. 466, 1905.

the sense of an *increase* but a *decrease*, so that there is a disappearance of ions. This result is, I think, what would be expected from our knowledge of the relation of calcium to clotting, and the observations of E. G. Martin must be explained in another way. My experiment was made on fowl's blood. As shown by Delezenne, if kept from contact with the tissues this blood remains unclotted for a long time. It was collected from the external jugular vein and placed in a U tube furnished with an electrode in each limb and immersed in a water-bath at  $10.2^{\circ}$  C. When the temperature had become constant the specific conductivity of the blood was 5382 gemmhos. A small piece of muscle from the fowl was then dropped into one side of the tube. The conductivity steadily fell to 4360 gemmhos, which value was attained in 40 minutes; no doubt the process was completed earlier than this, but it was not wished to disturb the apparatus until it was to be expected that clotting had occurred. The diminution of conductivity therefore amounted to 18.5 per cent. of the original value.

#### 6. *Action of enzymes*

I do not purpose here to enter into this subject in any detail, since it will be dealt with fully in a forthcoming paper. There is a certain amount of evidence that the splitting-off of inorganic constituents contributes to the rise of electrical conductivity observed in many cases, but the direct proof was found to be of considerable difficulty.

In the action of rennet on milk, I find a slight *increase* of conductivity. This is merely an additional fact showing the nature of this process to be quite different from that of clotting of blood. In all probability rennet-action is only an expression of pepsin-action in neutral or faintly alkaline medium.<sup>1</sup>

### VI. SPECIFIC ADSORPTION

The phenomena of adsorption show in this respect an approximation to true chemical combination.

It was shown by Schönbein<sup>2</sup> that when strips of filter-paper were immersed in solutions of various salts that the height to which the salts

1. Pawlow and Parastschuk. *Hoppe-Seyler's Zeitsch.*, 42, p. 415, 1904.  
2. Poggenдорff, *Ann.*, 114, p. 275, 1861.

rose was less in the case of calcium and barium than in that of potassium or strontium, the calcium, etc., being more completely held by the paper. In the case of a solution of iodine in  $\text{KI}$ , while the latter rose nearly as far as water, the iodine was kept back in the lower third. These facts were explained by Schönbein as capillary attraction, but, as Ostwald remarks, there is no doubt that it is really an adsorption process that causes the separation of the bodies in question, capillarity only occasions the transport of the separated fractions.

These results of Schönbein have been extended and employed as a means of analysis by Goppelsroeder.<sup>1</sup>

It is well known to histologists how certain tissues take up particular dyes in preference to others. In certain cases, as for example, the differences between paper and silk, as to their respective behaviour towards congo-red and methylene blue, this is probably to be accounted for by the fact of the electrical charge in the two dyes being opposite in sign combined with the negative charge of the substance to be stained, as already pointed out.

Acid fuchsin is used in Van Gieson's stain to show connective-tissue. In view of this, I thought it of interest to try the behaviour of gelatin in sheet towards this dye and toluidin blue respectively. The same amount of gelatin was found to take up 72 per cent. of the acid-fuchsin and only 32 per cent. of the blue. It is possible here that the electrolytes of the gelatin were responsible for the difference, since they would increase the adsorption of the acid dye and diminish that of the basic dye. This possibility is excluded when we compare the amounts of congo-red and acid fuchsin, both electro-negative dyes, taken up by gelatin or filter-paper. The following are the results of an experiment:

Equimolecular concentrations of dye.

Equal weights of gelatin and filter-paper.

Filter-paper from Congo-red	...	31 %
"    "    acid-fuchsin	...	32 %
Gelatin    "    Congo-red	...	49 %
"    "    acid-fuchsin	...	95 %

1. *Mitt. d. techn. Gewerbe Museums, Wien, 1899, and Studien über die Anvendung d. Capillaranalyse, Basel, 1904.*

Gelatin, then, has a specific adsorption affinity for acid fuchsin.

The preference exhibited by silk for methylene-blue over congo-red, previously described, may be explained in another way than by specific adsorption, viz., by the probable greater negative charge of silk.

That gelatin has a greater adsorption-affinity for calcium sulphate than it has for potassium chloride seems to be shown by the experiments on washing out electrolytes, given in the earlier part of this paper. It was found that KCl is more easily washed away than calcium sulphate.

## VII. THE APPLICATION OF THE FACTS OF ADSORPTION-PROCESSES TO VARIOUS PHENOMENA

The theoretical interest of adsorption-processes has been pointed out by Ostwald,<sup>1</sup> who suggests the possibility that a mechanical theory of chemical affinity may be developed on the basis of a complete study of these phenomena.

From a more practical standpoint, it will be found that they play a very important part in numerous processes. I may refer briefly to a few of these.

### 1. *The Soil*

The power of the soil in holding back soluble salts and other bodies is, no doubt, due to adsorption. By this means these bodies are prevented from being readily washed away by the rain.

### 2. *Purification of sewage*

In the filter-process of nitrification, it appears that the complex organic substances, which are inimical to the nitrifying organisms, are kept back by adsorption in the upper layers of the filter.<sup>2</sup>

### 3. *The ash-constituents of organic compounds*

I have already given evidence that these are present in an adsorbed form, and shown that the difficulty of removing the last fractions of inorganic electrolytes is adequately explained on this basis.

1. *Op. cit.*, p. 1098.

2. See Dr. Harriette Chick, *Proc. Roy. Soc.*, 77B, p. 247, 1906

It is scarcely necessary to give instances of this difficulty, and I will merely refer to three instances. Macallum<sup>1</sup> in searching for the cause of the silver reaction of tissues met with considerable difficulty in completely removing chlorides. Mellanby<sup>2</sup> states that there is a molecular combination between neutral salts and globulin. Lange<sup>3</sup> found that it was impossible to remove all ash-constituents from filter-paper by extraction with hydrochloric and hydrofluoric acids and washing.

#### 4. *Dyeing*

On the whole, I think it must be taken as the true explanation of this process that it is, in the main, an adsorption. The very slight diffusibility of most dyes through parchment-paper seems to me a considerable objection to Witt's theory of solid-solution. If the dye is dissolved in the paper it should be readily given off again to water on the opposite side in the same way as hydrogen passes through palladium in the experiments of Ramsay.<sup>4</sup> The theory to which my experiments lead is very much the same as that to which Picton and Linder have independently arrived.<sup>5</sup> According to these observers, there are two stages to be distinguished.

Stage I. The 'coagulation stage' in which single ionic interchange takes place between the 'fibre substance' (colloid) and the dye, resulting in the separation of insoluble dye derivatives retaining a feeble charge.

Stage II. The 'colour absorption' stage, in which coagula produced in stage I attract and retain the oppositely-charged particles of the dye substance.

My experiments show that no actual precipitation of the dye must take place except in the substance to be stained, and I should be inclined to modify the above theory by omitting stage I, and reading instead of 'coagula produced in stage I' simply 'colloids of fibre-substance.' These bodies usually having a negative charge, it is

1. *Proc. Roy. Soc.* 76 B, p. 225, 1905.

2. *Journ. of Physiology*, 33, p. 359, 1905.

3. *Ber. Deutsche. Chem. Ges.*, 1878, p. 823.

4. *Phil. Mag.*, 38, pp. 206, 218, 1894.

5. *Journ. Chem. Soc.*, 88, p. 1,935, 1905.

necessary, in order for them to take up an electro-negative dye, that the latter shall be discharged by the presence of a kation. When the dye is electro-positive, on the other hand, neutral salts are unnecessary and usually deleterious, since both classes of dyes are most sensitive to the kation which would be inhibiting in the case of positive dyes. In certain cases alkali increases the adsorption of these latter, presumably owing to the powerful action of the  $\text{OH}^-$  ion more than counteracting the opposite effect of the kation. The common practice of using basic dyes in strong solution of borax and acid dyes in acid solution is in agreement with this view. It appears, that in the case of certain acid dyes, such as congo-red, the concentration of the electrolyte used in practice is unnecessarily high.

### 5. *The Staining of Histological Preparations*

Here again the balance of evidence is, as it seems to me, in favour of the adsorption theory.<sup>1</sup> It may be that there are exceptional cases where true chemical combination takes place, but they appear to be rare.

This being so, it is obvious that the part played by electrolytes must be taken into account. If electrolytes are split off from living cells when they die or are injured it is clear why these cells readily take up acid dyes under such conditions; moreover, since electrolytes are unnecessary when the substance to be stained is electro-negative, it is also clear why living cells can be stained with basic dyes, if, as seems probable, the structures in question have a negative charge. The reaction of the tissues is on the alkaline side of neutrality and, as Hardy has shown, globulin is electro-negative in alkaline solution, and Pauli has shown the same for albumin.

I am not forgetting the work of Overton on the solubility of basic dyes in the 'Plasmahaut,' and the insolubility of acid dyes therein. The action of electrolytes must also, nevertheless, make itself felt.

An interesting case, which at the same time presents considerable difficulty as to its explanation, is the staining of granules in the axis-cylinder of nerve-fibres. These granules are stained by neutral-red as

1. See Alfred Fischer, '*Fixierung, Färbung, etc. d., Protoplasmas.*' Jena, 1899.

shown by Macdonald<sup>1</sup>, but only in the position and neighbourhood of an injured spot. They correspond to the distribution of potassium ions, and are interpreted by Macdonald as precipitates of the dye caused by potassium. He finds, in fact, that fairly strong solutions of potassium salts are capable of throwing down the dye. Since neutral-red is a basic dye these results seem at variance with the action of neutral salts on adsorption of these dyes as described in the preceding pages. I thought it of interest therefore to see whether neutral-red behaves differently to the basic dyes on which my experiments were chiefly performed. I found it, however, to behave quite in the same way. I did not, indeed, notice any tendency to precipitation even by 2.6 per cent. KCl. There are evidently, then, some other factors at work in the case of the staining by neutral-red of the granules produced by injury in the axis-cylinder of nerve-fibre.

Since congo-red showed itself to be particularly sensitive to electrolytes it would seem a suitable reagent for the detection of electrolytes, if split off from injured tissue. I have tested the behaviour of nerve-fibres to this dye. It is easily seen, under the microscope, that only the cut ends of the nerve are stained and not the uninjured parts of the fibre. I could not detect any appearance of granules, but did not look for them under a high power. It was to be noted also that connective-tissue fibres took the stain, as I imagine because they were non-living.

The fixation of adsorbed dye by heat has already been pointed out. This fact is of interest in connection with Altmann's method of using acid-fuchsin. The fixation of dye by electrolytes has also been mentioned.

Recently Emil Mayer<sup>2</sup> has shown that the affinity of the Nissl bodies of nerve-cells for basic dyes is abolished by previous treatment with neutral salts. This is in complete concordance with the results I have described.

It occurred to me that, considering the opposite action of neutral salts on electro-negative and electro-positive dyes it should be possible

1. *Proc. Roy. Soc.*, 76, page 325, 1905.

2. Hofmeister's *Beiträge*, 7, p. 560, 1906.

to vary the colour of paper stained in a mixture of two dyes of opposite character by the addition of electrolytes. This proved not to be so, the reason being the formation of dye-compounds, which appeared to be unaffected by electrolytes. One of these dye-compounds, that of eosin and methylene-blue, is well-known to histologists and is generally regarded as a salt of the methylene-blue base with the eosin acid. Similar compounds are formed by most pairs of acid and basic dyes. They are very insoluble in water though soluble in excess of either component. This latter fact suggests the possibility that they are not true compounds but adsorption-compounds of oppositely-charged colloids. As shown by Biltz mutual precipitation occurs in such cases and solution in excess of either colloid. On this account I have devoted some attention to these bodies. In the first place, if true chemical compounds are formed by a process of double decomposition the inorganic components should be found combined together in the solution. I tested this in the case of anilin-blue and methylene-blue by taking about 3 grams of the latter and the molecular equivalent of the anilin-blue. The precipitate was filtered off and the filtrate evaporated to dryness in order to estimate the chloride present. Unfortunately, by an omission to make a note of the amount of the filtrate from the silver chloride, the exact value was not arrived at. From the quantity of silver nitrate required to completely precipitate the chloride, it was obvious, notwithstanding, that practically the whole of the chlorine of the methylene-blue was contained in the filtrate. The precipitate was, therefore, a compound of the methylene-blue base with the anilin-blue acid, the sodium of the anilin-blue having at the same time combined with the chlorine of the methylene-blue. I should hesitate to state definitely whether this kind of combination was inconsistent with a process of adsorption, especially in the light of van Bemmelen's results; on the face of it, at the same time, it appears more like a true chemical compound. Dr. J. H. Scott informs me that he has noticed that a new absorption-spectrum makes its appearance when solutions of eosin and methylene-blue are mixed. I have repeated this observation in the following way: A solution of methylene-blue was taken of such a strength as

to show distinctly the two dark bands on the red side of the D-line in a certain cell. An equivalent molecular solution of eosin was taken, so that when mixed in equal volume there should be two molecules of methylene-blue to one of eosin. This eosin solution was placed in a cell of equal depth to that containing the methylene-blue and the two cells then placed before the slit of the spectroscope. The combined spectrum of the two dyes was seen. Equal volumes of the two solutions were then mixed, a portion of the mixture at once placed in both cells and these then observed. By taking a double layer it would seem that there would be no change in the spectrum unless chemical changes had taken place. What one sees is that the band of methylene-blue next the D-line has almost completely disappeared, leaving a faint shade. No change is to be seen in the band in the red, nor in the eosin band at F. The same disappearance of the one band of methylene blue may be observed when any other acid-dye such as anilin-blue or congo-red is taken instead of eosin. On the other hand, if thionin or toluidin-blue is taken instead of methylene-blue, no obvious change is produced in the spectrum until precipitation occurs, when, of course the bands become much fainter. I believe the explanation of the disappearance of the methylene-blue band is to be found in the fact that it is the first to disappear on mere dilution. Now when an oppositely charged colloid is added to a solution of the blue the precipitate does not fall at once and it might be supposed there was no important change ; but, if the experiment is made in a vessel through which a beam of bright light is passing, the track of the beam is barely visible in the methylene blue, but almost immediately on adding the other dye the beam begins to appear, gradually becoming brighter as the particles increase in size. In point of fact then, methylene blue is really taken out of solution, and the first band to disappear would be the one next the D-line. The other thiazin-dyes, so far as investigated, do not show the same behaviour on dilution, the various bands all disappear or fade in nearly the same proportion.

These dye-compounds, although almost insoluble in water, are soluble in alcohol, so that it might be supposed that by taking a strong

alcoholic solution of a methylene-blue compound, one should see both the methylene blue bands. This is not so, and is due to the fact that in alcoholic solution of methylene-blue the band next the D-line is not to be seen at all distinctly. If the solution showing only the other band is gradually increased in depth or concentration, it is seen that this band broadens out until it reaches the D-line, but there is no indication of a gap between the bands. A fact which confirms the interpretation I have given of the change in the methylene-blue spectrum produced by combination with an acid-dye, is that scharlach R. in weak alcoholic solution produces the same change, and also a precipitate of a dye-compound. Now scharlach R. is an indifferent dye, not forming salts, but forms an electro-negative colloidal solution; so that it would seem that here, at all events, the precipitate must be an adsorption compound. A similar state of affairs is to be observed in the case of rosolic acid. Here the possibility of double decomposition is also absent, except, of course, that by mass-action a small proportion of methylene blue rosolate might be formed. The only instance I have found where there is a real change in the spectrum is in the mixture of methylene-blue and picric acid; picric acid is, however, a powerful reagent, and much weight cannot be laid on this fact as far as concerns the question before us. In mixing methyl-violet and an acid-dye no change is to be observed in the absorption spectrum.

The absence of change in the absorption-spectrum of these dye-compounds does not, of course, exclude the possibility of their being true chemical compounds.

With regard to the properties of these bodies, they are indiffusible, as such, through parchment-paper. In dilute watery solution they appear to be dissociated; for, on dialyzing the eosin-methylene-blue compound, for example, it will be seen that after a time eosin begins to pass through, and is ultimately followed by the methylene-blue. Since this observation was made on a solution of the pure body, which had been thoroughly washed with cold water, the eosin and methylene-blue must have passed out as the free acid and base respectively. This dissociation is also shown by the fact that, at  $100^{\circ}$ , the solution is a fairly good conductor of the electrical current, and is electrolyzed by a constant current.

Although these compounds are, no doubt, colloids, they do not appear to carry a charge—or only a very small one. Electrolytes have a very slight effect in the sense of retarding adsorption. In the boundary apparatus the behaviour of anilin-blue-methylene-blue under electric stress is difficult to interpret, but since the phenomena are the same in neutral acid, or alkaline solution, it does not seem to be a question of electric charge. What happens is this : after the current has passed for some time it will be seen that electrolysis has occurred, so that on the anode side there is a layer of anilin-blue solution, and on the kathode side one of methylene-blue. The upper boundaries of these two are at the same level in both limbs, but the methylene-blue layer being deeper than the anilin-blue it makes the level of the unaltered compound on the kathode side lower than on the anode side, so that it appears as if negatively charged and moving to the anode. The behaviour to electrolytes, if any at all, is, on the contrary, in the sense of a positive charge ; so that I am inclined to think that the behaviour in the electric field is, in some way, due to different velocities of the two ions. In the case of the eosin-methylene-blue there was no similar difference in level of the unaltered dye, so that it may be that here the two ions are more nearly equal in velocity.

This absence of proof of any definite electric charge makes the compounds less interesting from the theoretical point of view in some respects. But it is, I suppose, what might be expected if these bodies are formed by the mutual neutralization of electro-positive and electro-negative colloids.

On the whole it seems impossible to give, in the present state of knowledge, a decided answer to the question as to the nature of these dye-compounds.

## 6. *Antitoxins*

It has been shown by Craw<sup>1</sup> that the combination between toxin and antitoxin follows more closely in its nature that of adsorption than that of chemical combination. The puzzling fact known as the 'Danysx-von-Dungern phenomenon' is, for example, satisfactorily

1. *Journ. of Hygiene*, 5, p. 115, 1905, and *Proc. Roy. Soc.*, 76 B, p. 179, 1905.

explained by what I have called in the earlier part of this paper the 'law of adsorption.' In illustration I may cite one experiment :

Two filter-papers of equal size (12 cm. diam.) were each cut into eight pieces. Two flasks, each containing 50 c.c. of a dilute congo-red solution were taken, and to one of these the whole of one paper was added at once, to the other piece by piece at intervals of about twelve hours. After all the paper had been added the amounts taken up from the solution were :

Added altogether	...	...	...	37 %
„ piece by piece at intervals	...	...	...	48 %

It is advisable in this experiment to wait a considerable time between the addition of each piece of paper on account of the slow attainment of equilibrium in the case of congo-red and paper.

#### 7. *The part played in enzyme-action*

The interest of adsorption phenomena in this region is in connection with the combination between enzyme and substrate, which so much recent work indicates as an indispensable condition of attack. The fact that enzymes are carried down when a colloidal precipitate is produced in a solution containing them, has been long known and used as a means of preparation. But whether the union between a more or less specific enzyme and the body hydrolyzed under its influence is to be looked upon rather as a true chemical combination is a matter of dispute.

Certain experiments have been made by Dauwe<sup>1</sup> as to the taking up of enzymes by colloids. These were done chiefly on pepsin and various proteins, a few on the taking up of pepsin by agar and of emulsin by coagulated egg-white. Dauwe comes to the conclusion that the process is not one of adsorption, since the same weight of boiled egg-white takes up the same amount of pepsin whether the egg-white is in large pieces or fine powder. He considers that the hypothesis of solid solution applies to the case better. It seems to me that this conclusion is scarcely justified. Coagulated egg-white is not a homogeneous solid body, but porous, like charcoal, and therefore its active surface must not

1. Hofmeister's *Beitrage*, VI. p. 426, 1905.

be restricted to the external surface of the pieces of various size. Porous solids like charcoal, even in lumps, adsorb gases, and that not only on their surfaces but throughout their substance. Dauwe, indeed, appears to have thought of this possibility, but regards adsorption as the same thing as solid solution in such cases.<sup>1</sup> The distinction is, surely, analogous to that between true solution and colloidal solution. Although recent investigations teach that we must not draw a hard and fast line between these two kinds of solution, there is no doubt that the molecules or particles in suspension change their properties as their dimensions increase beyond what are ordinarily called molecular, and that they begin to have the properties, due to surface, of matter in mass. In the same way, as I think, we ought to keep the name 'solid solution' for such cases as alloys of metals, and give the name adsorption not only to the taking up of substances from their solutions by surfaces, in the usual sense of the word, but also by such surfaces as those forming the walls of pores. The criterion by which any given case is to be decided, is, of course, the way in which the relative amounts taken up vary as the concentration of the body taken up changes.

Dauwe rejects the hypothesis of chemical combination on the ground that the enzyme taken up can be extracted again by an appropriate solvent, for instance, pepsin from coagulated egg-white by a solution of egg-white. He regards the process as one of solid solution, or relative solubilities of the enzyme in the substrate and water, and brings forward in support of this view the experiments of Reichel and Spiro<sup>2</sup> on the apparent loss of rennet in the process of clotting of milk. These observers showed that the disappearance of the enzyme was to be accounted for by the taking up of it by the clot. Now, as I understand their results, they are rather in favour of adsorption. If it were a case of solid solution the percentage loss would be the same whatever the rennet concentration, but if one looks at the table on page 481 of the second paper referred to, it is seen that the percentage loss steadily *increases* as the concentration of the

1. *Loc cit.*, p. 443.

2. Hofmeister's *Beiträge*, VI, p. 68, 1905, and VII, p. 479, 1905.

enzyme falls ; in other words the more dilute the solution the more adsorption takes place. This is particularly well shown by the last experiment on the table. The mathematical expression given is also in accordance with this interpretation.

If the union of enzyme and substrate follows the law of adsorption it would be expected that there would be found some effect of this law on the relation of activity of enzyme to its concentration. In my paper on *The Kinetics of Tryptic Action*,<sup>1</sup> I showed that the form of the function in question varied according to the stage of the reaction, but if we take the early stages in which the action is most rapid there seems to be some effect suggestive of an adsorption process. The initial linear stage is very short, so that we may omit it, and, if we compare the relative times taken by concentrations of trypsin varying as 8, 4, 2, and 1 to produce an increase of conductivity of 1800 gemmhos in caseinogen solution, we find the values are :

Relative trypsin content		Time taken in minutes
8	...	54
4	...	79
2	..	126
1	...	233

so that the lower concentrations of trypsin are relatively more active. It is somewhat remarkable that the value of the exponent found in another experiment was 1.42 to 1.67<sup>2</sup> and this is the same as that given by Reichel and Spiro in their paper for the 'Teilungsfactor' of rennet, viz., 1.5 to 1.67. Whether this is merely an accidental coincidence I am not prepared as yet to state, the subject being still under investigation.

I have myself made one or two experiments similar to those of Dauwe, which are perhaps of sufficient interest to give here. It was shown by W. A. Osborne that calcium caseinogenate does not pass through a porous clay filter, trypsin, on the contrary, does do so. If then, we mix solutions of trypsin and calcium caseinogenate, the

1. *Archives des Sciences Biologiques*. Tome XI, Suppl. p. 261. St. Petersburg, 1904.  
2. Page 26 of the reprint.

'compound' formed should prevent the appearance of trypsin in the filtrate. This I find to be the case. It may be said that what we have here is an instance of mutual action of colloids, but, as I think I have been able to show in the previous pages, there is no essential difference between this action and adsorption by solids from watery solution. On the other hand, it might be held by some, that the case is one of true chemical combination. To test this view I performed a similar experiment, taking caseinogen and malt amylase, and found that this enzyme is also held back by caseinogen, so that it appears to be merely adsorbed. This interpretation of the union of enzyme and substrate does not, of course, exclude a certain degree of specific relation between a particular enzyme and the substrate hydrolyzed by it, as we have seen there is considerable evidence of specific adsorption. It is probable, moreover, that it does not hold, in the same degree, for such enzymes as invertase, maltase, or lactase, when the substrate hydrolyzed is not colloidal, and where there seems to be a very close relationship between the chemical structure of the enzymes and the bodies split by them.

In another way the results described in the present paper are of interest in connection with enzymes. I refer to the action of electrolytes. From the work of Cole,<sup>1</sup> McGuigan,<sup>2</sup> and others, it follows that there is a certain opposition between the action of kations and anions. This indicates that enzymes are possibly electrically charged colloids. Victor Henri,<sup>3</sup> in fact, speaks of trypsin as a negative colloid. I have tested the behaviour of a solution of Grüber's trypsin in the boundary apparatus, and found that it does indeed move to the anode, but how far this negative charge is due to the enzyme itself it is naturally impossible to decide until we have in our hands a pure preparation of the enzyme.

It is possible, however, to attack the problem in another way. Caseinogen in solution in alkali is electro-negative, if trypsin is also negative neutral salts, and especially those of bivalent kations, such

1. *Journal of Physiology*, XXX, pp. 202 and 281, 1904.
2. *American Journal of Physiology*, X, p. 444, 1904.
3. *C. R. Soc. di Biologie*, LIX, p. 132, 1905.

as calcium, should increase the adsorption. In some preliminary experiments I have made there seems to be some action of this kind, but it is difficult to obtain the correct relative concentrations so as to avoid precipitation. An experiment was also made on adsorption of trypsin by paper as follows :—

Four crystallizing dishes were taken, each containing a circle of filter paper, in two of them there was a watery solution of trypsin, in the other two a solution of trypsin of the same strength in  $\frac{m}{350}$   $\text{CaSO}_4$ . After standing in a cool place for 24 hours, a paper from the watery solution was taken out, drained for 1 minute, placed in a flask, and heated to  $100^\circ$  in a steam sterilizer ; the same was done with a paper from the  $\text{CaSO}_4$  solution. 160 c.c. of 5 per cent. caseinogen in ammonia were added to each, and they were then placed in the thermostat at  $39^\circ$  ; when warmed to the first was added a drained paper from the  $\text{CaSO}_4$  trypsin, and to the second a similar one from the watery solution. The electrical conductivities were determined at intervals. There was not a very great difference between the two, but what there was showed that rather more trypsin had been taken up by paper under the action of  $\text{CaSO}_4$ . The times taken to reach a change of 800 gemmhos were 165 minutes for the trypsin from  $\text{CaSO}_4$  solution, and 192 minutes for that from watery solution. A similar experiment taking a  $\frac{m}{350}$  solution of toluidini-blue instead of  $\text{CaSO}_4$  was unsuccessful on account of mutual precipitation of the dye and trypsin. The precipitate, when filtered off and washed, showed itself to have considerable tryptic power. This subject requires further investigation.

The importance of the study of adsorption and surface-action in general in connection with the enzyme-action is emphasized by Bredig<sup>1</sup> in his article on 'Chemical Kinetics' in the *Ergebnisse* of Asher and Spiro.

#### 8. *Oligo-dynamic action*

It has been pointed out by Pauli<sup>2</sup> that the puzzling phenomena called 'oligo-dynamic action' by v. Naegeli, and consisting in toxic

1. *Ergebnisse d. Physiologie*, I, p. 211, 1902.

2. Hofmeister's *Beiträge*, VI, p. 257 (footnote), 1905.

actions of distilled water which has been in contact with polished metal, are, in all probability, due to the presence of colloidal metallic hydroxides. Various solids, such as paper, are known to remove this toxic property. It occurred to me, therefore, that perhaps the adsorption of congo-red which occurs to some extent, even from distilled water, might be due to these hydroxides. I found that the ordinary laboratory distilled water caused considerably more adsorption of congo-red than that which I had prepared myself, using a tin condenser. From this latter, nevertheless, 22 per cent. was taken up. By allowing filter-paper to soak in this water previously the value was reduced to 18 per cent. It did not seem possible to get below this—no doubt the electrolytes dissolved from the glass and the traces remaining in the paper were sufficient to account for the effect.

#### VIII. SUMMARY OF RESULTS

1. The hyperbolic form of the curve of adsorption is confirmed.
2. The curve of electrical conductivity of successive distilled water extracts of gelatin has the same form.
3. It is impossible to wash out all the electrolytes from gelatin except by a practically infinite number of changes of water, each change removing a less percentage than the previous one.
4. The electrolytes are, therefore, neither chemically combined nor merely admixed, but in the intermediate form known as adsorption.
5. When gelatin has been washed nearly free from electrolytes it is capable of diminishing the conductivity of solutions of electrolytes in which it is placed. This it does by adsorbing them in a non-ionized condition.
6. The rate at which congo-red is taken up by paper is greatly accelerated by rise of temperature, but the total amount taken up when equilibrium is attained is less the higher the temperature.
7. The temperature-coefficient of the reaction-velocity is extremely low, so that the theory of Nernst as to the part taken by diffusion in heterogeneous reactions seems to apply to the case of adsorption.

8. At low temperatures equilibrium is attained very slowly, at room temperature at least 24 hours being required.

9. The adsorption-compound of gelatin and inorganic electrolytes is also dissociated as the temperature rises.

10. Raising the temperature rapidly to  $100^{\circ}$  tends to fix congo-red in paper so that it is afterwards extracted by water with considerable slowness.

11. No evidence was obtained of any production of heat in adsorption.

12. The reaction between congo-red and cellulose is reversible, as is also that between gelatin and electrolytes.

13. Dyes forming colloidal solutions are extremely sensitive to electrolytes in regard to their adsorption. The effect seems to be proportional to the degree of their colloidal nature or size of colloid particles.

14. The action of electrolytes may be expressed as follows : In the case of electro-negative dyes, like congo-red, kations facilitate adsorption, anions depress it. In the case of electro-positive dyes like toluidin-blue kations depress, anions facilitate. But in both cases the effect of anions is very small compared to that of kations.

15. The effect of bivalent kations is considerably greater than twice that of univalent kations.

16. Salts of the heavy metals which form positively charged colloidal hydroxides in solution have a very powerful effect in promoting adsorption of electro-negative dyes.

17. There is evidence of the carrying-down with the adsorbed dye of the facilitating ion.

18. The presence of a stable colloid, like gelatin, protects congo-red from the action of electrolytes. A negative charge seems necessary for this action, since egg-albumin, although exercising a similar action in alkaline solution, had the opposite effect in acid solution.

19. The explanation of the action of electrolytes, as well as that of other electrically-charged colloids is to be found in the negative charge of non-conductors, like paper, when immersed in water. (Quincke).

20. The different behaviour of silk and paper towards electro-negative and electro-positive dyes, as also the influence of alcohol on the process, is to be explained by the results of Coehn on the influence of the respective dielectric constants on the charge.

21. When gelatin is precipitated by tannin its adsorbed electrolytes are split off.

22. There is no evidence of a sudden separation of electrolytes at the moment of death. There is a gradual one when a living tissue is warmed from  $11^{\circ}$  C. to  $56^{\circ}$  C.

23. In the process of clotting of blood there is a *diminution* of electrical conductivity, so that ions (probably  $\text{Ca}^{++}$ ) disappear from solution.

24. The adsorption affinity of gelatin for acid-fuchsin is greater than that for congo-red, while that of paper is the same for both.

25. A theory of dyeing is suggested on the basis of adsorption in relation to electrically-charged colloids.

26. An explanation, apart from different permeability of the cell, is suggested for the staining of living cells by basic dyes and the non-staining by acid dyes.

27. The nature of the compounds between acid and basic dyes is investigated. They appear to be uncharged colloids, but the evidence as to whether they are colloidal adsorption compounds or true chemical compounds is not decisive.

28. Evidence is brought forward to show that the union between enzyme and (colloidal) substrate is of the nature of adsorption. The adsorption of trypsin by paper is facilitated by calcium sulphate.

TABLE OF DYES

Name	Chemical nature	Molecular weight	Diffusion	Size of Particles	Electric migration	Electrical conductivity in gemmos	States of solution	Action of												
								Kations			Anions			Negative colloid	Positive colloid	Stable colloid				
								H	K	Ca	OH	Cl	SO <sub>4</sub>							
Picric acid	Colour acid	229.2	Rapid	Partially sub-microscopic	...	...	?	...	o	...	...	...	...	...	...	...	...	...	...	...
Rosolic acid	Colour acid	304.2	Very slow	Marked Tyndall phenomenon	To anode in weak alcohol	...	Negative colloid in weak alcohol	+	+	+	...	...	...	...	...	...	...	...	...	...
Methyl orange	Colour acid	327.37	Fairly rapid	...	? Ppt. on anode	...	?	...	...	...	...	...	...	...	...	...	...	...	...	...
Eosin	K salt of colour acid	647.9	Rapid	A-microscopic	? Ppt. on anode	2225 $\left(\frac{m}{100}\right)$	Negative colloid	+	...	+	...	...	...	...	...	...	...	...	...	...
Acid fuchsin	Na salt (acid) of colour acid	447.44	Slow	...	To anode	...	Negative colloid	...	...	+	...	...	...	...	...	...	...	...	...	...
Congo red	Na salt of colour acid	696.68	Very slow	Sub-microscopic	To anode	5660 $\left(\frac{m}{100}\right)$	Negative colloid	+	+	+	...	...	...	...	No ppt.	...	...	...	...	...
Anilin blue	Na salt of colour acid	734.34	Very slow	Sub-microscopic	To anode	7380 $\left(\frac{m}{100}\right)$	Negative colloid	...	+	+	...	...	...	...	...	...	...	...	...	...
Nigrosin	Na salt of colour acid	About 722	Very slow	Sub-microscopic	...	...	...	...	+	+	...	...	...	...	...	...	...	...	...	...
Scharlach R	'Indifferent' dye	380.36	Nil into weak alcohol	Sub-microscopic	To anode in weak alcohol	...	Negative colloid	...	+	Ppt.	...	...	...	...	...	...	...	...	...	...
Methylene blue	Chloride of colour base	319.81	Slow	A-microscopic	To kathode ?	549 $\left(\frac{m}{100}\right)$	Positive colloid	-	-	-	...	+	...	Ppt.	No ppt.	...	...	...	...	...



## NOTES TO TABLE OF DYES

The *molecular weight* of methyl-violet is given as that of the penta-methyl derivative.

*Diffusion.* The words 'very slow' mean that a certain amount had passed through parchment paper by the end of 24 hours.

The statements as to the ultra-microscopic behaviour of dyes in the column headed 'Size of particles' are taken from the work of Michaelis referred to in the text. 'Sub-microscopic' means that the solutions are resolvable into particles. 'A-microscopic' means that they are optically homogeneous.

*Electric migration.* It was difficult to be certain in the case of methylene blue since bleaching occurred by the electrolytic chlorine.

The measurements of *electrical conductivity* were taken at 39° C unless otherwise mentioned. They are not to be looked upon as absolutely correct, since the dyes were not specially purified, except the eosin methylene-blue compound.

*State of solution.* It is impossible to make definite statements as to certain dyes, e.g., eosin, since they have some properties of colloids, but not all.

The columns headed 'Kations' and 'Anions' refer to the action of these bodies on adsorption by paper. It is to be understood that in all cases the action of Anions is very much less than that of Kations.

The columns 'Negative' and 'Positive colloid' refer to actual precipitation, not adsorption.

The column 'Stable colloid' refers to the effect of this on the facilitation or inhibition of adsorption by kations.

## THE NATURE OF ENZYME ACTION

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### CATALYSIS

ALL investigators who have had occasion to consider the nature of the chemical changes taking place in living organisms must have been struck with the comparative ease with which highly stable bodies are split up under these conditions. Sugar is oxidised to carbon dioxide and water, egg-white is hydrolysed to amino-acids and other simple bodies. Under ordinary laboratory conditions, strong acids and high temperature or similar powerful agents are necessary to effect such changes. Schönbein may be mentioned as one of those who early called attention to this circumstance.

Now although, at first sight, the powers referred to may be regarded as a distinguishing characteristic of systems which are called "living," it must be remembered that there is a large and increasing class of phenomena, known to chemists as "catalytic," which manifest properties in many ways analogous. Oxygen and hydrogen, for instance, combine at ordinary temperatures so slowly that the production of water is not to be detected, and the application of the high temperature of a flame or electric spark is needed. But the presence of a minute

quantity of finely divided platinum is sufficient to cause combination to take place at ordinary temperatures. Again, the oxidations produced by hydrogen peroxide proceed in many cases at a very slow rate, which can be enormously increased by the addition of traces of iron or manganese.

What, then, are the characteristics of such phenomena? And are there to be found in the living organism bodies acting in a similar way to the platinum or iron in the above cases?

The essential property of a catalyst is that given in the well-known definition by Ostwald, in which it is stated to be a body which accelerates (or in some cases retards) a chemical reaction. The original definition also states that the chemical reaction in question is one that, left to itself, proceeds at an extremely slow rate. But it is obvious that it is theoretically immaterial what the original velocity of the reaction is: any foreign substance that changes the rate of the reaction is a catalyst. At the same time the practically important cases are those where the reaction is almost or quite inappreciable without the assistance of a catalyst. A further characteristic of these bodies is that they do not appear in the final products of the reaction and usually are to be found unaltered at the end. There are, however, exceptions to this last statement, as we shall see later, owing to the instability of the catalyst under the conditions of the reaction.

At the outset it is important to be quite clear as to the distinction between what may be called "trigger-action" and catalysis. Let us suppose a block of some solid material resting at the top of an inclined plane, which has a somewhat rough surface, and is at such an angle that the block would, if left to itself, slide down very slowly. This is prevented by a stop of some kind which can be removed, say by pulling a trigger. Now it is plain that it makes no difference either to the rate at which the block slides down or to the energy developed in its fall whether the trigger is very easily worked or is stiff and needs the expenditure of much energy to move it. But let us suppose that the block is slowly sliding and that we apply a catalyst, in the shape of grease, to the inclined plane. The block will now move faster and the rate will be proportional to the amount of grease used, although the energy developed will, as before, be unaltered. Here we see illustrated two additional properties of catalysts—viz., that the amount of

increase of velocity of reaction is, within limits of course, proportional to the concentration of the catalyst, and also that no energy is added to the system by its presence. The fact that when the falling block has reached the bottom of the slope the energy developed by its fall is the same whether it fell rapidly or slowly calls attention to yet another important property of catalysts, which consists in the fact that a small quantity will, in the end, produce as much effect as a larger one, provided that it be given a longer time to act, on the understanding that during this longer time the catalyst itself remains intact.

As a rule these bodies are very active, so that it is usually taken as one of their characteristics that they act in extremely low concentrations compared to those of the bodies acted upon.

When we now turn again to the living organism, with the properties of catalysts in our minds, we note that bodies answering to this description have for many years been prepared from various cells and tissues. To mention one or two only, we have diastase from malt, which converts starch to maltose; pepsin from the stomach, which splits up various proteins into peptones; peroxydase, which separates active oxygen from various organic peroxides; and so on. These various preparations were originally called "ferments," but owing to a certain confusion between the organism producing certain changes—yeast, for example—and the chemical substance by whose agency the changes are produced, it was suggested by Kühne to call these chemical agents "enzymes," expressing the fact that invertase—one of the earliest known—was contained in yeast (*ἐν ζύμῃ*). The name has come into general use, though "ferment" is also frequently used.

"Enzymes," then, are the organic catalysts met with in the living cell. They have certain properties in addition to those already mentioned, one of which is that of being destroyed by a temperature of from 50 to 70° C., probably due to their being colloidal in nature. In contradistinction to the changes produced by living protoplasm, their activities are not prevented by the presence of antiseptics, such as chloroform or toluol, although in certain cases these substances have a more or less retarding action.

Although, as yet, none of the enzymes has been prepared synthetically, and their only source is the living organism, it

seems to me unscientific to include this condition in their definition. Oppenheimer gives practically Ostwald's definition of a catalyst, as referred to above, with the addition of the proviso that an enzyme is a "catalytically active substance which is produced by living cells." But the origin of a substance does not concern us when investigating its properties, and, as Bredig points out, this fact does not come into consideration any more than the fact of the heart being composed of living cells affects the discussion of its properties as a pumping-engine.

As catalysts, then, the function of enzymes is to accelerate the velocity of reactions, so that the study of enzyme action in the main becomes one of velocity of reaction. Our attention must, accordingly, be directed first of all to this question. But, as will be seen, it is necessary, before we can profitably discuss the general laws of reactions as catalysed by enzymes, to consider shortly how far these reactions are to be regarded as reversible. In other words, is a synthetic process capable of acceleration by the same catalyst that accelerates the corresponding analytic one? and in what way is the position of equilibrium affected by the presence of a catalyst?

#### REVERSIBILITY

If we add ethyl acetate to water we find, on examination of the mixture after some time, that, in addition to ethyl acetate and water, there are present the products of hydrolysis of the former, viz. ethyl alcohol and acetic acid. By analysis of the solution at various intervals of time, we find, moreover, that a state of equilibrium is arrived at. In this condition, provided that no change takes place in other conditions, such as temperature, the relative proportions of ethyl acetate, acetic acid, and alcohol are always the same. Again, suppose that we start from the other end, so to speak, and take acetic acid and alcohol, instead of ethyl acetate, we find that the same relative proportion of the three bodies is present when equilibrium is established. We have to deal, therefore, with two opposite reactions. A little consideration will show that, as Van 't Hoff points out, these two reactions must be regarded as both proceeding simultaneously, but at varying rates in proportion to the concentration of the reacting bodies, until in the equilibrium position they possess the same velocity. In fact, suppose we

start with ethyl acetate in water, for a moment the reaction between ethyl alcohol and acetic acid will be absent, but as soon as traces of these bodies are formed by splitting of the ester the opposite reaction will commence, although at first slowly.

Now the hydrolysis of ethyl acetate is accelerated by the presence of various catalyts, such as acid ( $H^+$  ions), and the enzyme lipase. Is the opposite (synthetic) reaction also accelerated by these bodies?

Since the equilibrium position is given by the equality of the two opposite velocities, it is plain that if one only of these is changed the equilibrium position must alter. So that, if it is found experimentally that catalyts do not affect the final equilibrium position, it follows that both the opposite reactions must be correspondingly changed.

If the view taken in the introductory remarks as to the action of catalyts is correct, and that no energy is added or taken away from a reacting system by the presence therein of a catalyst, it would seem, *à priori*, necessary that the position of equilibrium should be unaffected. There is, moreover, direct evidence that such is the case. The question has been thoroughly investigated in Ostwald's laboratory by Koelichen in the case of this polymerisation of acetone by bases, and by Turbaba in that of the equilibrium between aldehyde and paraldehyde. It was found in both cases that the equilibrium position was unaffected by catalyts. Naturally the time taken to attain thereto varied considerably, according to the catalyst used.

Many cases are known where a catalyst which accelerates a particular reaction also accelerates the opposite one, when acting on the products of the former. The instance already given of ethyl acetate is one of these; hydrochloric acid not only accelerates the hydrolysis of the ester, but also its formation when acting on a mixture of acetic acid and alcohol.

But the problem which concerns us here is, do the colloidal organic catalyts, the enzymes, fall into line with the simpler inorganic ones in this respect? Practically this resolves itself into this question, does an enzyme under one set of conditions accelerate a particular reaction and under another set of conditions accelerate the opposite reaction? Does lipase, for example, behave like hydrochloric acid in the case just mentioned?

In certain cases synthetic actions of this kind have already been observed. I may refer to the production of ethyl butyrate by the action of lipase on butyric acid and ethyl alcohol in the experiments of Kastle and Loevenhart, of amygdalin from glucose and mandelic-nitrile-glucoside by the maltase of yeast (Emmerling), of salicin by emulsin, and probably of saccharose by invertase from their respective products of hydrolysis (Visser), of a disaccharide from glucose by maltase (Croft Hill), and of isolactose from galactose and glucose by lactase (Emil Fischer and Frankland Armstrong). There is some evidence also of a similar action in the case of the enzymes which act upon proteins.

We may take it, then, that, so far as investigated from this point of view, enzymes have shown themselves to be capable of accelerating both of the opposing components in reversible reactions.

There are, at the same time, certain cases which need a little more consideration; but before proceeding further it will simplify matters if a few words be said as to the terminology of the subject. It was suggested by Duclaux that the termination "-ase" should be taken as expressing an enzyme, and that this termination should be added to the body acted on by the enzyme—*e.g.* lactase is the enzyme hydrolysing lactose. It is, of course, inconvenient to displace some of the old-established names, such as "pepsin" and "trypsin," but, as far as possible, Duclaux's recommendation should be acted upon. Again, an English name for the substance split by the enzyme is badly wanted. "Substrate" is used by the Germans, and, in default of a better, it will be used in the present article. "Hydrolyte" is used by some authors; but this name does not include cases where the action is not one of hydrolysis but of intra-molecular splitting or oxidation.

It has been the custom to speak of an enzyme which attacks starch or protein, for example, as "amylolytic" or "proteolytic" respectively; but, as Prof. Armstrong has pointed out, these names are incorrectly formed. "Amylolytic," in analogy with "electrolytic," should mean a decomposition by means of starch, and, to avoid this confusion, Prof. Armstrong advises the use of the termination "-clastic" instead of "-lytic" in speaking of enzyme action.

After this digression I now return to the discussion of some

recent work on the synthetic action of enzymes. It will be noticed that, in the list of cases given above, isolactose and not lactose is stated to be formed by the action of lactase on glucose and galactose. Accordingly, the sugar synthesised by the enzyme is not the one which it hydrolyses, but the optical isomer of the latter. Similarly, Croft Hill found that maltose is only produced in small amount by the action of maltase on glucose, and that a new disaccharide (revertose) makes its appearance. Frankland Armstrong states that this revertose is identical with isomaltose, as Emmerling had also concluded. Frankland Armstrong is, indeed, of the opinion that the rule is that with a particular enzyme that body is synthesised which is *incapable* of being hydrolysed back by the enzyme in question.

It will be obvious that this conception involves many difficulties. To follow the evidence on which it rests, and which consists in the mode of action of maltase and emulsin respectively on glucose, a brief explanation of the stereochemical relations of the glucosides is necessary. Glucose itself is now looked upon as having the structure of a glucoside—*i.e.* the internal anhydride structure of a lactone. It therefore exists in two optically isomeric forms known as  $\alpha$ - and  $\beta$ -glucose. In water these two forms, which differ in their rotatory power, exist together in equilibrium; according to Tanret, in 10 per cent. solution there is 3.7 per cent. of the  $\alpha$ -form and 6.3 per cent. of the  $\beta$ -form. When a solution of glucose in methyl-alcohol, in which also it exists in both  $\alpha$  and  $\beta$  forms, is acted on by hydrochloric acid, two stereo-isomeric methyl-glucosides are formed, corresponding to the  $\alpha$ - and  $\beta$ -glucoses. Of these the  $\alpha$ -compound alone is hydrolysed by the maltase of yeast, and the  $\beta$ -compound alone by emulsin—the enzyme found so frequently in the higher plants, and which hydrolyses so many of the natural glucosides, such as amygdalin. These latter, then, naturally must be regarded as  $\beta$ -glucosides, while maltose (glucose-glucoside) is an  $\alpha$ -glucoside; isomaltose is presumably the glucose- $\beta$ -glucoside.

If we take a solution of glucose, therefore, and act upon it by maltase, we should expect, from all that we know of reversible reactions, that a certain amount of maltose would be synthesised; and this, in fact, is what Croft Hill originally believed to take place. Later he found that only a small part of the bi-hexose consisted of maltose, the remainder being, as

he thought, a new sugar, revertose. Moreover, as already mentioned, Frankland Armstrong thinks it doubtful whether maltose is produced at all by the action of maltase, and that revertose is really isomaltose. Emulsin, on the other hand, which does not hydrolyse maltose, does produce this sugar, though apparently in very small quantity.

If we accept this point of view, we are landed in many difficulties, as well as having to reject many experimental results of other observers. Croft Hill, for example, found that the same equilibrium point was reached whether he started from glucose or from a mixture consisting chiefly of maltose—viz. for a 40 per cent. solution, equilibrium was arrived at when the percentage of maltose was 15 and that of glucose 85. This would seem impossible if the synthetic product were isomaltose, on which the enzyme present has no action. Since there is no agent capable of hydrolysing the isomaltose formed, there is no reason why the glucose should not be completely transformed to this disaccharide. Again, as Croft Hill showed, the relative percentages of glucose and "maltose" varied according to the concentration of the original solution taken; in a 10 per cent. solution, for example, the percentage of maltose is only 5 at the equilibrium point. If, therefore, we were to take a 40 per cent. solution of glucose and act upon it with maltase until the 15 per cent. of the synthetic disaccharide has been produced and then dilute to four times its volume, if the synthetic sugar formed were isomaltose it should remain unchanged, whereas, if maltose, it would, of course, be hydrolysed back to glucose by the enzyme present. This experiment has been performed by Croft Hill with the result of hydrolysis back to glucose, according to the latter of the two above alternatives.

The results of Visser, who found that emulsin synthesised salicin, the same glucoside which it hydrolyses, and that invertase is able to synthesise cane-sugar, are also at variance with Frankland Armstrong's results.

It must be admitted, therefore, that further evidence is necessary before we can accept this point of view of the synthesis by enzymes of bodies which they do not hydrolyse.

Croft Hill seems to suggest the possibility of more than one enzyme being present in the preparations used. This suggestion must be kept in mind, especially as the methods used by Frankland Armstrong depend on the destruction of glucose or

maltose by fermentation with living yeasts. The importance of the synthetic aspect of enzyme action has been pointed out by Croft Hill. The various enzymes present in living cells may indeed be responsible for the building-up processes taking place therein. Take such a case as the formation of starch from sugar in the green leaf. Even supposing that less than 99 per cent. of the glucose or maltose produced in photosynthesis is converted by the intracellular amylase into starch, the fact that starch is insoluble and is at once deposited out of the reacting system renders possible a considerable amount of conversion in a reasonably short time.

Before passing on to the subject of the next section it is advisable to refer to another kind of equilibrium, which is of a somewhat more complex nature than that already discussed, and which is met with in certain instances, especially where the products of the reaction have a specific retarding action on the enzyme. Such a case is that of amygdalin and emulsin, as investigated by Tammann. These cases differ from what may be called "true" chemical equilibrium in that the enzyme itself forms part of the system when in equilibrium. This is shown by the fact that addition of more enzyme to a solution which has arrived at a stationary condition causes further progress of the reaction. It is not correct to call these cases "false" equilibrium, since they show all the properties of a true equilibrium, such as alteration by adding more substrate, removing products of reaction, dilution or concentration or change of temperature, with also that characteristic already referred to, viz. additional change by adding more enzyme. It is in such instances that the question of compounds between enzyme and substrate or products of reaction comes more prominently under our notice; but this aspect of the phenomena will be discussed later.

#### VELOCITY OF REACTION

Practically, all enzyme actions with which we have to deal are unimolecular, or, in other words, their velocity may be represented by the rate of change of one molecule into two or more. The great majority of them, in fact, consist in the splitting up of a molecule by introducing into it the elements of water.

To simplify matters we will commence by neglecting the

reversible nature of these reactions and assume that they proceed to completion.

In such cases the rate at which the change proceeds will be, at any moment, proportional to the amount of unaltered substrate present at that moment. A simple application of the calculus shows us that the time course of the reaction will therefore form a logarithmic curve, when put into the graphic form.

What is the nature of the corresponding curve when enzyme actions are concerned? Several cases have already been investigated; but it can only be regarded as somewhat unfortunate that the first of such reactions to be completely analysed, chiefly by Victor Henri, turns out to be an anomalous one. I refer to the action of invertase on cane-sugar. When the values of the velocity constant in this instance are calculated by application of the unimolecular formula to the experimental data, it is found that the values so found steadily *increase*, instead of remaining the same all through. In nearly all other examples worked out up to the present time the corresponding values steadily *decrease*. Such are maltase and lactase (E. F. Armstrong), emulsin (Tammann), lipase (Kastle), and trypsin (by myself). Amylase is stated by some to follow the same law as invertase.

In the greater number of cases there are, then, some influences at work which either diminish the amount of enzyme acting or in some way make it less active. The chief cause seems to be the increasing concentration of the products of reaction, since by adding these, or certain of them, it is found that the reaction is slowed. There are several possible ways in which this effect may be produced. In the first place there is some evidence that there is a kind of combination taking place between the enzyme and certain of the products of its action; this would result in a part of the enzyme being withdrawn from participation in the reaction. In the second place, as insisted on by Moore, the reversible nature of these reactions has not been sufficiently taken into account; even where the reaction appears to proceed to completion it is probable that this means that the equilibrium point is very near one end, and the tendency for the reaction to run in the opposite direction will show itself more and more as this equilibrium point is approached. In the cases of invertase and emulsin investigated by Visser from this point of view, it was found that the

occurrence of the reverse reaction was capable of accounting for the greater part of the deviation from the unimolecular logarithmic law, so that much more regular values were found for the velocity constant when calculated from expressions including terms taking this reverse reaction into account. It did not seem, nevertheless, that the whole of the falling-off from the logarithmic curve could be explained in this way. Visser introduces the idea of "intensity" of action of the enzyme which is in some way diminished as the reaction proceeds. In some instances, in which the enzyme is one sensitive to changes of chemical reaction, it seems possible that the influence of the products of reaction may be partly accounted for in this manner. Trypsin is very sensitive to alkali, and the various amino-acids formed by its action may, by combining with alkali, diminish the intensity of the tryptic action. This is a point requiring investigation.

The consideration just mentioned leads us back to the peculiarity of the velocity of reaction of invertase. This is found neither to proceed in accordance with the strict logarithmic law nor to fall away from it, but, on the contrary, to become more rapid than the law requires. In other words, the activity of the enzyme appears to be *increased* as the reaction proceeds. Two suggestions have been made to account for the similar state of affairs when cane-sugar is inverted simply by the action of water at 100°. Kullgren has shown that an acid is produced, and as we know that the reaction is catalysed by  $H^+$  ions, this appears sufficient to account for the experimental results. Now it is possible that the hydrolysis produced by invertase may also be accompanied by the formation of acid, and, as the activity of this enzyme is increased considerably by  $H^+$  ions in moderate concentration, the explanation of Victor Henri's results may be found here. There is also another possibility suggested by Mellor and Bradshaw in connection with inversion of cane-sugar by water at 100°—namely, that the glucose and fructose as first formed may be in those modifications which have the higher rotatory powers, and that only at a later period, when equilibrium is attained, is the normal rotation of glucose arrived at. If this is the case with invertase action, it is obvious that if readings are taken of the change as it progresses in the tube of the polarimeter itself, these readings will indicate a higher concentration of inverted sugar than

really exists. Both of the possibilities mentioned are capable of experimental test.

On the whole, then, we may take it that when proper account is taken of the reversible nature of enzyme action and of the action of some of the products of the reaction on the activity of the enzyme itself (positive or negative auto-catalysis), the unimolecular logarithmic formula, deduced from the law of mass action, satisfactorily applies to the phenomena in question.

When we proceed to investigate the rate of change more in detail, we find that this simple law does not apply to the initial and final stages of the reaction—at all events, in the cases which have been investigated up to the present time. (I refer to those of lactase and maltase as investigated by Frankland Armstrong, and of trypsin as investigated by myself.) Although the greater part of the reaction follows the logarithmic law, with the qualifications above referred to, the initial stage and the final stage follow a linear course. In other words, it is found that equal amounts of the substrate are hydrolysed in equal times, notwithstanding the change in its concentration which is taking place. As Frankland Armstrong explains this result, it is due to the combination which takes place between the enzyme and the substrate as a necessary preliminary to the hydrolysis of the latter. As to the nature of this combination and the evidence for its existence, more will be said later; for the present we may assume that some kind of intimate association does take place. When, therefore, at the commencement of the reaction, the concentration of the substrate is considerably in excess of that of the enzyme, practically the whole of the latter is in combination with substrate; and since it will not be set free to attack a further quantity of substrate until the first is hydrolysed, it is obvious that as long as the substrate is in considerable excess equal amounts of it will be split in equal times. As the concentration of the substrate diminishes, the amount at any time in active association with enzyme will be proportional to this concentration, so that the rate of change will follow the law of mass action. In the final stage the amount of change is again equal in equal times, due now to the enzyme being in excess, so that it is able to attack the whole of the substrate available.

Moore has pointed out that, by taking into consideration the reversibility of the reaction and also the alteration of the intensity of the action of the enzyme, due to some effect of

certain products of the reaction, it is possible to frame a mathematical expression which not only includes the above three stages of the hydrolytic part of the reaction, but also a stage of zero change at the equilibrium point and a reversed velocity beyond this point. The expression is undoubtedly a complex one, although capable of simplification in particular cases. It is, moreover, satisfactory to find that such an equation can be formed on the basis of experimental data.

#### *Concentration of Enzyme.*

When we come to consider the various influences that are capable of changing the rate of a reaction as catalysed by enzymes, the first point that suggests itself is, how is this velocity related to the amount of enzyme present? We have already seen that in the case of inorganic catalysts it has been shown that the final equilibrium point is unaffected by the amount of catalyst present. The time taken to reach this point is alone affected. With regard to enzymes, it has been shown by Croft Hill that the same law applies to the case of maltase, and by Visser that it applies to invertase and emulsin.

Various statements have been made from time to time with respect to the relation of the concentration of enzyme to the rate of the change produced by it. Indeed, when we consider the different states of affairs in the three stages of the reaction, we understand the cause of the disagreement amongst observers as to this question. The relation cannot, in fact, be the same at all stages of the reaction. In the first stage, so long as the enzyme is in small proportion to the substrate, the rate of change is in direct proportion to the amount of enzyme present; in the second (logarithmic) stage the ratio is some exponential function of the enzyme concentration, so that when this is doubled, for example, the rate of change is not also doubled, but is usually multiplied by about  $2^{1\frac{1}{2}}$  to  $2^{\frac{3}{2}}$ ; whereas in the last stage, in which the enzyme is in excess, it is clear that the rate of change will be independent of the amount of enzyme present. These facts come out quite unmistakably in the cases in which they have been looked for, such as lactase (E. F. Armstrong, trypsin (the present author), and others. The bearing of the exponential function of the second stage on the nature of the combination between enzyme and substrate will be seen later.

*Effect of Temperature*

Increase of temperature is well known to have a considerable effect on the rate of ordinary chemical reactions, the usual factor being between 2 and 4 for  $10^{\circ}$ : for example, the velocity at  $40^{\circ}$  is from 2 to 4 times that at  $30^{\circ}$ . In the case of enzymes the corresponding factor has been determined for emulsin by Tammann to be 7.14 between  $60$  and  $70^{\circ}$ , for trypsin by myself to be 5.3 between  $20$  and  $30^{\circ}$ , and for the catalase of blood by Senter to be 1.5 between  $0$  and  $10^{\circ}$  C. But whereas the curve expressing this fact becomes steeper as the temperature rises in the case of ordinary chemical reactions, it is found that enzymes show a phenomenon known as the "optimum" temperature—viz. a particular temperature at which during a given time a greater amount of substrate is acted upon than at any temperature either above or below this. To understand the meaning of the "optimum" it is sufficient to bear in mind two facts—viz. that enzymes are rapidly destroyed at high temperatures, probably because of their colloidal character; and that, in order to determine the degree of their activity, it is necessary to allow the action to proceed for a considerable time at the raised temperature. This being so, we are unable to observe directly the rate of change at the beginning of the action at any new temperature, and at the higher temperatures, before sufficient change has occurred to enable measurements to be made, a considerable part of the enzyme has been destroyed. This is shown very distinctly by the observations of Frost Blackman on the respiration of plants. If curves are drawn expressing the evolution of carbon dioxide at various temperatures and at various times after the commencement of exposure to these temperatures, and these curves are continued so as to show the rate at the first moment of action of the particular temperature, it is found that the points so fixed lie on a curve exactly like that of Van 't Hoff for ordinary chemical reactions, so that the higher the temperature the greater the initial rate of change, and the optimum temperature is merely an expression of the fact that at a certain temperature the increased velocity due to this raised temperature is more than sufficient to counteract the rapid destruction of the enzyme. It follows also from these experiments that the apparent optimum temperature will vary considerably, according to the time which has elapsed between

the commencement of the exposure to the temperature and the period at which the observation is made.

The fact that during the brief life of an enzyme at a high temperature its activity is so enormously increased makes caution necessary in experimental work. It is sometimes the practice, in order to stop the action of an enzyme at a given moment, to raise the temperature of the reacting mixture as rapidly as possible to  $100^{\circ}$  or thereabouts. This cannot be done instantaneously, and during the necessary interval of time the enzyme becomes exceedingly active, so that considerable further change may take place. That such change, in point of fact, does occur, was found by Delezenne in the case of papain, and by myself in the case of trypsin. This method of stopping further action is, therefore, inadmissible in accurate investigations. It is better to freeze solid as rapidly as possible, or, when such admixture is immaterial to subsequent work, to add some substance which stops the action of the enzyme, such as alkali to solutions containing pepsin or invertase.

From the point of view of the theory of enzyme actions, the large temperature coefficient has some importance. As pointed out by Senter, it shows that the interpretation of the form of the velocity curve as due to diffusion in a heterogeneous system, on the lines of the theory of Nernst, does not agree with the experimental facts. The temperature coefficient of diffusion processes is low, whereas that of enzyme actions is unusually high.

#### *Accelerators and Retarders*

Of Ehrlich's two classes of bodies acting on the living cell enzymes belong to that one which consists of bodies of high molecular weight, like the foodstuffs and bacterial toxins, which are directly assimilated by the protoplasm and built up into its giant molecule. The other class—to which drugs belong, as well as the various chemical messengers, or hormones, produced by the organism—are of simpler chemical constitution, and act by the physico-chemical characters of their molecule as a whole. The first class of bodies alone gives rise, when injected into the living organism, to the production of antagonistic bodies—antitoxins or anti-enzymes, etc. Of these latter several are known to be normally present in the blood, such as anti-trypsin and anti-rennet. Others have been produced by hypodermic injec-

tion of the respective enzymes. Anti-trypsin has also been shown by Weinland to exist in the bodies of intestinal worms, which are thus protected from the action of the pancreatic juice. It is stated by this observer that the intestinal epithelium also contains a similar substance; but recent work by Hamill has failed to confirm this statement, although the mucous membrane of the stomach was found to contain an anti-pepsin, as affirmed by Weinland. These anti-bodies appear not to destroy the enzyme, since a mixture of trypsin and anti-trypsin, at first inactive, slowly recovers its proteoclastic power, apparently by gradual disappearance of the anti-body. The mode of combination of toxin and anti-toxin has been shown by Craw to be of the nature of adsorption, the characteristic of which process is that it partakes of some of the properties of physical union and some of those of chemical combination—it is, in fact, as Ostwald puts it, a combination in varying proportion. The dyeing of cotton by Congo-red is such a process. If two pieces of cotton of the same size are dyed in solutions of Congo-red, one of which is twice the strength of the other, it is found that the cotton does not take up equal quantities of dye from both, as it would if a true chemical compound were formed, nor does it take up twice the amount from the stronger solution, as it would if the process were purely physical, such as solid solution; it is found, in fact, that relatively more is taken up from the more dilute solution; so that the amount taken up from that of twice the strength is not double that taken up from the weaker, but less than this—indeed,  $\times 2^{\frac{1}{x}}$ , where  $x$  is greater than 1 and usually less than 2.

It must suffice here to refer to the action of various electrolytes, especially that of  $\text{OH}^-$  and  $\text{H}^+$  ions, to which various enzymes are differently sensitive. Pepsin is paralysed by  $\text{OH}^-$  ions, while being increased in activity by  $\text{H}^+$  ions. It appears probable, from the researches of the present writer, that the action of neutral salts may chiefly consist in facilitating or otherwise the mutual adsorption of the enzyme and substrate.

The enzymes connected with oxidation processes are extremely sensitive to traces of certain metallic salts. Laccase has been shown by Bertrand to be considerably increased in activity by the presence of manganese. A similar relation of tyrosinase to iron has been pointed out by Miss Durham. It is not certain whether these enzymes are unable to exert this activity in the absence of the metals referred to; if this is the

case, manganese and iron would be for them "co-enzymes," to which we may now turn our attention.

#### CO-ENZYMES

It was noticed by Magnus that an extract of liver containing lipase became inactive on dialysis. On investigation it was found that the activity was restored by adding a portion of a boiled extract of liver, or even a similar extract after proteins had been precipitated by uranyl-acetate. The activating body is soluble in absolute alcohol but not in ether, and is not present in the ash of liver. It is, therefore, a non-colloidal substance, but not inorganic. What may be called, then, the lipoclastic system of the liver consists of two components—the one, destroyed by boiling, may be regarded as the enzyme proper, but it is inactive except in the presence of the dialysable body, the "co-enzyme."<sup>1</sup>

A similar state of affairs has been shown by Harden and Young to exist in the case of the alcoholic enzyme of yeast, zymase. The press juice of yeast may be separated by dialysis, or by filtration through gelatin, into two parts, each by itself inactive, but becoming active when mixed. The filtrate or dialysate always contains phosphates, and it was found that the addition of phosphate to the inactive residue had a similar activating effect to that of the filtrate itself. A remarkable fact is that the amount of carbon dioxide (and alcohol) produced is proportional to the amount of phosphate added—viz. one molecule of carbon dioxide for each atom of phosphorus. The authors are not prepared as yet to state whether phosphate is the only co-enzyme concerned.

Certain experiments of Cohnheim indicate that the pancreas produces a co-enzyme for the glycolytic oxidase of muscle. The difficulty in the investigation of this phenomenon is that the pancreatic extract in 90 per cent. alcohol, which was added to watery extracts of muscle, caused an inhibition of sugar oxidation, if present even in slight excess. This may, perhaps, be the reason why other observers have not been able to confirm Cohnheim's results. The matter cannot be looked upon as decided at present, but it appears from Cohnheim's

<sup>1</sup> According to recent work (Loevenhart, "Proc. Am. Physiolog. Soc.," in *Amer. Journ. of Physiology*, vol. xv. p. xxvii. 1906), the co-enzyme in this case is bile salts.

data that there is something in his pancreatic extracts which considerably increases the destruction of sugar in muscle extracts, when added to these in a certain proportion.

### ZYMOGENS

Since all enzymes are products of cell activity, it follows that, in all probability, some intermediate stage of their formation from cell protoplasm may be separated from the cells producing them. Such a body is the mother-substance of pepsin, prepared from the gastric mucous membrane by Langley and Edkins. This "pepsinogen" is itself inactive, but is transformed into active pepsin by the action of acid. The similar body formed by the pancreatic cells (trypsinogen) is secreted as such, and remains inactive until it comes into contact with a specific enzyme (enterokinase), found by Pawlow and his pupils in the small intestine. This enterokinase is secreted by the epithelium of the small intestine, and converts the trypsinogen into active trypsin.

These zymogens must be carefully distinguished from the enzymes above mentioned as being inactive apart from their respective co-enzymes. In this latter case the process of activation is a reversible one, in the sense that an active system can be rendered inactive by removing the co-enzyme and restored to activity by replacing the latter. An active enzyme, however, once produced from its zymogen, cannot be reconverted into its precursor; the process appears to be one of hydrolysis, by which a new body is formed. The system of inactive enzyme and co-enzyme is rather an association of two different bodies, probably of the nature of adsorption.

### CHEMICAL AND PHYSICAL PROPERTIES

Enzymes, as mentioned already, are colloids, and therefore exhibit the characteristic properties of this state of matter. Such are indiffusibility, precipitation by heat, greater or less instability, and probably an electric charge of their suspended particles.

It is impossible to make any definite statement as to their chemical nature, except of a negative character. The really active substance is present in the usual preparations in so small an amount that it appears almost hopeless to discover its chemical constitution. As colloids they readily carry along with

them, in a state of adsorption, constituents of the solutions from which they are precipitated. It is not surprising, then, to find that proteoclastic enzymes show certain reactions of proteins, invertase apparently contains carbohydrate, and so on. But when the solutions are more and more carefully purified it is found that, although powerfully active preparations can be obtained, these solutions show fewer and fewer characteristic reactions. Pekelharig's pepsin, for instance, showed only a minority of the usual protein tests, and was found to contain no phosphorus. This last fact definitely excludes the view taken by some that enzymes in general are nucleo-proteids.

At the same time it seems, from evidence to be mentioned immediately, that there is some very close similarity between a particular enzyme and its substrate, so that, when it is found that invertase cannot be prepared free from mannose, it may well be that this sugar is a necessary part of its molecule.

The facts referred to in the earlier pages of this article as to the relations between certain enzymes and optically isomeric substrates led Emil Fischer to put forward his famous simile of lock and key relationship.

In connection with this possible similarity of chemical structure it is of interest that the statement has recently been made that lipase is soluble in ether, and therefore probably of a fatty nature. The lipase of the cytoplasm of castor-oil seeds has also been shown by Nicloux to be destroyed by contact with water.

#### MODE OF ACTION

It is evident from what has already been said that there is a very intimate association between an enzyme and its appropriate substrate. A few further facts pointing to some kind of combination may be mentioned here. Enzymes in presence of substrate or products of reaction are considerably less sensitive to the action of heat. It appears also that a particular enzyme has a special affinity for certain constituents of the substrate, as, for instance, invertase for fructose (E. F. Armstrong), trypsin (according to Emil Fischer) for tyrosin, leucin and, to a less degree, alanin. Now these bodies are precisely those which exercise the greatest inhibitory power on the velocity of the (hydrolytic) reaction ; in fact, glucose does not

affect the action of invertase, whereas fructose has a considerable action of this kind. If an enzyme accelerates the reverse reaction, as well as the usual hydrolysis or splitting-up, it is easy to understand why it is capable of combining with products of reaction in addition to combining with the unaltered substrate; if we suppose that in order to exert its catalytic influence it must enter into some such intimate connection with the bodies to be acted upon, such "compounds" must be formed between enzyme and products before synthesis of the latter will occur.

Enzymes, being colloids, are particularly prone to form adsorption compounds, so that we naturally look for evidence as to the nature of the compounds in question. The fact that, at all events in the logarithmic stage of the reaction, increasing the concentration of the enzyme to say twice its value does not double the reaction velocity, but something less than this, is considerable evidence in this direction. A relationship of this nature is characteristic of adsorption phenomena. It also tends to confirm the view of Victor Henri that the enzyme is shared by the substrate and the water present in such a manner that the proportion taken up by the former is greater the lower the concentration of the enzyme. If we put:  $a$  = the quantity of enzyme adsorbed from a solution of concentration = 1, and  $b$  = that adsorbed from a similar solution of concentration = 2, then  $a = b \times 2^{\frac{1}{x}}$ , in which  $x$  is what is called the "proportionality factor," and is always greater than unity, which value it would have if the process were a purely physical one, such as solid solution, for example. The higher the value of  $x$  the more nearly the process approximates to a chemical combination, in which case it is obvious that  $a = b$ , and therefore  $2^{\frac{1}{x}}$  in the above expression becomes unity or  $x = \text{infinity}$ . In the case of trypsin  $x$  varies from 1.5 to 1.67, and according to Schütz and Borissow's "law of squares"—which is, however, merely a special case— $x = 2$ . I find in some experiments made recently that in the case of Congo-red and paper  $x$  is usually less than 2, about 1.6 in fact, but it varies considerably according to the amount of electrolyte present. It would appear, then, that the union of enzyme and substrate follows a similar law to that of ordinary adsorption phenomena.

A particularly interesting case as regards the question before us is that of the lipase of the liver as investigated by Dakin.

It was found that this enzyme, acting on the optically inactive mandelic esters, hydrolysed more rapidly the dextro component than the lævo one. In this way the percentage of dextro-mandelic acid in the products of the reaction was greater at first than that of the lævo-mandelic acid; as the reaction proceeded the relative amounts became closer, until finally both were present in equal quantity and the acid was optically inactive. These facts can only be satisfactorily explained on the hypothesis that the enzyme is itself optically active and forms addition compounds with the ester. In the words of Dakin, "The dextro and lævo components of the inactive ester first combine with the enzyme, but the latter is assumed to be an optically active asymmetric substance, so that the rates of combination of the enzyme with the d. and l. esters are different. The second stage in the reaction consists in the hydrolysis of the complex molecules of (enzyme + ester). Since the complex molecule (enzyme + d. ester) would not be the optical opposite of (enzyme + l. ester), the rate of change in the two cases would again be different. Judging by analogy with other reactions one might anticipate that the complex molecule which is formed with the greater velocity would be more rapidly decomposed. In the present case it would appear that the dextro component of the inactive mandelic ester combines more readily than the lævo component with the enzyme, and that the complex molecules (d. ester + enzyme) are hydrolysed more rapidly than (l. ester + enzyme), so that if the hydrolysis be incomplete dextro acid is found in solution and the residual ester is lævo-rotatory."

We have as yet no definite information as to what happens after the combination between enzyme and substrate has taken place. In the case of inorganic catalysts the most acceptable explanation is that the intermediate compound is split up again with the formation of the particular products of the reaction and the liberation of the catalyst in its original form. The intermediate formation of ethyl-sulphuric acid in the production of ether and that of nitrosyl-sulphuric acid in the old chamber process of manufacture of sulphuric acid may be given as illustrations. Practical experience has found that in the latter process the nitric acid gradually disappears in the form of bye-products, so that the catalyst is not completely restored. This fact is of importance in connection with the view taken by some investigators that certain bodies, such as Buchner's zymase, are not

enzymes, since there is a proportionality between the amount of enzyme and the total amount of change produced. This fact is probably to be explained by the disappearance of the enzyme during the reaction. A similar state of affairs is, indeed, to be seen in the case of trypsin, though in a less marked degree. It is found that small quantities of this enzyme will not effect the same amount of hydrolysis as larger quantities, even when allowed a very long time for the action. Experiment shows that trypsin does disappear slowly even in the presence of substrate or products.

Ostwald has insisted that, in order that the formation of an intermediate compound should be regarded as an adequate explanation, it is necessary to show that the two reactions, formation of intermediate compound and splitting-up of this compound, taken together progress at a velocity greater than that of the reaction without the presence of the catalyst. This proof has actually been afforded by Brode. When hydrogen peroxide acts upon hydriodic acid, this latter is decomposed at a certain rate, which rate is greatly accelerated by the presence of a trace of molybdc acid. Now Brode has been able to show that permolybdc acids are formed by action of the peroxide on molybdc acid and that these permolybdc acids, themselves produced at a considerable velocity, react on the hydriodic acid with very great velocity, molybdc acid being formed again, ready for a further activity. These two reactions, then, together progress at a greater rate than the action of the peroxide by itself upon the hydriodic acid.

When we consider the extraordinarily minute concentration in which enzymes exert their activity, there seems some justification for such a view as that of Arthus and de Jager—viz. that the properties known as those of enzymes are not associated with definite chemical individuals, but may be conferred, to a greater or less degree, on various kinds of bodies. In the process of purification of certain enzymes solutions have been obtained which, while very active, contain only an infinitesimal amount of solid matter. On the other hand Brode has shown that, in the reaction above described, the presence of 1 gram molecule of molybdc acid dissolved in 31,000,000 litres of water is capable of obvious catalytic action. In this case the catalyst is a definite chemical compound.

Space will not allow of the discussion of other theories of

enzyme action, such as the molecular vibration theory of Liebig, or that of Barendrecht as to the "radiations" emitted by enzymes. I must content myself with calling to mind what is so well insisted on by Bredig—viz. that enzyme solutions are, as colloids, heterogeneous systems, and that it is necessary always to remember that surface-action and adsorption undoubtedly play a considerable part in the reactions taking place therein.

It may perhaps be objected that the view advocated in the preceding pages, that the combination between enzyme and substrate or products is to be looked upon as of the nature of adsorption, does not sufficiently take account of the specific nature of enzyme action. It may be said that caseinogen, for example, would be expected to form adsorption compounds indifferently with either trypsin or amylase. Adsorption, however, is not a purely physical process, and is undoubtedly more or less specific. The "adsorption affinity" of gelatin is considerably greater for acid-fuchsin than for Congo-red. But we know as yet too little about the essential nature of the process to warrant further discussion.

One more experimental result may be mentioned to conclude this section. It was found by Korschun, in investigating the relations between rennet and its anti-body, that by filtration through porous clay a solution of rennet could be separated into fractions which by appropriate dilution of the stronger fractions could be brought to the same strength as regards combination with the anti-body, but which differed considerably in their power of coagulating milk. In other words, the original solution appeared to contain a modified form of the enzyme analogous to Ehrlich's "toxoids"; that is, a part of the enzyme had lost its characteristic activity while still retaining its power of combining with the anti-body. I have myself met with some facts which point to the production of a similar modification of trypsin by warming to about 25° for a day or two; I have suggested calling these modified enzymes "zymoids." The facts afford support to Ehrlich's view that the combining power and the fermentative activity are functions of distinct side-chains.

#### COMPLEX SYSTEMS

It may be of interest if, in conclusion, I give a brief indication of some recent work on systems in which enzymes play an

essential part and which simulate as a whole the properties of enzymes.

It has long been known that certain preparations can be obtained from tissues of both plants and animals which have the power of carrying active oxygen to certain oxidisable substances, such as guaiaconic acid, salicylic aldehyde, tyrosin, etc., and thereby causing the oxidation of these substances. These various phenomena were very obscure until the researches of Bach and Chodat threw light upon them. These observers show that there are three distinct classes of bodies involved. Firstly, organic peroxides of the type of substituted hydrogen peroxide; these are not enzymes, although they are decomposed by boiling. Secondly, peroxydases, enzymes which act upon the peroxides and produce active oxygen, which is capable of oxidising certain bodies when these are present. The system, peroxide and peroxydase, forms the oxidising agent, or oxydase as it was originally called. In addition to these two classes there is a third, also of enzyme nature, viz. the catalases, which decompose hydrogen peroxide with evolution of inactive or molecular oxygen and appear to act only on this particular peroxide. These are widespread in both plants and animals, but their function is not altogether clear, with the exception of one important case to be mentioned immediately. The "hæmase" of blood investigated by Senter is one of these catalases. Although the point of view taken by Bach and Chodat undoubtedly tends to clear up much obscurity, it does not explain all the facts known. The markedly specific nature of certain "oxydases," such as tyrosinase and aldehydase, is difficult to understand, and the mechanism by which the peroxide takes up atmospheric oxygen after being decomposed by the peroxydase, and thus reconstitutes itself, is as yet unknown.

Another complex system is that responsible for the activity of the green leaf in the forming of starch and oxygen from carbon dioxide and water. Important advances have recently been made in this region by Ussher and Priestley. They show that the system concerned consists of three partners—the protoplasm of the chlorophyll corpuscle, the chlorophyll itself, and a catalase. By means of the pigment, acting as both chemical and optical sensitiser, light energy is employed to cause reaction between carbon dioxide and water in such a manner that formaldehyde

and hydrogen peroxide are formed. Now both these bodies are poisonous and, if allowed to accumulate, the reaction would soon come to an end. The formaldehyde is, however, rapidly polymerised by the protoplasm of the chloroplast, and the hydrogen peroxide is split up into oxygen and water by the catalase. We see then why the reaction as a whole does not occur in non-living preparations or extracts of green leaves. Formaldehyde is, indeed, produced in the presence of chloroform on exposure to light, but since no polymerisation occurs the chlorophyll is destroyed by it, and no further reaction is possible. The production of hydrogen peroxide and formaldehyde even takes place in light in leaves killed by boiling, and in this case, since the enzyme (catalase) is destroyed, as well as the protoplasm, the hydrogen peroxide also contributes to the destruction of the chlorophyll.

#### CONCLUSION

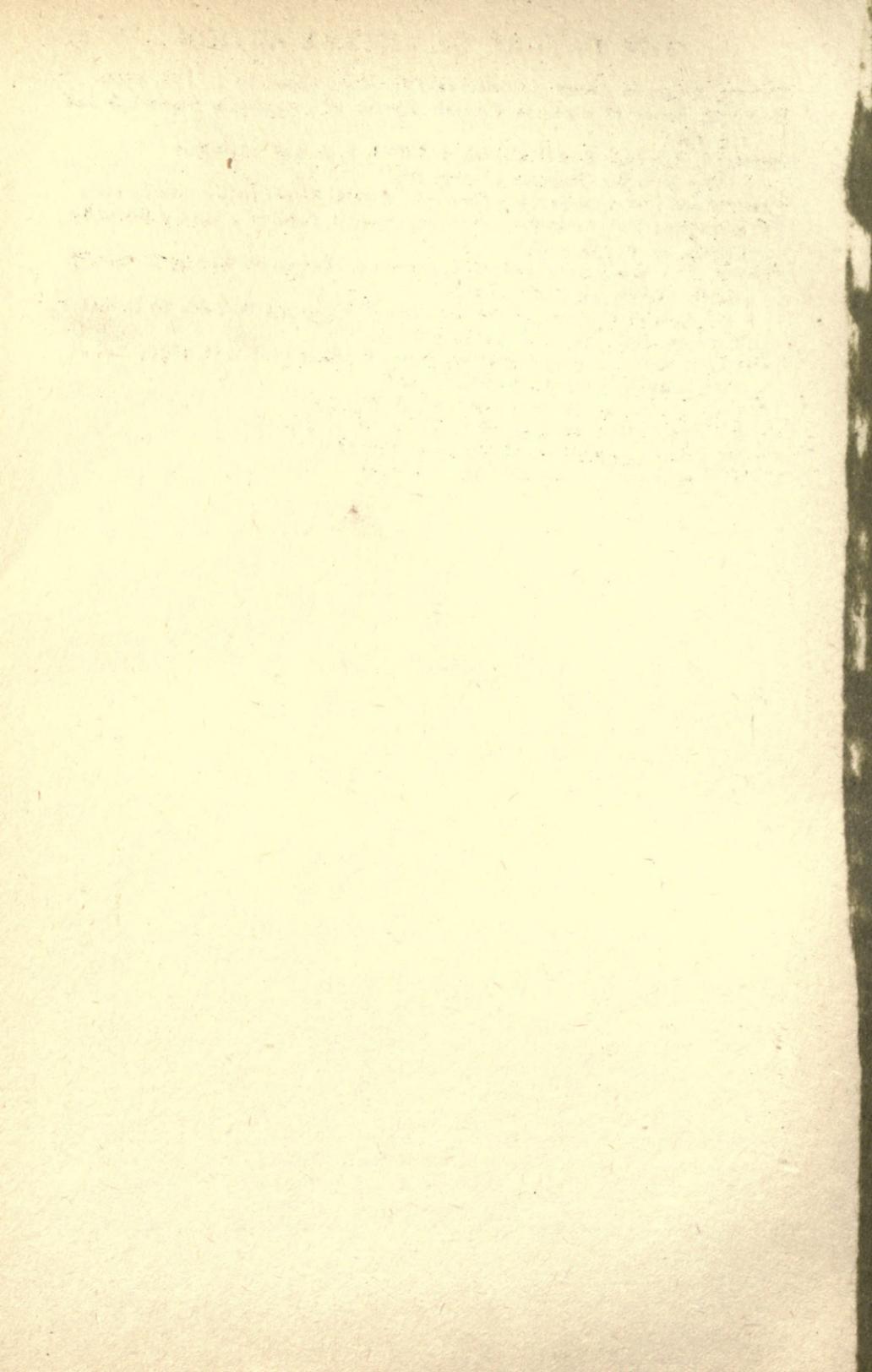
The living organism is enabled by the use of enzymes to bring about, under ordinary conditions of temperature and moderate concentrations of acid or alkali, many chemical reactions which would otherwise necessitate high temperature or powerful reagents. These enzymes are catalysts of a colloidal nature, and obey the usual laws of catalytic phenomena. Certain properties in which they differ from most inorganic catalysts are to be explained by this colloidal condition. One such property is destruction by heat and comparative instability as the temperature rises, thus affording an explanation of the so-called optimum temperature. The facility with which additive or adsorption compounds are formed with substrate or products is also due to the colloidal character of these enzymes.

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ON THE IDENTITY OF TRYPSINOGEN AND ENTEROKINASE RESPECTIVELY, IN VERTEBRATES. BY  
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BAYLISS and Starling<sup>(1)</sup> have shown that secretin is not specific for each type of animal but is a definite chemical substance common to all classes of vertebrates. It became of interest, therefore, to ascertain if any similar relationship existed between the pancreatic juice and the enterokinase of different animals, that is to say, whether trypsinogen and enterokinase are specific for each animal or are the same substances throughout the vertebrata. To this end the following procedure was adopted.

*Methods.* Enterokinase was prepared from such widely different animals as the dog, cat, rabbit, rat, pigeon, frog, tortoise and fish (dace). The mucous membrane of the upper portion of the small intestine of these animals was lightly scraped, the scrapings ground up with sand and extracted with chloroform water for 36 hours. This extract was filtered, first through paper and then through a Berkfeld filter. A slightly opalescent solution free from all solid particles was thus obtained.

Pancreatic juice, prepared by the injection of secretin into the jugular vein, a cannula having been previously inserted into the pancreatic duct, was obtained from a dog, a cat and a rabbit, and was collected in sterilised flasks. To these juices equal volumes of 2% sodium fluoride solution was added. (In the experiments described below pancreatic juice, or, shortly, P.J., means a mixture of equal volumes of sodium fluoride solution and pure pancreatic juice.)

A modification of Mett's tubes containing, instead of coagulated egg-albumin, 10% gelatine dissolved in 1% sodium fluoride solution and coloured with methyl violet was used to indicate the development of proteolytic activity in the solutions.

The following mixtures were then made in small flasks:—

		mm. of gelatine digested	
		(1)	(2)
A.	2 c.c. Dog's P.J. + 1 c.c. Dog's Ek.	= 14	4·5
	„ + 1 c.c. Cat's Ek.	= 14	4·5
	„ + 1 c.c. Rat's Ek.	= 14	4·5
	„ + 1 c.c. Rabbit's Ek.	= 13·5	4·5
	„ + 1 c.c. Frog's Ek.	= 10	4·5
	„ + 1 c.c. Pigeon's Ek.	= 14	4·5
	„ + 1 c.c. Tortoise's Ek.	= 13	5·0
	„ + 1 c.c. Dace's Ek.	= 14	4·5
„ + 0 c.c. Ek.	= 0	0	
B.	2 c.c. Rabbit's P.J. + 1 c.c. Dog's Ek.	= 13	6
	„ + 1 c.c. Cat's Ek.	= 13	6
	„ + 1 c.c. Rat's Ek.	= 11	5·5
	„ + 1 c.c. Rabbit's Ek.	= 12	6
	„ + 1 c.c. Frog's Ek.	= 7	5·5
	„ + 0 c.c. Ek.	= 0	0
C.	2 c.c. Cat's P.J. + 1 c.c. Dog's Ek.	= 13	
	„ + 1 c.c. Cat's Ek.	= 12	
	„ + 1 c.c. Rat's Ek.	= 13	
	„ + 1 c.c. Rabbit's Ek.	= 13	
	„ + 1 c.c. Frog's Ek.	= 13	
	„ + 1 c.c. Pigeon's Ek.	= 12·5	
	„ + 1 c.c. Tortoise's Ek.	= 13	
	„ + 1 c.c. Dace's Ek.	= 13	
„ + 0 c.c. Ek.	= 0		

Gelatine tubes and a drop of toluol were added to each flask and the whole left for 16 hours. At the end of this time the amount of gelatine digested is shown in the first column opposite each mixture. New tubes were then added to series *A* and *B* and were measured after the lapse of 6 hours. The results are given in column 2.

Delezenne<sup>(2)</sup> has already shown that pancreatic extracts obtained from various animals can be activated by dog's enterokinase. From the above experiments we see that not only is this the case, but that enterokinase from a variety of animals is capable of activating pancreatic juice obtained from different sources. It will be noticed that in *A* and *B* the amount of digestion at the end of 16 hours in the tubes activated by frog's enterokinase is less than that in the other tubes. That this is due merely to the small quantity of enterokinase in the solution and not to any real difference in the enterokinase itself is proved by the fact that on the addition of new tubes digestion proceeds equally in all the

tubes; further, new and stronger frog's enterokinase used in experiment C activates cat's pancreatic juice just as readily as does the enterokinase of the other animals.

We are justified, therefore, in concluding from the above experiments that both enterokinase and trypsinogen are not specific for each animal but are always the same definite chemical individuals although occurring in widely different classes of animals.

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ON THE MECHANISM OF PROTECTION OF INTESTINAL WORMS, AND ITS BEARING ON THE RELATION OF ENTEROKINASE TO TRYPSIN. BY J. MOLYNEUX HAMILL, M.A., M.B. (*Cantab.*), B.Sc. (*Lond.*), *Sharpey Scholar*.

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THE discovery of antiferments naturally suggested that the resistance of intestinal parasites to digestion might be due to the presence of an antiferment in their tissues. This view has been advocated by Weinland<sup>(2)</sup>, who considers that the resistance of intestinal parasites to digestion is due to the presence of antiferments, especially anti-trypsin, in their tissues. On the other hand, Dastre and Stassano<sup>(3)</sup>, who have investigated the nature of the antibody in *Ascaris*, have come to the conclusion that it is not anti-tryptic but anti-kinasic in action. In dealing with these different results, it is necessary to take into account the different views which are held as to the interaction of enterokinase and trypsinogen.

According to Pawlow, enterokinase acts as an enzyme, converting trypsinogen into trypsin in the same way that trypsin converts proteids into simpler products. Delezenne<sup>(1)</sup>, however, assigns quite a different rôle to enterokinase; according to him the conversion of trypsinogen into trypsin is brought about by a combination of the enterokinase with the trypsinogen, the resulting complex being trypsin. He endeavours to bring the activation of trypsinogen by enterokinase into line with the interaction between complement and immune-body. He regards enterokinase as a complement, and trypsinogen as an immune-body, either of these substances alone is unable to attack proteid, but when all three, enterokinase, trypsinogen, and proteid are brought together, the trypsinogen, acting as an amboceptor, allows the enterokinase to attack and break up the proteid molecule.

The presence, in *Ascaris*, of an antibody which is antikinasic in character would be a considerable argument in favour of Delezenne's view. It becomes of importance, therefore, to obtain definite evidence as to the precise nature of the antibody, whether it is antikinasic or merely antitryptic in action.

I propose, then, in this paper, first of all to inquire into the nature and mode of action of the antibody in the juices of intestinal worms, and then to pass on to a consideration of some of its properties.

*Methods.* Preparation of worm extract. Worms (*Ascaris*), obtained from the intestinal tracts of horses, were washed and then ground up as thoroughly as possible with sand. The pasty mass thus obtained was covered with chloroform water, well shaken, and allowed to stand for 36 hours. The liquid portion was then strained off through muslin, filtered through paper, and finally passed through a Berkfeld filter. In this way a clear yellowish aqueous extract of worms was obtained quite free from any cells. A little toluol was added to guard against putrefaction. The extract so prepared will retain its activity with but little impairment for weeks, or even months.

The trypsin solutions were made by treating 3 grams of pancreatin ("Rhenania") with 100 c.c. of 2% sodium carbonate solution and filtering. The solutions were always prepared as required and used at once.

In order to measure the proteolytic activities of my solutions I used a modification of Mett's tubes employed by Fermi in which the albumin is replaced by 10% gelatine containing 1% sodium fluoride as a preservative. The addition of a little methyl violet to the gelatine facilitates the measurement of the amount digested at the end of the experiment. This method, although not perfect, gives results quite sufficiently accurate for an investigation of this character, and is much superior to Dastre and Stassano's cubes of albumin and Weinland's flocks of fibrin. Its use, however, is restricted to temperatures in the neighbourhood of 15° C.

Enterokinase solutions and pancreatic juice were obtained from dogs in the way already described in my previous paper<sup>(7)</sup>.

## I. THE MODE OF ACTION OF THE ANTIBODY.

*Is the antibody antitryptic?* In order to determine this the following mixtures were made:—

2 c.c. Trypsin + 0 c.c. W. extract = 13	2 c.c. Trypsin + .6 c.c. W extract = 6
„ „ + .1 c.c. „ = 12	„ „ + .7 „ „ = 4.5
„ „ + .2 „ „ = 11	„ „ + .8 „ „ = 3
„ „ + .3 „ „ = 10	„ „ + .9 „ „ = 1
„ „ + .4 „ „ = 9	„ „ + 1.0 „ „ = 0
„ „ + .5 „ „ = 8	

Each mixture was made up to 3 c.c. with water. Gelatine tubes and a drop of toluol were added to each. The digestion in mm. after 16 hours is given opposite each mixture.

That the trypsin used contained no enterokinase free to such a degree as to be able to activate pancreatic juice was proved by adding 1 c.c. trypsin to 2 c.c. pancreatic juice; and 1 c.c. to 2 c.c. water. Gelatine tubes were digested with equal rapidity in both mixtures, thus proving that the pancreatic juice had not been activated. Similar experiments with other commercial trypsins have yielded exactly similar results; no trace of free enterokinase could be detected in any of them. Nevertheless, Dastre and Stassano<sup>(3)</sup>, adhering to the conception of the dual nature of trypsin, believe that enterokinase exists in trypsin, although modified to some extent in combination. They assert that the slowing and ultimate cessation of proteolysis which occurs as successive portions of proteid are added to an activated pancreatic juice (trypsin) are due to the using up of one or other of the components of the trypsin, and that such exhausted trypsin can be restored to activity by the addition of enterokinase or inactive pancreatic juice.

To test this the following experiments were performed:—

5 grs. of gelatine were added to 20 c.c. trypsin; and 20 c.c. of the same trypsin were kept as a control. Toluol was added to each. After the lapse of a week the following mixtures were made and gelatine tubes added.

Dig. in. mm.	Dig. in mm.
2 c.c. Ex. T. + 1 c.c. Ek = 1	2 c.c. Ex. T. + 1 c.c. PJ = 1
2 c.c. Ex. T. + 1 c.c. B.Ek = 1	2 c.c. Ex. T. + 1 c.c. B.PJ = 1
2 c.c. C.T. + 1 c.c. Ek = 4.5	2 c.c. C.T. + 1 c.c. PJ = 4.5
2 c.c. C.T. + 1 c.c. B.Ek = 4.5	2 c.c. C.T. + 1 c.c. B.PJ = 5.0

Control. 2PJ + 1 drop Ex. T. = 0.

The tubes were measured at the end of 16 hours.

Ex. T. = "exhausted" trypsin; C.T. = control trypsin; B.Ek = boiled enterokinase; B.PJ = boiled pancreatic juice.

We see that it is a matter of complete indifference whether we add enterokinase and pancreatic juice, or boiled enterokinase and boiled pancreatic juice to the exhausted trypsin. Obviously, therefore, enterokinase and pancreatic juice are incapable of restoring proteolytic activity to an "exhausted" trypsin solution. Bayliss<sup>(5)</sup> has shown that dilution

of a tryptic digestion mixture which has reached a standstill will enable further proteolysis to occur; any increase of proteolytic activity observed by Dastre and Stassano was probably due to mere dilution of the mixture by enterokinase or pancreatic juice. Furthermore, Bayliss and Starling<sup>(6)</sup> have brought forward a mass of evidence in support of the view that trypsin is not equivalent to a combination of enterokinase and trypsinogen, as Dastre and Stassano believe, but is an entirely new substance differing in every way from both enterokinase and trypsinogen. They have shown also that enterokinase plays no part in the proteolytic activities of trypsin.

It is obvious, therefore, that the inhibiting effect of worm extract on digestion in an experiment such as that described on page 481 where no enterokinase in any form whatever is present, can be due only to a substance depressing the proteolytic activities of trypsin; that is to say, the inhibitory effect of the antibody is antitryptic in nature.

*Is the antibody also antikinasic?* It is possible that the antibody in the worm extract, in addition to its antitryptic power, may also possess the power of inhibiting the activities of enterokinase; that is to say, may be antikinasic. Thus, when enterokinase, pancreatic juice, and worm extract are mixed together, the prevention of the development of proteolytic power in the solution might conceivably be due to the non-conversion of trypsinogen into trypsin owing to the inhibitory effect of the worm extract on the normal activities of the enterokinase.

In attempting to settle this point we are met at the outset by the difficulty of determining whether the arrest of digestion is due to inhibition of the activity of trypsin already formed, or to the inhibition of the activity of the enterokinase, thus preventing the transformation of trypsinogen into trypsin.

In addition to this, the matter is complicated still further by our ignorance of the quantities of the active substances in the solutions with which we are dealing. I have succeeded, however, in devising experiments in which these obstacles have been overcome.

But before describing these, let us examine the evidence which Dastre and Stassano adduce in favour of the antikinasic power of worm extract. These observers<sup>(4)</sup> mixed worm extract and enterokinase and kept them at 37° C. for 3 to 4 hours. They then added pancreatic juice and a cube of albumin; the cube did not undergo digestion. This was their crucial experiment, and from it they concluded that the worm extract had destroyed the enterokinase and had thus prevented the

activation of the pancreatic juice. It is well known that enterokinase in alkaline solution is rapidly destroyed at  $37^{\circ}\text{C}$ .; Dastre and Stassano guarded against this source of error by omitting the usual 5% sodium carbonate when mixing the enterokinase and worm extract. But their worm extract contained 2% sodium fluoride. Sodium fluoride is distinctly alkaline, and is itself sufficient to destroy enterokinase as the following experiments show.

Enterokinase solution was prepared in the way already described. Some was kept just as prepared (Ek.). To the rest was added sodium fluoride up to 2% (Ek<sup>Na</sup>). The worm extract used was neutral. The following mixtures were then made:

		Gel. digested in mm. after 16 hours.
A at $15^{\circ}\text{C}$ . for 2 hrs.	{	(·3 c.c. Ek <sup>Na</sup> +1 c.c. W)+1 c.c. PJ = 3·0
	{	(·3 c.c. Ek <sup>Na</sup> +1 c.c. H <sub>2</sub> O)+1 c.c. PJ = 5·0
	{	(·3 c.c. Ek <sup>Na</sup> )+1 c.c. PJ+1 c.c. W = 3·0
	{	(·3 c.c. Ek+1 c.c. W)+1 c.c. PJ = 3·0
	{	(·3 c.c. Ek+1 c.c. H <sub>2</sub> O)+1 c.c. PJ = 5·5
	{	(·3 c.c. Ek)+1 c.c. PJ+1 c.c. W = 3·0
B at $37^{\circ}\text{C}$ . for 2 hrs.	{	(·3 c.c. Ek <sup>Na</sup> +1 c.c. W)+1 c.c. PJ = 0
	{	(·3 c.c. Ek <sup>Na</sup> +1 c.c. H <sub>2</sub> O)+1 c.c. PJ = 0
	{	(·3 c.c. Ek <sup>Na</sup> )+1 c.c. PJ+1 c.c. W = 0
	{	(·3 c.c. Ek+1 c.c. W)+1 c.c. PJ = 3·5
	{	(·3 c.c. Ek+1 c.c. H <sub>2</sub> O)+1 c.c. PJ = 5·0
	{	(·3 c.c. Ek)+1 c.c. PJ+1 c.c. W = 3·0

The brackets indicate that their contents have been kept together for 2 hours and then the substances outside the brackets added. In series A the substances in brackets were kept at  $15^{\circ}\text{C}$ . for 2 hours. In series B the substances in brackets were kept at  $37^{\circ}\text{C}$ . for 2 hours. Gelatine tubes and toluol were added to all and the amount of digestion measured at the end of 16 hours.

From this we see that activation occurs in all the tubes except those in which the sodium fluoride enterokinase had been kept for 2 hours at  $37^{\circ}\text{C}$ . Dastre and Stassano's fundamental experiment is thus inconclusive; enterokinase exposed to the action of worm extract for some time, even at  $37^{\circ}\text{C}$ ., will activate pancreatic juice unless destroyed by substances like sodium fluoride before the pancreatic juice has been added. It is not permissible, therefore, to interpret the experiments which follow this one in their paper and which are intended to confirm it, by assuming, as these observers have done, the existence of antikinase in the worm extract; nor, indeed, is it necessary, since all the phenomena recorded can be accounted for quite satis-

factorily on the assumption that worm extract possesses antitryptic powers only.

I will now pass to experiments devised with the object of proving in a positive manner that worm extract can function as an antitryptic agent only.

*Series (a).* .5 c.c., 1 c.c., 1.5 c.c., and 2 c.c. W were each added to separate lots of 1 c.c. Ek and the whole left for 4 hours at 15° C. 1 c.c. Ek without W (W0) was kept as a control. At the end of 4 hours, 2 c.c. PJ were added to each and the volume of liquid made equal in each flask by the addition of water.

*Series (b).* .5 c.c., 1 c.c., 1.5 c.c., and 2 c.c. W were each added to separate lots of 2 c.c. PJ at the same time as the corresponding operation was performed in (a) and left for 4 hours at 15° C. 2 c.c. PJ without W (W0) were kept as a control. At the end of 4 hours 1 c.c. Ek was added to each and the volume of the liquid made equal in each flask by the addition of water. Gelatine tubes and toluol were added to (a) and (b). After the lapse of 20 hours the tubes were measured with the following result:

<i>Series (a)</i>		<i>Series (b)</i>	
	Mm. of gel. digested		Mm. of gel. digested
W0	= 12	W0	= 12
W.5	= 11	W.5	= 11
W1.0	= 10	W1.0	= 10
W1.5	= 7	W1.5	= 7
W2.0	= 2	W2.0	= 3

Digestion proceeds equally in both (a) and (b). If the antibody were antikinasic in nature we should expect to find a difference in digestive power between (a) and (b) such as Bayliss and Starling<sup>(6)</sup> obtained when using an antikinasic serum prepared by injecting animals subcutaneously with enterokinase solutions. We find, however, that it is a matter of complete indifference in which order the worm extract is added to the mixtures; that, in fact, the worm exerts its activity only when trypsin is formed; that is to say, is antitryptic only.

Further investigation on entirely different lines leads us to the same conclusion, namely, that worm extract possesses no antikinasic power. If worm extract be added to a trypsin solution in such quantity as just to inhibit the activities of the latter, addition of enterokinase to this neutral mixture should (if the worm antibody has any affinity for enterokinase) cause a redistribution of the antibody in such a way that it shares itself between the enterokinase and the trypsin in proportion to its affinity for these two substances. If this occurred, some of the trypsin would become unmasked, and gelatine tubes added to the hitherto neutral solution would now undergo digestion. This was tested in the following way:

It was found by trial that gelatine tubes placed in a mixture of 2 c.c. trypsin and 1.5 c.c. worm extract showed no sign of digestion at the end of five days. Therefore, a mixture of 2 c.c. trypsin and 1.5 c.c. worm extract was taken as a neutral or balanced mixture. To two lots of such a mixture (2 c.c. trypsin + 1.5 c.c. W) were added 1 c.c. Ek and 2 c.c. Ek respectively. A third portion without any Ek was kept as a control. The volumes in all the flasks having been made equal by the addition of water, gelatine tubes and toluol were added. At the expiration of four days no digestion had taken place in any of the flasks; at the end of the fifth day 2 mm. of gelatine had disappeared in each flask.

It will be noticed that the neutrality of the mixtures persists for a time, but that eventually their tryptic activities become manifest. The mere disappearance of the inhibitory effect need not detain us in this connection. It is, however, important to notice that it persists for the same length of time in all the flasks, and that digestion, when it does begin, proceeds with equal rapidity in all three mixtures. The presence or absence of enterokinase in no way affects the balance between antibody and trypsin.

If then, as this experiment seems to show, the antibody devotes all its energies to the suppression of tryptic activity, the addition of a small quantity of a balanced mixture of pancreatic juice, enterokinase, and worm extract to pancreatic juice should cause activation of the latter; whereas, if the antibody were purely antikinasic no such activation should occur.

To test this, Ek, W, and PJ were mixed together, the amounts of W being varied till a balance was attained. Finally, a mixture of 1 c.c. Ek + 2.5 c.c. W + 2 c.c. PJ was found to remain inactive for five days. Therefore, two lots of 1 c.c. Ek + 2.5 c.c. W + 2 c.c. PJ were made up; one, to which gelatine tubes were added, was kept up as a control; half of the other lot was added to 2 c.c. PJ after the lapse of 10 hours, and, at the same time, a control of  $\frac{1}{2}$  c.c. Ek + 1.2 c.c. W + 3 c.c. PJ was set up. Gelatine tubes were added to both mixtures. After the lapse of 16 hours digestion was equal in both flasks (6 mm.). The balanced control showed no digestion.

This experiment proves that in the balanced mixture the enterokinase was not appropriated, either in whole or in part, by the antibody; that is to say,  $\frac{1}{2}$  c.c. of active enterokinase must have been added to the 2 c.c. PJ in order that digestion by both it and the control tube should have been equal. Thus, here also the inhibition must have been purely antitryptic in character.

## II. THE PROPERTIES OF THE ANTIBODY.

*The action of heat on worm extract.* Worm extract prepared in the way already described was used in these experiments. Its reaction was neutral, or faintly acid; never alkaline. Four separate lots of worm extract were taken; the first was allowed to remain at 15° C. for 6 hours, the second was kept at 37° C. for 6 hours, the third was kept for the same time at 50° C., and the fourth was boiled for 5 minutes. With these the following mixtures were made:—

W extract at 15° C. for 6 hours.

Digestion in mm.

2 c.c. T+2 c.c. W=5·0—5·5

2 c.c. T+3 c.c. W=4·5—5·0

2 c.c. T+0 c.c. W= 11—11

W extract at 37° C. for 6 hours.

Digestion in mm.

2 c.c. T+2 c.c. W=5·0—5·5

2 c.c. T+3 c.c. W=4·5—4·5

2 c.c. T+0 c.c. W= 11—11

W extract at 50° C. for 6 hours.

2 c.c. T+2 c.c. W=5·5—5·5

2 c.c. T+3 c.c. W=5·0—4·5

2 c.c. T+0 c.c. W= 11—11

W extract boiled for 5 minutes

2 c.c. T+2 c.c. W=5·0—5·0

2 c.c. T+3 c.c. W=4·5—5·0

2 c.c. T+0 c.c. W= 11—11

T=trypsin solution; W=worm extract. Gelatine tubes and toluol were added. The numbers opposite each mixture give the amount of digestion in mm. at each end of the tubes 20 hours later.

These experiments clearly show that the antibody in worm extract prepared as above described is capable of withstanding a considerable rise of temperature. Even boiling for five minutes does not in the least impair its activity. Weinland<sup>(2)</sup> has stated that the inhibitory power of worm extract is abolished at 100° C., and Dastre and Stassano<sup>(4)</sup> confirm his observation. As we shall see, their results are obtained only if the medium is alkaline.

*The action of heat on worm extracts in acid and alkaline media.*

In these experiments the acid worm extract was made by the addition of 3 or 4 drops of strong hydrochloric acid to about 5 c.c. of the extract. The alkaline extract was made by the addition of 1 c.c. of 2N.Na<sub>2</sub>CO<sub>3</sub> to 1 c.c. of worm extract. Thus the alkalinity of the mixture was equivalent to that of N.Na<sub>2</sub>CO<sub>3</sub>. Portions of both alkaline and acid extracts were kept for 6 hours at 15° C., at 37° C., and at 50° C., and some was boiled for 2 minutes. The following mixtures were then made, the acid worm extract being first neutralised when cool:

Acid W at 15° C. for 6 hours.  
2 c.c. T+2 c.c. W=5·0—5·5

Acid W at 37° C. for 6 hours.  
2 c.c. T+2 c.c. W=5·0—5·5

Acid W at 50° C. for 6 hours.  
2 c.c. T+2 c.c. W=5·0—5·0

Acid W boiled.  
2 c.c. T+2 c.c. W= 5—5

Control.  
2 c.c. T+2H<sub>2</sub>O = 8—8

Alk. W at 15° C. for 6 hours.  
2 c.c. T+2 c.c. W=5·0—5·5

Alk. W at 37° C. for 6 hours.  
2 c.c. T+2 c.c. W=5·0—5·5

Alk. W at 50° C. for 6 hours.  
2 c.c. T+2 c.c. W=9·0—9·0

Alk. W boiled.  
2 c.c. T+2 c.c. W=9·0—9·5

Control.  
2 c.c. T+2 c.c. alk.=9·0—9·5

Gelatine tubes and toluol were added and the digestion, measured in mm. after 16 hrs. appears opposite each mixture. T=trypsin solution. W=worm extract. Alk. (in control) =N. Na<sub>2</sub>CO<sub>3</sub>.

Thus we see that whilst acid worm extract may be heated with impunity even to the boiling point, alkaline worm extract, though preserving its inhibiting power well during prolonged exposure to 37° C., rapidly loses it as the temperature rises. I have repeatedly boiled acid worm extracts for 10 or 15 minutes without in any way impairing their activity; whereas extracts, rendered even faintly alkaline, at once lose their power when raised to the boiling point for a few moments only.

We are now in a position to understand why simple heating to 100° C. destroyed the antibody in Weinland's extracts. In preparing these extracts he used a solution of disodium hydrogen phosphate so that the extracts had a strongly alkaline reaction. That this is the cause of the results which he obtained is sufficiently obvious from the following experiment:

Digestion in mm.	
2 c.c. T+2 c.c. W	= 4·0
2 c.c. T+2 c.c. BW	= 4·0
2 c.c. T+2 c.c. H <sub>2</sub> O	=10·5
2 c.c. T+2 c.c. Wph.	= 4·0
2 c.c. T+2 c.c. BWph.	= 9·5
2 c.c. T+2 c.c. H <sub>2</sub> Oph.	= 9·5

*Explanation.* BW=worm extract boiled for 3 minutes.

The three lowest mixtures of the series are the same as the upper three except that each contains 5% Na<sub>2</sub>HPO<sub>4</sub>. Gelatine tubes and toluol were added, and the digestion in each tube was measured after 20 hours.

We see that worm extract to which disodium hydrogen phosphate has been added is just as powerful an inhibitor of tryptic digestion as ordinary worm extract. This phosphate solution, however, when boiled, completely loses its anti-action, whereas ordinary worm extract after similar treatment retains its activity unimpaired.

*Diffusibility of the antibody.* This was investigated in the following way. 15 c.c. of worm extract were dialysed against running water for four days, whilst 15 c.c. of the same extract were kept as a control in a flask with a little toluol. The 15 c.c. of dialysed worm extract became diluted to 43 c.c., therefore the 15 c.c. of control extract were also diluted to 43 c.c. and the following mixtures were made:—

Dig. in mm.	
2 c.c. T + 2 c.c. W	= 3·5
2 c.c. T + 2 c.c. DW	= 7·0
2 c.c. T + 2 c.c. H <sub>2</sub> O	= 7·0

Control worm extract = W. Dialysed worm extract = DW. Gelatine tubes and toluol were added. The tubes were measured after 16 hours.

From the fact that the worm extract loses all its inhibitory action after dialysis we can conclude that the antibody is not colloidal in nature, but, on the contrary, possesses the power of diffusibility to a marked degree.

*The action of alcohol on worm juice.* For these experiments worms were thoroughly ground up with sand, mixed with kieselguhr, and subjected to a pressure of 300 kgs. per sq. cm. in a Buchner's press. A clear yellowish juice, acid in reaction, was thus obtained. Two volumes of absolute alcohol were added to the juice and the heavy precipitate which formed was filtered off. The precipitate was washed six times by shaking with a mixture of two parts of alcohol and one of water; the filtered aqueous extract was used in the experiments below as AP<sub>1</sub>. The alcoholic filtrate was evaporated nearly to dryness *in vacuo* at a temperature of 40° C. to get rid of the alcohol. The residue redissolved in water is spoken of below as AE<sub>1</sub>.

The solution AP<sub>1</sub> was now taken, some was kept neutral (AP<sub>1</sub> neut.); some of this neutral solution was boiled (BAP<sub>1</sub> neut.); some was acidified with HCl, boiled for 3 minutes, allowed to cool and then carefully neutralised with ammonia (BAP<sub>1</sub> acid); finally, some was made just alkaline with ammonia and boiled for 3 minutes (BAP<sub>1</sub> alk.) The following mixtures were then set:—

	Dig. in mm.
2 c.c. T + 2 c.c. AP <sub>1</sub>	= 3·0
2 c.c. T + 2 c.c. BAP <sub>1</sub> neut.	= 3·5
2 c.c. T + 2 c.c. BAP <sub>1</sub> acid	= 3·0
2 c.c. T + 2 c.c. BAP <sub>1</sub> alk.	= 8·0
2 c.c. T + 2 c.c. H <sub>2</sub> O	= 8·0

Gelatine tubes and toluol were added, the tubes were measured after 16 hours.

The solution AE<sub>1</sub> was also examined for inhibitory action in the same way as AP<sub>1</sub>.

The following mixtures were made :

	Dig. in mm. after 16 hrs.
2 c.c. T + 2 c.c. AE <sub>1</sub>	= 3·0
2 c.c. T + 2 c.c. BAE <sub>1</sub> acid	= 3·0
2 c.c. T + 2 c.c. BAE <sub>1</sub> alk.	= 7·0
2 c.c. T + 2 c.c. H <sub>2</sub> O	= 7·0

From these two experiments we see that both the precipitate and filtrate, obtained by the addition of two volumes of absolute alcohol to the worm juice, contain antibody. Prolonged washing of the precipitate with alcohol of this strength does not remove the inhibitory substance. The probable explanation of this is that some of the antibody is carried down by the precipitate and held there by a process analogous to adsorption, and in this state 66 % alcohol is unable to remove it from the precipitate.

Since the alcoholic filtrate is acid in reaction, we can evaporate the filtrate to dryness on the water-bath instead of boiling off the alcohol *in vacuo* as described above. The aqueous extract obtained by treating the residue with water is strongly inhibitory, as is seen from the following experiment :—

	Dig. in mm. after 16 hrs.
2 c.c. T + 2 c.c. AE <sub>1</sub>	= 3·0
2 c.c. T + 2 c.c. BAE <sub>1</sub> acid	= 3·0
2 c.c. T + 2 c.c. BAE <sub>1</sub> alk.	= 7·7
2 c.c. T + 2 c.c. H <sub>2</sub> O	= 7·7

If we now take the alcoholic filtrate obtained by treating worm juice with 2 volumes of absolute alcohol (AE<sub>1</sub>) and add to it a further quantity of alcohol (2 volumes) a very fine precipitate amounting to hardly more than a turbidity is produced. Eventually, however, the scanty precipitate settles to the bottom of the vessel, leaving the alcoholic solution quite clear. No further precipitate is produced by adding more alcohol. The precipitate was filtered off and washed with alcohol. It dissolved readily in water, the solution so obtained being here called AP<sub>2</sub>.

The alcoholic filtrate from this precipitate was evaporated almost to dryness *in vacuo* at 40° C. The residue dissolved in water is called AE<sub>2</sub>.

The solution AP<sub>2</sub> was now taken; some was made acid with HCl, boiled for 3 minutes and then cooled and neutralised (BAP<sub>2</sub> acid); some was made faintly alkaline with ammonia and boiled for 2 minutes (BAP<sub>2</sub> alk.); and some was kept untouched (AP<sub>2</sub>).

The aqueous AE<sub>2</sub> was treated in the same way as AP<sub>2</sub> and the following mixtures were made:—

	Mm. digested		Mm. digested
2 c.c. T + 2 c.c. AP <sub>2</sub>	= 2·5	2 c.c. T + 2 c.c. AE <sub>2</sub>	= 6·0
2 c.c. T + 2 c.c. BAP <sub>2</sub> acid	= 2·5	2 c.c. T + 2 c.c. BAE <sub>2</sub> acid	= 6·0
2 c.c. T + 2 c.c. BAP <sub>2</sub> alk.	= 6·5	2 c.c. T + 2 c.c. BAE <sub>2</sub> alk.	= 7·0
2 c.c. T + 2 c.c. H <sub>2</sub> O	= 7·0	2 c.c. T + 2 c.c. H <sub>2</sub> O	= 7·0

Toluol and gelatine tubes were added and the digestion measured after 16 hours.

We see from these experiments that the fraction AP<sub>2</sub> precipitated by the addition of further alcohol is extremely active in inhibiting tryptic digestion; further, practically all the antibody is thrown down by this concentration of alcohol, since the filtrate possesses only an insignificantly small inhibiting action.

To the clear alcoholic filtrate AE<sub>2</sub>, three volumes of ether were added. A scanty granular precipitate was thrown down. This was filtered off, washed with ether, and dried. It dissolved easily in water, forming a clear solution, EP. The ether filtrate was evaporated nearly to dryness *in vacuo*, and the residue dissolved in water, EE.

The following mixtures were then made:—

	Dig. in mm. after 16 hrs.
2 c.c. T + 2 c.c. H <sub>2</sub> O	= 7·0
2 c.c. T + 2 c.c. EP	= 7·0
2 c.c. T + 2 c.c. EE	= 6·0

Gelatine tubes and toluol were added and the digestion measured after 16 hours.

The ether extract is thus practically without inhibitory power, the ether precipitate is quite inactive. The solution AE<sub>1</sub> gives the biuret reaction and Fehling's test. AE<sub>2</sub> gives Fehling's test but no biuret reaction. The solution EE gives no Fehling's test or, at the most, gives a very slight reduction. The precipitate EP however reduces Fehling's solution very strongly.

We are able, therefore, by means of the addition of alcohol, to precipitate first of all most of the proteid from the worm juice. Both this precipitate and the alcoholic filtrate obtained from it exert a

markedly inhibitory action on tryptic digestion. The alcoholic filtrate can be evaporated to dryness on the water-bath and the residue still retains its activity. From this weak alcoholic filtrate a further scanty precipitate can be thrown down by the addition of more alcohol. This precipitate is intensely active; its aqueous solutions react to heat in acid and alkaline media just as does the original worm juice. Practically the whole of the antibody is precipitated by this strength of alcohol (85%), since the filtrate from it is almost inactive. By the addition of ether to this filtrate another precipitate is obtained, but this is inactive; it probably represents merely the carbohydrate element in the worm juice.

From the foregoing observations the antibody appears to be a definite chemical entity of no very great complexity. It is readily diffusible, very thermostable in acid solution, but easily destroyed when rendered alkaline, resembling in this respect such a substance as, say, secretin. Like some of the vegetable albumoses it requires a high concentration of alcohol to precipitate it from solution. It is possible that in structure the antibody may prove to be of the same order of complexity as some of the amino-acids. To this point, however, I hope to revert in a future paper.

#### SUMMARY.

1. The antibody in the tissue juices of intestinal worms is anti-tryptic in its action as stated by Weinland and not antikinasic as Dastre and Stassano assert.
2. The antibody, in neutral or acid extracts of intestinal worms, is uninjured by boiling; if, however, the extracts be made even faintly alkaline their anti-action is immediately destroyed on boiling.
3. The antibody is soluble in weak alcohol, but is precipitated when the concentration is increased to 85%. The precipitate dissolves easily in water and the resulting solution strongly inhibits the proteolytic activities of pancreatic juice. The antibody diffuses readily through colloid membranes.

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ON THE ALLEGED ADAPTATION OF THE PANCREAS  
TO LACTOSE. BY R. H. ADERS PLIMMER, D.Sc.

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It has been shown by E. Fischer and W. Niebel<sup>(1)</sup> and by Portier<sup>(2)</sup> that the pancreas of animals such as the dog, ox, horse, calf, and pig, contains no ferment capable of hydrolysing lactose. This result was confirmed for adult animals in 1899 by Weinland<sup>(3)</sup>, who, however, asserted that the presence or absence of lactase in the pancreas depended on the previous diet of the animal. He found that lactase was a constant constituent of extracts of pancreas made from sucking animals, and that the effect of feeding dogs for some weeks with milk was to cause the appearance of this ferment in the pancreas. This observation of Weinland's was confirmed by Bainbridge<sup>(4)</sup>. Bainbridge extended his observations also to the pancreatic juice, and found that here too the administration of lactose to adult animals caused the secretion of lactase in the juice. Bainbridge carried out an elaborate series of experiments with the view of determining the exact mechanism by means of which the adaptation of the pancreas to the presence of lactose in the alimentary canal was effected, and concluded that the administration of lactose caused the formation of some substance in the epithelial cells of the intestine which was absorbed by the blood and carried to the pancreas, where it gave rise to the formation of lactase. The effect of secretin on such a pancreas was to cause the turning out of all the ferments which it contained, including the lactase.

Recently, however, Bierry<sup>(5)</sup>, in the course of a research on the distribution of lactase, has examined extracts of the pancreas and the pancreatic juice, in adult dogs fed on biscuit or meat, as well as in other dogs which had been fed on lactose. He states that under no circumstances can the lactose-splitting ferment be found either in the pancreas or in the pancreatic juice, and ascribes the results of previous observers to the insufficiency of the methods of analysis employed by them.

In view of the uncertainty of the earlier results of Vasilieff and of Walther on the qualitative adaptation of the pancreatic juice to the food-stuffs present in the intestine, the adaptation of the pancreas to lactose, described by Weinland and Bainbridge, acquired great importance, as presenting direct evidence of the adaptation of the pancreas to the food. The question of the accuracy of their observations must therefore largely influence our attitude to the views put forward by Pawlow, with regard to the correlation of the total activities of the pancreas with the nature of the food. At the suggestion of Prof. Starling I have therefore submitted the whole question to a fresh investigation.

The credibility of the conclusions arrived at by Weinland and Bainbridge depends on the trust which can be placed on their methods. Those employed by Weinland have been justly criticised by Bainbridge. In Weinland's experiments an infusion of the pancreas was treated with a given proportion of lactose, and then placed in the incubator with the addition of toluol for some hours. The presence or absence of lactase in the infusion was judged by (1) submitting the mixture to the action of *Saccharomyces apiculatus*, which, while fermenting glucose, has no action on lactose: (2) by estimating the rotatory power of the solution after separation of the proteids it contained by means of Brücke's reagent. In no case were control observations carried out on mixtures in which the pancreatic infusions had been boiled, but the presence or absence of change was judged of by comparing the rotation with the rotation which would be expected from the amount of lactose added to the fluid. (3) In a certain number of cases the osazones were prepared from the mixture. For the identification of these osazones Weinland trusted chiefly to their microscopic form, no determination being made either of the melting point of the purified crystals or of their content in nitrogen.

Of these three methods Weinland placed most reliance on the polarimetric. In his dilute solutions the differences of readings that he observed were well within the limits of experimental error. Two minutes might make a difference of 10% inversion. Moreover, if any proteolysis had taken place in the solution amino-acids would be formed and would not be precipitated by Brücke's reagent. In the strongly acid filtrate obtained from this reagent, nearly all the possible amino-acids which might be present would have a dextro-rotation, and therefore might account for the whole of the increased rotatory power observed in these various experiments. The presence of dextrose in the

pancreatic infusions was not excluded, and therefore one can not rely implicitly on the fermentation test applied by him.

Later experiments <sup>(6)</sup> on feeding with galactose instead of lactose also produced a small increase in rotatory power, but only a slight evolution of gas on fermentation with *Sacch. apiculatus*, but in this case Weinland considered that his result was negative.

In Bainbridge's experiments most of these sources of error were avoided. In almost all his experiments a control experiment was made with boiled juice or boiled infusion. He rightly discarded the polarimetric method, and adopted as his criterion the reducing powers of the solutions as determined by Pavy's solution. The osazone method employed by him was unsatisfactory, since in no case was there isolation of the osazone or determination of its melting point and its nitrogen content. Moreover, although Pavy's method can be applied with considerable accuracy to the determination of reducing sugar, its accuracy depends on the scrupulous observance of identical conditions in all the determinations. Small alterations in the time of boiling or in the volume of fluids used may produce differences in the results, which are serious when, as in Bainbridge's experiments, deductions are to be drawn from differences in the reducing power of two solutions amounting to only .2 to .9 c.c. in a total quantity of fluid of between 4 and 8 c.c.

The method employed by Bierry consisted in the preparation of the osazones after separating proteid-substances by means of mercuric nitrate. He considered that this was the most delicate method, but found that it was inaccurate unless 20 per cent. of lactose were hydrolysed. Unfortunately no data are given of the osazone obtained, except that it was soluble in a mixture of equal parts of acetone and water, in which glucosazone is insoluble. The possibility that less than 20 per cent. inversion had occurred is not excluded, since the length of time during which the ferment was allowed to exert its action is not stated.

In my reinvestigation of the subject I have endeavoured to avoid the above sources of fallacy. Of the various methods of determining the question of inversion there is no doubt that, as Bainbridge has pointed out, a comparison of the reduction before and after conversion is the most delicate.

Of the methods for determining the reducing power of a solution, practically the only one which is free from sources of error, depending on the experimenter, is that in which the copper oxide produced is collected and weighed. I have therefore made use of Allihn's method for the determination of the reducing power of the solutions. Moreover, before

the determination is carried out all proteids and allied bodies must be separated from the solution. For this purpose I have used mercuric nitrate as employed by Bierry. The method of boiling after acidification employed by Bainbridge is inadequate since it only removes the greater part of the coagulable proteid, leaving the proteoses and other disintegration products still in the solution. Since my object is merely to determine the presence or absence of lactase in the given juice or tissue extract, I have always allowed ample time for the action of the ferment to take place, generally leaving the solutions in the incubator for two or three days. In every case two experiments have been made side by side, one consisting of the mixture of the juice or extract with lactose, and the other consisting of exactly the same amounts of lactose and of boiled juice or extract. Both these solutions have been subjected throughout to exactly the same procedure. Finally, in each case the osazones have been made and the glucosazone, if present, separated from the lactosazone, and the identity of these substances determined both by the melting point as well as by an estimation of their content in nitrogen by Dumas' process. By adopting these methods it is easy to detect the presence of a lactose-splitting ferment. In every case, when such a ferment is present, we can after one to three days' incubation get a big difference in the copper reducing power of the two solutions as well as definite production of glucosazone. In every case I have also taken polarimeter readings of the two fluids. This method, however, is much less delicate than the other two, and suffers moreover from the drawback that it is impossible to separate amino-acids from the solutions, so that the final rotatory power of the solutions may differ apart from any difference in their sugar content.

I have investigated the question of the presence of lactase after feeding with milk and milk sugar in five animals, namely 4 dogs and 1 kitten. The dogs were fed with meat and biscuit and in addition about two pints of milk and two ounces of milk sugar per diem. This diet was continued from two to five weeks. At the end of this period the animals were anæsthetised, and pancreatic juice, excited by injection of secretin, was collected by Prof. Starling for a period of 4 to 6 hours. The animals were then killed, the pancreas cut out and pounded up with sand. The pounded organ was then suspended in toluol water until the next day, when it was centrifuged, and the opalescent supernatant fluid taken and handed to me to determine the presence or absence of lactase. In each case the pancreatic juice and the pancreas infusion were divided into two parts; one part was kept at 100° C. for 20 to 30 minutes in

order to destroy any lactase which might be present. Exactly equal volumes of each were then treated with the same bulk of 5 % lactose solution, and 2 % of toluol added. The mixtures were then incubated at 38° C. in corked bottles for periods varying from one to three days. As is well known the pancreatic juice is strongly alkaline. In the first experiment therefore the sample was taken at the end of 24 hours, and the remaining mixture of juice and lactose was neutralised and allowed to incubate for a further 24 hours.

In the third experiment the whole of the pancreatic juice was neutralised at the commencement of the experiment by means of hydrochloric acid, and was then treated as already described. The kitten was about 5 weeks old and had been about a fortnight away from its mother, but had received milk during the whole time.

The two mixtures after being incubated for the various times were treated with 10 c.c. of mercuric nitrate solution, prepared in the manner described by Patein and Dufau<sup>(7)</sup>. After allowing to stand for about 12 hours, in order that the precipitates should settle, they were filtered and the same quantity of the filtrate of each was neutralised with the same amount of 10 per cent. caustic soda. By this means most of the mercury was precipitated as oxide, the filtrate from this precipitate containing only small traces. Exactly equal quantities of the filtrates were then treated with hydrogen sulphide to remove the last traces of the mercury, and the excess of hydrogen sulphide was removed by means of copper sulphate solution. The solutions were then made up to a definite volume, generally 250 c.c. and filtered. The faintly acid filtrates, so obtained, were then employed for determining the rotatory power in a 2 decimetre tube and the reducing power by Allihn's method, the cuprous oxide being collected on a modified Soxhlet filter as described by Plimmer and Bayliss<sup>(8)</sup>, washed with alcohol and ether, dried at 110° C. and weighed. In every case at least two determinations of the cuprous oxide were made, and since these differed only by about 2 milligrammes in weight, the reduction to copper or oxidation to cupric oxide was not carried out.

In order that there should be the least possible error in all these processes the same pipettes were used throughout. They were always thoroughly cleansed, and dried with alcohol and ether before the liquids were taken up into them for measurement; only when the mixtures were finally made up to a definite volume, different measuring flasks were employed, otherwise the volumes taken from each solution were always exactly the same. By this means a perfect control experiment was made.

The only difference in the two mixtures consisted in the amount of copper sulphate added to remove the hydrogen sulphide ; a slight excess of this compound in the solutions could make no difference to the rotatory power since its colour was scarcely apparent, nor to the reducing power by Allihn's method, where an excess of copper sulphate in the Fehling's solution taken is always present.

Before preparing the osazones the solutions were again neutralised, whereby the excess of copper was precipitated as hydrate and filtered off ; in some cases, however, a dark blue solution resulted, from which the copper separated on adding the phenylhydrazine hydrochloride and sodium acetate. This mixture was used in preference to the phenylhydrazine and glacial acetic acid, since an excess of the latter might cause hydrolysis of the lactose, as often happens with cane-sugar. For every gramme of lactose in solution one and half times its quantity of phenylhydrazine hydrochloride and twice its quantity of sodium acetate were added ; and the mixtures were heated on the water-bath for about 2 hours after filtering from a small amount of resin and copper, if any. The osazones never came down whilst the solutions were hot, but on cooling crystals were always obtained. These were filtered off and washed thoroughly with cold water. They were then washed with boiling water, in which they completely dissolved leaving behind a resin, and on cooling they again separated ; in this way they were recrystallised, and after again filtering off and washing with cold water, they were dried at  $110^{\circ}$  C. and in many cases analysed.

In order to show that this method which I have adopted is adequate to display the presence of lactase, if any ferment existed in the solution I give also protocols of experiments made with extracts of mucous membrane of the small intestine, in which, as is well known, lactase is certainly present. Here the osazone obtained was not completely soluble in boiling water ; the insoluble portion was therefore dissolved in dilute alcohol for recrystallisation and analysed after drying at  $110^{\circ}$  C.

The results are best seen from the table, in which are included the figures obtained with a 2 per cent. lactose solution before and after hydrolysis with acid ; 20 c.c. of the solutions were in most cases taken to determine the reducing power. The additional data are given in the protocols.

Table of Results.

Exp.	Animal	Organ	Time of action	Rotations		Reductions			Diff. mgm. glucose	Amount of Inversion		
				Control	Diff.	Grs. Cu <sub>2</sub> O	=glucose in mgm.	Grs. Cu <sub>2</sub> O			=glucose in mgm.	
I.	Dog	Pancreatic juice	24 hrs.	1° 27'	1° 26'	+1'	Grs. Cu <sub>2</sub> O 0.3250 0.3208	=glucose in mgm. 138.2	Grs. Cu <sub>2</sub> O 0.3236 0.3210	137.4	+ 0.8	—
		Pancreatic juice neutralised	24 hrs.	1° 9'	1° 10'	-1'	Grs. Cu <sub>2</sub> O 0.2936 0.2940	124.6	Grs. Cu <sub>2</sub> O 0.2940 0.2952	124.9	- 0.3	—
II.	Dog	Pancreas	48 hrs.	1° 39'	1° 37'	+2'	Grs. Cu <sub>2</sub> O 0.4332 0.4350	194.5	Grs. Cu <sub>2</sub> O 0.4316 0.4330	193.6	+ 0.9	—
III.	Kitten	Pancreas	53 hrs.	1° 44'	1° 42'	+2'	Grs. Cu <sub>2</sub> O 0.4210 0.4222	187.9	Grs. Cu <sub>2</sub> O 0.4214 0.4212	187.7	+ 0.2	—
V.	Dog	Pancreatic juice neutralised	76 hrs.	1° 52'	1° 52'	0	Grs. Cu <sub>2</sub> O 0.4700 0.4742 0.4756	216.3	Grs. Cu <sub>2</sub> O 0.4724 0.4702 0.4732	215.5	+ 0.8	—
VI.	"	Pancreas	76 hrs.	1° 40'	1° 40'	0	Grs. Cu <sub>2</sub> O 0.4216 0.4234	188.4	Grs. Cu <sub>2</sub> O 0.4200 0.4180	186.5	+ 1.9	2.4 per cent.
VII.	"	Pancreatic juice	96 hrs.	1° 28'	1° 29'	-1'	Grs. Cu <sub>2</sub> O 0.4160 0.4158	184.8	Grs. Cu <sub>2</sub> O 0.4134 0.4130	183.5	+ 1.3	1.6 per cent.
VIII.	"	Pancreas	80 hrs.	1° 40'	1° 38'	+2'	Grs. Cu <sub>2</sub> O 0.4220 0.4240	188.6	Grs. Cu <sub>2</sub> O 0.4210 0.4218	187.8	+ 0.8	—
IV.	Kitten	Intestine	53 hrs.	1° 52'	1° 42'	+10'	Grs. Cu <sub>2</sub> O 0.4546 0.4524	205.1	Grs. Cu <sub>2</sub> O 0.4264 0.4272	190.6	+14.5	17.7 per cent.
IX.	Dog	Intestine	64 hrs.	0° 33'	0° 24'	+9'	Grs. Cu <sub>2</sub> O 0.1730 0.1724	70.9	Grs. Cu <sub>2</sub> O 0.1418 0.1448	58.0	+12.9	51.8 per cent.
X.	Kitten	Intestine	72 hrs.	1° 59'	1° 50'	+9'	Grs. Cu <sub>2</sub> O 0.4858 0.4870	223.5	Grs. Cu <sub>2</sub> O 0.4750 0.4738	216.9	+ 6.6	7.1 per cent.
XI.	Dog	Intestine	72 hrs.	1° 56'	1° 41'	+15'	Grs. Cu <sub>2</sub> O 0.5896 0.5922	250.6	Grs. Cu <sub>2</sub> O 0.4150 0.4172	185.0	+65.6	82.7 per cent.
		Hydrolysis by acid	...	...	...	...	Grs. Cu <sub>2</sub> O 0.2038 0.2040	84.5	Grs. Cu <sub>2</sub> O 0.1420 0.1456	58.3	+ 26.2	104.8 per cent.

Exp. I. 60 c.c. pancreatic juice.

100 c.c. 5% lactose solution + 25 c.c. juice + toluol. Control 100 c.c. 5% lactose solution + 25 c.c. boiled juice + toluol. Incubated at 38° for 24 hours. 25 c.c.  $\frac{N}{10}$  H<sub>2</sub>SO<sub>4</sub> then added to each.

50 c.c. removed and precipitated by 10 c.c. mercuric nitrate. Filtered; 50 c.c. filtrate neutralised with 5 c.c. 10% NaOH and filtered. 45 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 100 c.c. Filtered; and filtrate used for rotation, reduction, and osazones after neutralising and filtering from Cu(OH)<sub>2</sub>.

Remainder of neutralised mixtures incubated for 24 hours longer and then precipitated by 10 c.c. mercuric nitrate solution. Filtered; 100 c.c. filtrate neutralised with 6 c.c. 10% NaOH and filtered. 90 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed by CuSO<sub>4</sub>. Made up to 250 c.c. Filtered; and filtrate used for rotation, reduction, and osazones after neutralising and filtering from Cu(OH)<sub>2</sub>.

The osazones both of the experiment and the control were completely soluble in boiling water. They melted at 210—212° C. and 209—210° C. respectively; 0.1954 grm. osazone obtained from the experiment gave 16.4 c.c. N at 14° C. and 772 mm. Hence N = 9.98%.

Exp. II. 125 c.c. toluol water extract of pancreas of milk- and lactose-fed dog.

{ 100 c.c. 5% lactose solution, 50 c.c. extract + 2 c.c. toluol } Incubated at 38° C.  
{ 100 c.c. 5% lactose solution, 50 c.c. boiled extract + 2 c.c. toluol } for 48 hours.

To each 10 c.c. mercuric nitrate solution added. Filtered; 140 c.c. filtrate neutralised with 6.5 c.c. 10% NaOH and filtered. 125 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. Filtered; and filtrate used for rotation, reduction, and osazones after neutralising and again filtering.

The osazones from both solutions were completely soluble in boiling water and both melted at 208—210° C.

0.2626 gm. osazone of the experiment gave 23 c.c. N at 14° C. and 770 mm. Hence N = 10.44%.

0.2560 gm. osazone of the control gave 21.9 c.c. N at 13° C. and 762 mm. Hence N = 10.41%.

Exp. III. 120 c.c. toluol water extract of pancreas of kitten 4—6 weeks old.

{ 100 c.c. 5% lactose solution, 50 c.c. extract, 2 c.c. toluol } Incubated at 38° C.  
{ 100 c.c. 5% lactose solution, 50 c.c. boiled extract, 2 c.c. toluol } for 53 hours.

To each 10 c.c. mercuric nitrate solution added. Filtered; 140 c.c. filtrate neutralised with 6 c.c. 10% NaOH and filtered. 120 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. Filtered; and filtrate used for rotation, reduction, and osazones after neutralising and filtering.

The osazones in both cases were completely soluble in boiling water.

Exp. IV. 120 c.c. chloroform water extract of intestine of kitten.

{ 100 c.c. 5% lactose solution, 50 c.c. extract, 2 c.c. toluol } Incubated at 38° C.  
{ 100 c.c. 5% lactose solution, 50 c.c. boiled extract, 2 c.c. toluol } for 53 hours.

To each added 10 c.c. mercuric nitrate solution. Filtered. 140 c.c. filtrate neutralised with 6 c.c. 10% NaOH and filtered. 120 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. Filtered; and filtrate used for rotation, reduction, and osazones after neutralising and again filtering.

The osazone of the experiment was not completely soluble in boiling water. The insoluble portion was recrystallised from dilute alcohol. It melted at 205—207° C.

0.1020 gm. gave 13.9 c.c. N at 14° C. and 768 mm. Hence N = 16.2%.

The osazone of the control was completely soluble in boiling water.

Exp. V. 70 c.c. pancreatic juice of milk- and lactose-fed dog. (2 c.c. = 2.33 c.c. 0.4% HCl). Neutralised with 77 c.c. 0.4% HCl.

{ 100 c.c. 5% lactose solution, 60 c.c. juice, 2 c.c. toluol	} Incubated at 38° C. for 76 hours.
{ 100 c.c. 5% lactose solution, 60 c.c. boiled juice, 2 c.c. toluol	

To each added 10 c.c. mercuric nitrate solution. Filtered. Filtrate not completely precipitated, so that another 30 c.c. mercuric nitrate added to 160 c.c. filtrate. Filtered; 180 c.c. filtrate neutralised with 24 c.c. 10% NaOH and filtered. 190 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. Filtered; and filtrate used for rotation, reduction, and osazones after neutralising and filtering.

The osazones of the experiment and the control were completely soluble in boiling water. They melted at 208—210° C. and 209—211° C. respectively.

0.2628 gm. of osazone of the experiment gave 22.3 c.c. N at 13° C. and 770 mm. Hence N = 10.17%.

Exp. VI. 150 c.c. toluol water extract of pancreas of milk- and lactose-fed dog.

{ 100 c.c. 5% lactose solution, 60 c.c. extract, 2 c.c. toluol	} Incubated at 38° C. for 76 hours.
{ 100 c.c. 5% lactose solution, 60 c.c. boiled extract, 2 c.c. toluol	

To each added 10 c.c. mercuric nitrate solution. Filtered; 140 c.c. filtrate neutralised with 6 c.c. 10% NaOH and filtered. 125 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. Filtered; and filtrate used for rotation, reduction, and osazones after neutralising and again filtering.

The osazones in both cases were completely soluble in boiling water.

Exp. VII. 25 c.c. pancreatic juice of milk- and lactose-fed dog.

{ 100 c.c. 5% lactose solution, 10 c.c. juice, 2 c.c. toluol	} Incubated at 38° C. for 4 days.
{ 100 c.c. 5% lactose solution, 10 c.c. boiled juice, 2 c.c. toluol	

To each added 10 c.c. mercuric nitrate solution. Filtered; 100 c.c. filtrate neutralised with 5.5 c.c. 10% NaOH and filtered. 90 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. Filtered; and filtrate used for rotation, reduction, and osazones, after neutralising and again filtering.

The osazones in both cases were completely soluble in boiling water.

Exp. VIII. 175 c.c. toluol water extract of pancreas of milk- and lactose-fed dog.

{ 100 c.c. 5% lactose solution, 75 c.c. extract, 2 c.c. toluol	} Incubated at 38° C. for 80 hours.
{ 100 c.c. 5% lactose solution, 75 c.c. boiled extract, 2 c.c. toluol	

To each added 10 c.c. mercuric nitrate solution. Filtered. 150 c.c. filtrate neutralised with 6 c.c. 10% NaOH and filtered. 135 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. Filtered; and filtrate used for rotation, reduction, and osazones after neutralising and again filtering.

The osazones in both cases were completely soluble in boiling water.

Exp. IX. 70 c.c. toluol water extract of mucous membrane of intestine of dog.

{ 100 c.c. 5% lactose solution, 25 c.c. extract, 2 c.c. toluol	} Incubated at 38° C. for 64 hours.
{ 100 c.c. 5% lactose solution, 25 c.c. boiled extract, 2 c.c. toluol	

To each added 15 c.c. mercuric nitrate solution. Filtered. 120 c.c. filtrate neutralised with 8.1 c.c. 10% NaOH and filtered. 20 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 100 c.c. Filtered; and filtrate used for rotation and reduction. Remainder of solution treated in same way but used for osazones after neutralising and again filtering.

The osazone of the experiment was not completely soluble in water. The insoluble portion was recrystallised from dilute alcohol. It melted at 195—198° C.

0.1038 gm. gave 12 c.c. N at 11° C. and 768 mm. Hence N = 13.93%.

The osazone of the control was completely soluble in boiling water.

Exp. X. 120 c.c. toluol water extract of mucous membrane of intestine of kitten passed through Berkfeld filter.

{ 100 c.c. 5% lactose solution, 50 c.c. extract, 2 c.c. toluol	} Incubated at 38°C.
{ 100 c.c. 5% lactose solution, 50 c.c. boiled extract, 2 c.c. toluol	

To each added 10 c.c. mercuric nitrate solution. Filtered. 150 c.c. filtrate neutralised with 7.5 c.c. 10% NaOH and filtered. 140 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. Filtered; and filtrate used for rotation, reduction, and osazones, after neutralising and again filtering.

The osazone of the experiment was completely soluble in boiling water except for a very small quantity.

The osazone of the control was completely soluble in boiling water.

Exp. XI. 210 c.c. toluol water extract of mucous membrane of intestine of dog.

{ 100 c.c. 5% lactose solution, 100 c.c. extract, 2 c.c. toluol	} Incubated at 38°C.
{ 100 c.c. 5% lactose solution, 100 c.c. boiled extract, 2 c.c. toluol	

To each added 10 c.c. mercuric nitrate solution. Filtered. 170 c.c. filtrate neutralised with 5.5 c.c. 10% NaOH and filtered. 150 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. Filtered; and filtrate used for rotation and reduction.

The osazone of the experiment was not soluble in boiling water.

The osazone of the control was completely soluble in boiling water.

The table of results clearly shows that neither the pancreatic juice nor the extracts of pancreas of milk and lactose-fed dogs contain lactase. Except in Exps. VI and VII, the difference in reducing power is always less than 1 mgm. glucose, but here it was about 2 mgm. This is no doubt due to experimental error, since, where inversion has certainly occurred there is a difference varying from 7 mgm. to 66 mgm. which represents changes of 7—83 per cent., whereas in the above experiments the difference only represents a change of about 2.5 per cent.

Exp. X clearly shows that the reduction method of determining the presence of lactase is by far the most delicate. A difference of 6.6 mgm. glucose or 7 per cent. inversion could be determined, whereas only a very small quantity of glucosazone could be obtained. This by itself would be insufficient to definitely show the presence of lactose. Bierry's statement that a change of less than 20 per cent. cannot be detected with certainty by the osazone method is thus confirmed.

The melting points of the glucosazone obtained in Exps. IV and IX are too low. This can easily be accounted for by the presence of galactosazone, which would also be formed and which would lower the melting point considerably. As has been frequently shown the melting points of osazones when impure are no criterion of their composition, so that no stress can be laid upon these data. The estimation of nitrogen by Dumas' method, however, definitely shows whether the osazone of a monosaccharide or a disaccharide is present, since glucosazone contains

15.64 per cent. of nitrogen and lactosazone 10.41 per cent. The value 13.93 which was obtained in Exp. IX points to the presence of a mixture of glucosazone and lactosazone. (It may be here stated that Kjeldahl's method of estimating nitrogen is not applicable to osazones. Glucosazone prepared from glucose gave 10.7 per cent. nitrogen, and lactosazone from lactose gave 7.7 per cent.)

One must therefore conclude with Bierry that the statements of Weinland and Bainbridge were founded on fallacious methods of experiments, and that under no circumstances is there any adaptation of the activity of the pancreas to the presence of lactose in the food. The general importance of such a conclusion I have already indicated. The ideas of Pawlow with regard to the power of adaptation possessed by this gland are founded on the results of Vasilieff and of Walther which, however, as Bainbridge showed, do not prove his point as regards lipase, and must be unreliable as regards the trypsin from the fact that at the time the experiments were made the importance of enterokinase for the activation of the juice was yet unknown. Popielski, a former pupil of Pawlow, has himself concluded that there is no qualitative adaptation in the pancreas, but that the composition and amount of this secretion is determined solely by the intensity and duration of the stimulus (or as we should now express it, the amount of secretin produced in a unit of time and the duration of its in-flow into the blood). My experiments therefore would incline us to accept Popielski's view, or at least to require further evidence for the qualitative adaptation of the pancreas to the food-stuffs.

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ON THE PRESENCE OF LACTASE IN THE INTESTINES OF ANIMALS AND ON THE ADAPTATION OF THE INTESTINE TO LACTOSE. BY R. H. ADERS PLIMMER, D.Sc.

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IN a recent communication to this *Journal*<sup>(1)</sup>, I showed that neither the pancreas nor the pancreatic juice of dogs, even when they were fed on milk and lactose, contained the enzyme lactase. These results definitely proved that there was no adaptation of this organ to a particular diet, a result which might have been expected, as the intestines of dogs contain the ferment in considerable quantity.

Since, however, the pancreas never at any time in the life of an animal contains lactase, whereas the intestines of, at any rate, young animals contain this ferment, while those of adult animals in many cases have lost their power of hydrolysing lactose, adaptation of this organ might be reasonably expected to occur, when the animals are subjected to this stimulus by feeding, a supposition put forward by Emil Fischer and W. Niebel<sup>(2)</sup> in 1895.

Experiments in this direction have already been carried out by Weinland<sup>(3)</sup> and by Portier and Bierry<sup>(4)</sup>. Weinland found that the adult rabbit and the fowl (the intestines of which do not contain lactase), when fed on milk and lactose produced this enzyme in their intestines. Portier and Bierry, using the duck in their experiments, in one case obtained no evidence of adaptation, but in another case found that adaptation had occurred. In their communication they stated that they would confirm this, but so far as I have been able to find out, no confirmation has appeared. The same observers also made experiments on the production of the ferment inulase, an enzyme not present in the intestines of animals, by feeding animals on Jerusalem artichokes, which contain inulin. The results were likewise negative and they confirm those of Richaud<sup>(5)</sup>, who found that inulin was easily hydrolysed by the acid of the gastric juice and thus converted into lævulose which could be assimilated.

Besides these experiments on the adaptation of the pancreas and intestine, a paper by Neilson and Terry<sup>(6)</sup> on the adaptation of the salivary glands of dogs to amylase has recently appeared. The results obtained by these authors, however, are not very conclusive and further experiments on this point would be of great interest.

Our present knowledge of the presence of lactase in the intestines of animals rests chiefly upon the experiments by Weinland. The earlier work by Dastre<sup>(7)</sup>, Röhmann and Lappe<sup>(8)</sup> and E. Fischer and Niebel was limited to the dog, calf, ox and horse. More extended observations were made by Portier<sup>(9)</sup>, who examined, in addition to the above animals, the rabbit, the pig and birds. Lactase was found in the intestine of the old rabbit, but not in that of the pig and birds. Then, a year later, Weinland took up the subject and examined the intestines of young and old dogs, young and old rabbits, young and old pigs, young and old oxen, young and old sheep, old horse, young goat, new-born child and fowl. Lactase was found to be present in all except the old rabbit, old ox, old sheep and fowl. His results, in the main, confirm those of the previous observers, of whom in this connection must also be mentioned Pantz and Vogl<sup>(10)</sup> with regard to the new-born child. The recent work of Langstein and Steinitz<sup>(11)</sup> on the presence of lactase in the excreta of children suffering from gastro-enteritis is a confirmation of this. Porcher<sup>(12)</sup> has also found lactase in the excreta of young mammals. The experiments of Bierry and Gmo-Salazar<sup>(13)</sup> upon animal lactase were rather in respect to its localisation than to its presence in young and old animals. Finally, a recent note by Mendel<sup>(14)</sup>, who found lactase present in the alimentary tract of the embryo and suckling pig, but not in the adult pig, completes the observations on the presence of lactase in the intestines of animals. The net result of the work of these observers is, that lactase is present in the intestines of all young animals but is not found in all old animals. Doubt still remains with regard to the old rabbit and old pig; the former having been found by Portier but not by Weinland to contain lactase; the latter, according to Weinland, contained lactase, but according to Portier and to Mendel it is not present.

It is necessary here to call attention to the methods which have been employed for the detection of the lactase. Portier used the osazone method, which has been shown by Bierry to be incapable of detecting a change of less than 20 per cent., and consequently, if less hydrolysis had occurred, the possibility of detecting it would be excluded.

Weinland used the polarimetric method, observing the difference in rotation of a solution of lactose at a period of about 6 hours after the addition of the extract of mucous membrane, and comparing it with that which would be given by the solution not submitted to this treatment. The differences thus obtained were so small, amounting to  $0.1-0.2^\circ$ , and corresponding to less than 3 per cent. inversion, that real conclusions cannot be based on his results. Though the solutions were also submitted to the action of *Saccharomyces apiculatus*, which ferments glucose but not lactose, the results obtained in this way are doubtful since the presence of glucose was not excluded by a proper control with boiled extract.

In consequence of this, I have thought it advisable to try to confirm Weinland's observations and to amplify them as much as possible, more especially as the question of adaptation is biologically of such great importance. The results which I have obtained are embodied in a table, from which it will be seen that I can find no evidence of the adaptation of the intestine.

The method which I consider the best for the determination of the presence of lactase is the reduction method, which is not only the most sensitive and most accurate but which at the same time gives an idea of the quantity of ferment present. It was not used by any of the above observers except Röhmann and Lappe. It has been supplemented in many cases by the other two methods as controls. Of the reduction methods, Allihn's, which consists in weighing the precipitated cuprous oxide, either as such, or when reduced to metallic copper, is that which is the most accurate and most free from personal error, and this method has been used in the following experiments exclusively.

The manner in which these experiments have been performed is essentially the same as I have previously described, which consisted in adding a definite volume of an extract of the mucous membrane of the intestine of the animal, removed as soon after death as possible,—immediately in the case of the smaller animals which were kept in the laboratory—to a known volume of lactose solution. The mucous membrane was scraped off, ground up with sand and treated for 6—24 hours with toluol water; before use, the extract was filtered through cotton wool or lint. The presence of unbroken cells, which passed through, may be premised to be advantageous, as it has been proved by Dastre, Pregl, Bierry and others, that lactase is not found in the intestinal juice but is present only in the cells of the mucous membrane. No attempt was made to isolate or prepare an extract of

lactase, such as was obtained by Porcher<sup>(15)</sup>, since my object was simply to determine the presence or absence of this ferment in the mucous membrane of the intestine. A control experiment was made in every case, which consisted in adding to the same volume of lactose solution an exactly equal quantity of boiled extract. A small quantity of toluol, generally 2 c.c., was added to each, and the two flasks, carefully corked, were incubated at 36—38° C. for 2—4 days, which, in the case of animal lactase, is essential, as I shall show later, since the ferment, when present, is either small in quantity or slow in its action. After the period of incubation, the same volume of mercuric nitrate solution (prepared by Patein and Dufau's method) was added to each to precipitate the protein and the flasks were allowed to stand for about 12 hours, so that the precipitate of protein should settle. The two solutions were then filtered and the same volumes of the filtrates were neutralised with the same quantity of 10 per cent. caustic soda, run in from a burette. The greater part of the mercury was thus removed, the remainder being separated from the solutions by passing hydrogen sulphide gas into the same volumes of the filtrates. Excess of the latter was removed by copper sulphate solution, the two solutions made up to a definite volume (generally 250 c.c.) and filtered. The filtrates, so obtained, were then used for the reduction by Allihn's method, 20 c.c. being generally employed, the cuprous oxide being collected on a weighed filter, as previously described, washed with water, alcohol and ether, dried at 110° C. and weighed. In every case, both the experiment and the control were treated in precisely the same way, the pipettes used in measuring out the volumes of the solutions being always the same and thoroughly cleansed and dried before taking up the solutions into them. As perfect a control experiment as possible was thus made. The only difference consisted in the amount of copper sulphate present in the solutions. This, however, was insufficient to colour the solutions except very faintly and would not interfere with the reduction where excess of copper sulphate is already present. For further confirmation of the results, in the earlier experiments, the rotations of the two solutions were also noted and the osazones were prepared after exactly neutralising with caustic soda, which completely removed the slight excess of copper sulphate present. (It not exactly neutralised and traces of copper still remain in solution, the separation of the osazone is not complete and on recrystallisation is totally lost.) In the later experiments, this confirmation was not considered necessary and would have required too much time to carry out during

the course of the large number of determinations which have been made. The results by the reduction method are quite sufficient to show how far the hydrolysis of the lactose had proceeded. It may be stated that unless the reduction showed 15—20 per cent. inversion, no osazone insoluble in boiling water could be isolated. A few examples will serve to show how this procedure was carried out:—

(1) 120 c.c. toluol water extract of mucous membrane of fowl fed for 14 weeks on milk and lactose mixed with the ordinary food.

100 c.c. 5 % lactose solution, 50 c.c. extract, 2 c.c. toluol	} Incubated at 38° C.
100 c.c. 5 % lactose solution, 50 c.c. boiled extract, 2 c.c. toluol	

Then, to each was added 10 c.c. mercuric nitrate solution. Filtered. 130 c.c. filtrate neutralised with 5.5 c.c. 10 % NaOH and filtered. 120 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. and filtered. 20 c.c. filtrate used for reductions. Rotations of the experiment and the control were +1° 46' and +1° 45' respectively. The osazones, prepared after exactly neutralising and filtering from copper hydroxide, were completely soluble in boiling water and melted at 211° C. and 212° C. respectively.

(2) 60 c.c. toluol water extract of mucous membrane of large ♀ rat, not fed on milk.

50 c.c. 5 % lactose sol., 25 c.c. extract, 2 c.c. toluol	} Incubated at 36—38° C.
50 c.c. 5 % lactose sol., 25 c.c. boiled extract, 2 c.c. toluol	

Then, to each was added 5 c.c. mercuric nitrate solution. Filtered. 65 c.c. filtrate neutralised with 3.0 c.c. 10 % NaOH and filtered. 60 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. and filtered. 20 c.c. filtrate for reductions.

(3) 60 c.c. toluol water extract of mucous membrane of intestines of two newly-born guinea-pigs.

50 c.c. 5 % lactose sol., 25 c.c. extract, 2 c.c. toluol	} Incubated at 36° C.
50 c.c. 5 % lactose sol., 25 c.c. boiled extract, 2 c.c. toluol	

Then, to each was added 5 c.c. mercuric nitrate solution. Filtered. 70 c.c. filtrate neutralised with 3.5 c.c. 10 % NaOH and filtered. 65 c.c. filtrate treated with H<sub>2</sub>S. Excess of H<sub>2</sub>S removed with CuSO<sub>4</sub>. Made up to 250 c.c. and filtered. 20 c.c. filtrate for reductions.

With the exception of the larger animals, all were kept in the laboratory so that their actual diet for the various periods was known. In the case of the animals fed on milk and lactose, the fowls received it made up into a paste with their corn, which was previously ground; they ate the food quite well and put on a great deal of fat. The rabbits received it mixed up with bran or oats, the rats and guinea-pigs were fed with bread soaked in milk to which a little lactose was added. Neither the rabbits nor the guinea-pigs cared for this food, but they ate it quite well in the morning before receiving their other food, which consisted of oats and green leaves.

The following table gives the reduction values obtained with the quantity of glucose corresponding to the average reduction values in

each experiment taken from the figures published by E. Pflüger<sup>(16)</sup>. The percentage of hydrolysis is given in the last column, from which the actual results of the experiments can be readily seen.

Table of Results.

Animal	Time of incubation in days	Grs. $Cu_2O$	=	Mgm. glucose	Control		Percentage of inversion	
					Grs. $Cu_2O$	= Mgm. glucose		
(1) Kitten ...	... 2½	0.4546 0.4524	=	205.1	0.4264 0.4272	=	190.6	17.7
(2) Kitten ...	... 3	0.4858 0.4870	=	223.5	0.4750 0.4738	=	216.9	7.1
(3) Cat ...	... { 3 7	0.1860	=	76.7	0.1700	=	69.7	23.4
		0.2010 0.2024	=	83.6	0.1700	=	69.7	46.5
(4) Dog ...	... 2½	0.1730 0.1724	=	70.9	0.1418 0.1448	=	58.0	51.8
(5) Monkey ...	... 3	0.5962 0.5960	=	253.1	0.4334 0.4352	=	194.3	70.6
(6) Old calf ...	... 4	0.4332 0.4330	=	194.0	0.4186 0.4180	=	186.1	9.9
(7) Sheep ...	... 3	0.4290 0.4286	=	191.7	0.4264 0.4250	=	190.0	2.1
(8) Frog ...	... 4	0.5222 0.5190	=	244.5	0.5210 0.5214	=	244.8	0
(9) Fowl ...	... 2	0.4310 0.4323	=	193.4	0.4326 0.4324	=	193.6	0
(10) Fowl ...	... 3	0.4402 0.4406	=	197.9	0.4336 0.4348	=	194.6	3.9
(11) Fowl, fed 6 weeks with milk and lactose	3	0.4576	=	208.1	0.4558	=	206.5	1.9
		0.4598			0.4560			
		0.4588						
(12) Fowl, fed 9 weeks with milk and lactose	4	0.4694	=	214.4	0.4704	=	214.3	0.1
		0.4704			0.4692			
(13) Fowl, fed 14 weeks with milk and lactose	3	0.4278	=	190.8	0.4242	=	189.1	2.1
		0.4264			0.4236			
(14) Rabbit ..	... 5	0.4742 0.4750	=	217.0	0.4394 0.4386	=	197.2	23.4
(15) Rabbit ...	... 3	0.5136	=	240.0	0.4536	=	205.0	39.8
		0.5132			0.4526			
(16) Rabbit, kept 3 weeks without milk and lactose	3	0.4664	=	212.6	0.4494	=	202.9	11.2
		0.4668			0.4492			
(17) Rabbit, fed 3 weeks with milk and lactose	3	0.5350	=	225.4	0.4522	=	204.5	23.7
		0.5342			0.4530			
(18) Rabbit, kept 15 weeks without milk and lactose	3	0.2834	=	120.3	0.2318	=	97.3	55.1
		0.2848			0.2338			
(19) Rabbit, fed 15 weeks with milk and lactose	3	0.2942	=	124.7	0.2348	=	98.4	62.3
		0.2940			0.2358			
(20) Large intestine of Rabbit (16)	3	0.4564	=	206.7	0.4544	=	205.6	1.3
		0.4562			0.4544			
(21) Large intestine of Rabbit (17)	3	0.4584	=	207.9	0.4550	=	206.1	2.0
		0.4580			0.4552			
(22) Rat, intestines of 6 foetal, 2 days before birth	3	0.2536	=	106.8	0.2545	=	106.9	0
		0.2543			0.2564			
					0.2521			

Table of Results (continued).

Animal	Time of incubation in days	Grs. $\text{Cu}_2\text{O}$	=	Mgm. glucose	Control		Percentage of inversion
					Grs. $\text{Cu}_2\text{O}$	= Mgm. glucose	
(23) Rat, intestines of 9 foetal, 12 hours before birth	3	0.2520 0.2528	=	106.0	0.2310 0.2310	= 96.1	24.0
(24) Rat, small ...	3	0.2674 0.2676	=	112.8	0.2490 0.2488	= 104.5	18.5
(25) Rat, large ♀	2½	0.2406 0.2410	=	100.8	0.2316 0.2324	= 96.9	9.4
(26) Rat, large ♀	3	0.2258 0.2250	=	94.0	0.2214 0.2202	= 92.0	5.1
(27) Rat, large ♂	3	0.2452 0.2436	=	102.4	0.2352 0.2356	= 98.4	9.5
(28) Rat, large ♀, fed 12 weeks with milk and lactose	2	0.2300 0.2280	=	95.6	0.2230 0.2214	= 92.6	7.5
(29) Rat, large ♀, fed 13 weeks with milk and lactose	2½	0.2400 0.2390	=	100.3	0.2334 0.2324	= 97.3	7.2
(30) Rat, large ♀, fed 19 weeks with milk and lactose	3	0.2310 0.2298	=	96.2	0.2240 0.2218	= 92.9	8.3
(31) Rat, large ♀, fed 24 weeks with milk and lactose	3	0.2412 0.2418	=	101.1	0.2408 0.2400	= 100.6	1.2
(32) Guinea-pig, old	4	0.4374 0.4378	=	196.4	0.4354 0.4346	= 195.0	1.7
(33) Guinea-pig, old	3	0.2084 0.2072	=	86.3	0.2048 0.2048	= 85.0	3.6
(34) Guinea-pig, 2 animals, 1 day old	3	0.2738 0.2742	=	115.7	0.2376 0.2382	= 99.6	37.7
(35) Guinea-pig, 3 animals, 3 days old	4½	0.3056 0.3070	=	130.5	0.2234 0.2246	= 93.4	92.7
(36) Guinea-pig, 5 weeks old	3	0.2494 0.2476	=	104.3	0.2466 0.2488	= 103.9	0.9
(37) Guinea-pig, 7 weeks old	3	0.2378 0.2386	=	99.6	0.2344 0.2352	= 98.1	3.6
(38) Guinea-pig, 9 weeks old	3	0.1980 0.1974	=	81.8	0.1972 0.1960	= 81.4	1.1
(39) Guinea-pig, fed 5 weeks with milk and lactose	3	0.2202 0.2222	=	92.2	0.2194 0.2206	= 91.6	1.5
(40) Guinea-pig, fed 7 weeks with milk and lactose	3	0.2086 0.2104	=	87.0	0.2078 0.2094	= 86.6	1.1
(41) Guinea-pig, fed 8 weeks with milk and lactose	3	0.2284 0.2304 0.2280	=	95.6	0.2280 0.2302 0.2284	= 95.5	0.2
(42) Guinea-pig, fed 9 weeks with milk and lactose	3	0.2134 0.2112	=	88.3	0.2128 0.2126	= 88.4	0
(43) Guinea-pig, fed 9 weeks with milk and lactose	3	0.2242 0.2228	=	93.2	0.2228 0.2236	= 93.0	0.5
(44) Guinea-pig, fed 10 weeks with milk and lactose	3	0.2202 0.2208	=	91.9	0.2208 0.2196	= 91.7	0.5
(45) Guinea-pig, fed 11 weeks with milk and lactose	3	0.2094 0.2092	=	86.9	0.2148 0.2136	= 89.1	0
(46) Pig ...	2	0.2124 0.2130	=	88.4	0.1860 0.1856	= 76.6	36.0
(47) Pig ...	3	0.2538 0.2548	=	106.8	0.1868 0.1880	= 77.3	89.1
(48) Pig ...	3	0.2710 0.2722	=	114.6	0.2170 0.2178	= 90.5	62.1

Table of Results (continued).

Animal	Time of incubation in days	Grs. $Cu_2O$	=	Mgm. glucose	Control		Percentage of inversion	
					Grs. $Cu_2O$	=		Mgm. glucose
(49) Pig, filtered through Berkefeld filter	3	0.2516 0.2512	=	105.5	0.2498 0.2492	=	104.7	1.8
(50) Pig, fed 3 months with milk, upper half intestine	3	0.3072 0.3044	=	130.2	0.2220 0.2198	=	92.0	96.9
(51) Pig, fed 3 months with milk, lower half intestine	3	0.3052 0.3074	=	130.4	0.2332 0.2346	=	97.8	77.8
(52) Pig, fed 3 months with milk, upper half intestine, filtered through Berkefeld filter	3	0.2476 0.2474	=	103.8	0.2464 0.2452	=	103.0	1.8
(53) Pig, fed 3 months with milk, lower half intestine, filtered through Berkefeld filter	3	0.2464 0.2464	=	103.3	0.2436 0.2430	=	101.9	3.2
(54) Pig, fed 3 months with milk, pancreas	3	0.2352 0.2372	=	98.8	0.2244 0.2258	=	93.9	12.2
(55) Pig, fed 3 months with milk, pancreas + solution of glucose instead of lactose	3	0.3576 0.3582	=	155.1	0.3530 0.3544	=	152.9	
(56) Pig, pancreas	...	0.2770 0.2788	=	117.9	0.2666 0.2684	=	112.8	10.6
(57) Pig, pancreas, solution of glucose instead of lactose	3	0.1874 0.1870	=	77.3	0.1858 0.1872	=	76.9	

In all the experiments the period of incubation of the extracts of intestine with lactose lasted from 2—4 days, a length of time which is necessary for the animal lactase to exert its influence, especially when only a small quantity is present and particularly when only about 20—30 per cent. inversion occurs. The following experiment, made to determine the rate of action of lactase in the intestine of the cat, shows clearly that this length of time of incubation is necessary. (Weinland incubated his extracts only for a few—about 6—hours.)

150 c.c. 5% lactose solution, 150 c.c. extract, 2 c.c. toluol. After certain intervals of time 25 c.c. were removed and precipitated by 5 c.c.  $Hg(NO_3)_2$ . Filtered. 20 c.c. filtrate neutralised with 2.5 c.c. 10% NaOH and filtered. 15 c.c. filtrate treated with  $H_2S$ . Excess of  $H_2S$  removed with  $CuSO_4$ . Made up to 50 c.c. and filtered. 20 c.c. for reductions.

Time	Grs. $Cu_2O$	=	Mgm. glucose	Percentage of hydrolysis	Time	Grs. $Cu_2O$	=	Mgm. glucose	Percentage of hydrolysis
0	0.1700		69.7	0	72 hours	0.1860		76.7	23.4
6 hours	0.1750		71.9	7.4	96 "	0.1886		77.8	27.1
26 "	0.1788		73.6	13.0	120 "	0.1920		79.3	32.1
46 "	0.1816		74.7	16.7	146 "	0.1932		79.8	33.8
54 "	0.1848		76.1	21.4	170 "	0.2024 0.2010	0.2017	83.6	46.5

Another experiment was made with the intestine of the dog, but here two scrapings of the same length of intestine were made, a surface and a deep scraping, in order to determine, if possible, whether lactase, like enterokinase, is localised in any particular layer of cells. Both were well ground up and made up to the same volume with toluol water (200 c.c.) and filtered; 90 c.c. of each were incubated with 100 c.c. lactose solution, 30 c.c. samples being removed at intervals and treated in the usual manner.

Surface Scraping.				Deep Scraping.			
Time	Grs. Cu <sub>2</sub> O	= Mgm. glucose	Percentage of hydrolysis	Time	Grs. Cu <sub>2</sub> O	= Mgm. glucose	Percentage of hydrolysis
0	0.2310	96.5	0	0	0.2344	98.0	0
16 hours	0.2430	101.8	12.8	16 hours	0.2654	111.8	32.9
24 "	0.2520	106.0	22.9	24 "	0.2714	114.6	39.5
39 "	0.2664	112.4	38.4	39 "	0.2832	119.8	51.9
47 "	0.2714	114.6	43.6	47 "	0.2894	122.6	58.6
64 "	0.2752	116.4	47.9	64 "	—	—	—

These results would seem to show that lactase is localised rather in the deeper than in the surface layers of the mucous membrane, but it must be remembered that the quantity of solid matter is much greater in the former than in the latter and the volume of toluol water used to make up to 200 c.c. is much less. The following experiment, however, shows that more hydrolysis occurs with the surface scraping :

{ 100 c.c. lactose sol., 100 c.c. extract surface scraping, 2 c.c. toluol	} Incubated at 38° C. for 3 days.
{ 100 c.c. lactose sol., 100 c.c. boiled extract surface scraping, 2 c.c. toluol	
{ 100 c.c. lactose sol., 100 c.c. extract deep scraping, 2 c.c. toluol	
{ 100 c.c. lactose sol., 100 c.c. boiled extract deep scraping, 2 c.c. toluol	

The four mixtures were then treated in precisely the same manner with Hg(NO<sub>3</sub>)<sub>2</sub> &c.; and the following figures were obtained on reduction with 20 c.c. solution.

Surface Scraping.				Control		Percentage of hydrolysis
Grs. Cu <sub>2</sub> O	= Mgm. glucose	Grs. Cu <sub>2</sub> O	= Mgm. glucose			
0.5896	} 0.5908	250.6	0.4150	} 0.4161	185.0	82.7
0.5922		0.4172				
Deep Scraping.						
0.5754	} 0.5763	244.0	0.4172	} 0.4177	185.8	73.1
0.5772		0.4182				

The conclusion is from these experiments that lactase is distributed fairly equally through the whole of the mucous membrane of the intestine.

It has been shown above that neither the frog nor the fowl have lactase in their intestines, and we may conclude that animals lower than mammals do not possess this ferment. Of the mammals, the carnivora and omnivora have lactase present during the whole of their lives, but the herbivora only when they are young, with the exception of the rabbit; my result in this instance differs from that of Weinland but confirms Portier's. With regard to the pig, neither Portier nor Mendel could find lactase in its intestine, whereas it was found by Weinland whose results are confirmed by my own. Further, I have been able to confirm Bierry and Gmo-Salazar's observation that the ferment does not pass through a Berkefeld filter and is therefore localised in the cells of the mucous membrane of the intestine; hence the necessity of using extracts such as I have employed. In the cases of the dog, sheep, monkey, calf, pig, only a portion of the mucous membrane of the intestine was used, as the quantity in these animals was too great for convenient manipulation, in all other cases the whole was employed.

As one was able to employ the whole of the intestines of the smaller animals, namely the rat, guinea-pig, and rabbit, a real control was possible for determining whether adaptation had occurred. With the guinea-pig, where the lactase disappears entirely in about 5 weeks after birth, the result that adaptation does not occur is very obvious, the amount of hydrolysis which actually took place, from 0·2—1·5 per cent., being without doubt due to experimental error. In the rabbit and the rat a certain quantity of lactase is always present, but this quantity—from the amount of hydrolysis—did not increase when they were fed on milk and lactose, and we have thus further evidence that adaptation does not occur. As before mentioned, this evidence could only be obtained by employing the whole of the intestine of the animal in every case, so that a comparison was possible. If adaptation occurred, the percentage of hydrolysis should increase instead of remaining the same as when the animal is not fed with milk, and it never increased to the amount given by the young animal which might at any rate be expected. Thus, a young rat showed 18·5 per cent. hydrolysis and adult rats, fed for various periods, showed 7·2—8·3 per cent. hydrolysis, the same as that given by adult rats which had not been fed on milk.

The results with the fowl are confirmatory of those with the guinea-pig, rat and rabbit, but the fowl, never having had lactase in its intestine would not be so likely to show any adaptation as would mammals. If, however, adaptation occurred it would be less extraordinary in mammals than in the fowl, and adaptation in the fowl would be the most conclu-

sive evidence that adaptation could occur at all, and would bring down Darwin's environment theory to too narrow an issue, when such processes require indefinitely long periods of time. In the experiments on this subject the animals which permanently have lactase are those which are highest in the scale, namely man, monkey, the carnivora and omnivora, &c. The more functions an animal can perform, the higher it must be in the scale of organised beings. It does not seem impossible therefore that adaptation could be experimentally attained by prolonging the stimulus for very much longer periods, *i.e.* through many generations of animals instead of on one animal.

It might be thought that the amount of stimulus given to the smaller animals by feeding was insufficient, as the guinea-pigs and rabbits did not care about bread and milk diet. Through the kindness of Drs C. J. Martin and Dean, to whom I should like to express my thanks, an adult pig was kept for me at the Lister Institute and fed for 3 months on 4 gallons (= about 20 litres) of milk per day. This experiment was commenced before I had examined the intestine of a pig for lactase, the previous observers, Portier and Mendel, having been unable to detect the presence of this enzyme, whereas Weinland found it and I could not accept his results. In the meanwhile I examined other pigs' intestines for lactase and found it present, so that the above experiment was really of no use for determining whether adaptation of the intestine occurred. The experiment, however, gave me the opportunity of again investigating whether adaptation of the pancreas occurred. It showed 12.2 per cent. hydrolysis. Nearly the same amount of hydrolysis, 10.6 per cent., was given by the pancreas of an ordinary pig not fed on milk, and hence it may be concluded that adaptation does not occur even with this great stimulus. The roughly 10 per cent. hydrolysis in each case is due to contamination from the intestine, since the animals had to be killed in the slaughter-house where it is impossible to prevent contamination when dealing with such large masses of material, and where other animals have previously been killed. The hydrolysis would have been much greater if it were really due to the pancreas itself, which weighed about 150 gms. It was thought that the increase in the amount of reducing sugar might be due to reducing sugar from the pancreas itself, but experiments carried out in exactly the same manner with the pancreas alone and with a solution of glucose, instead of lactose, showed no difference in the amount of reducing sugar after the period of incubation.

It may therefore be concluded that neither the pancreas nor the

intestines of animals can be made to adapt themselves to any particular diet.

In addition to these results I have been able to determine at what period in the life-history of the rat lactase first appears. Embryos about 2 days before birth had no lactase in their alimentary canal, whereas about 12 hours before birth lactase had appeared.

The guinea-pig, as the figures show, already loses its lactase in 5 weeks after birth, and it would be interesting to know whether by continuous milk-feeding from birth, the enzyme could be kept from disappearing. Experiments in this direction are being carried out and some also in order to determine how soon before birth the enzyme appears.

Throughout these experiments I have been greatly helped by Miss Lane-Claypon, to whom I owe my thanks.

In conclusion, I wish to acknowledge my indebtedness to the Government Grant Committee of the Royal Society for a grant which has defrayed the expenses of this research.

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**Nitrogenous metabolism in normal individuals.** By J. M. HAMILL and S. B. SCHRYVER.

In view of the recent experiments by Chittenden, who has shown that it is possible to maintain nitrogenous equilibrium on diets containing relatively small amounts of protein, it was of interest to investigate the nitrogenous metabolism of normal individuals, living in their ordinary way and performing their normal avocations. The subjects of the experiments were seven workers in the laboratory of University College. The total nitrogen and uric acid were determined in 24 hour urines over a period of six days. The results are given in the following tables.

Subject	Day	Quantity of urine c.c.	Sp. gr.	Total N gm.	Uric acid gm.	Uric acid N gm.	$\frac{\text{Uric acid N}}{\text{Total N}} \times 100$
H.	1	1650	1030	20.8	1.02	0.340	1.6
	2	1700	1020	12.8	0.78	0.260	2.0
	3	1750	1018	17.8	0.77	0.256	1.5
	4	1250	1022	16.3	0.72	0.240	1.5
	5	1450	1022	17.4	0.73	0.243	1.4
	6	950	1020	13.8	0.64	0.213	1.5
P.	*1	1100	1019	9.0	0.35	0.116	1.3
	2	1540	1019	16.2	0.76	0.253	1.5
	3	1720	1018	16.5	0.79	0.263	1.6
	4	2200	1013	16.3	0.81	0.270	1.6
	5	1350	1021	14.6	0.83	0.273	1.9
	6	1900	1016	15.9	0.80	0.266	1.7
E.S.	1	1760	—	11.9	0.59	0.196	1.6
	2	1890	1013	9.8	0.50	0.166	1.7
	3	1840	1015	9.1	0.42	0.140	1.5
	4	2020	1013	9.4	0.38	0.126	1.4
	5	1660	1016	8.5	0.43	0.143	1.7
	6	1680	1015	8.9	0.37	0.123	1.4
S.S.	1	1100	1030	18.4	0.80	0.266	1.4
	2	1050	1028	15.3	0.67	0.223	1.4
	3	1520	1022	18.1	0.89	0.296	1.6
	4	1260	1020	16.5	0.82	0.273	1.6
	5	1170	1026	15.8	0.77	0.256	1.6
	6	1140	1027	15.3	0.64	0.213	1.4

\* P. 1. is incorrect since all of the urine was not collected on that day. All the first day values are somewhat high owing to a heavy meal being taken before the beginning of the experiment.

Subject	Day	Quantity of urine c.c.	Sp. gr.	Total N gm.	Uric acid gm.	Uric acid N gm.	$\frac{\text{Uric acid N}}{\text{Total N}} \times 100$
F.S.	1	2100	1015	15.8	0.72	0.240	1.5
	2	1500	1017	13.2	0.55	0.183	1.4
	3	1350	1015	12.2	0.55	0.183	1.5
	4	1480	1016	13.7	0.57	0.190	1.4
	5	1160	1016	10.8	0.46	0.153	1.5
	6	1320	1016	12.3	0.55	0.183	1.5

Subject	Day	Quantity of urine c.c.	Total N gm.	Uric acid gm.	Uric acid N gm.	$\frac{\text{Uric acid N}}{\text{Total N}} \times 100$
C.	1	900	13.9	0.64	0.213	1.6
	2	1150	14.8	0.55	0.186	1.3
	3	1290	12.7	0.65	0.216	1.7
	4	2100	15.0	0.66	0.220	1.4
	5	1670	11.2	0.56	0.186	1.6
	6	960	10.0	0.46	0.153	1.5
B.	1	1140	11.17	0.381	0.127	1.1
	2	750	11.0	0.582	0.194	1.8
	3	1410	14.7	0.616	0.205	1.4
	4	920	10.8	0.638	0.209	1.9
	5	900	10.7	0.582	0.194	1.8
	6	1140	10.4	0.455	0.151	1.4

Average per day for 6 days is given in the following table.

Subject	Weight (kilos)	Total N	Uric acid N	$\frac{\text{Uric acid N}}{\text{Total N}} \times 100$
E.S.	60	9.6	0.149	1.55
C.	78	12.9	0.195	1.51
F.S.	74	13.0	0.189	1.46
H.	94	16.5	0.258	1.56
S.S.	68	16.5	0.254	1.54
P.	72	14.7	0.240	1.63
B.	61	11.4	0.180	1.57

Average 1.54

Average per individual 13.5 gr. N excreted per day.

Allowing for a loss of 10% N in faeces, this is equivalent to 93 grams of protein per day.

There are several points of interest which are demonstrated by these tables. The first of these is the constancy of the ratio of the uric acid nitrogen to the total nitrogen. In five cases out of seven, the deviation from the average of 1.54% is well within the limits of experimental error; in the other two cases the deviation does not exceed 0.09%.

Another point of interest is the relatively low total nitrogen excreted. This is a point of considerable importance from the sociological standpoint. The following numbers are taken from Rowntree's *Poverty, a study in town life*, a study of social conditions in the town of York.

Social condition	Amount of protein used daily <sup>1</sup>
Working class families of total weekly earnings under 26s.	89 grams
"                    "                    "                    over 26s.	119 "
Servant keeping classes . . . . .	126 "
Workhouses (including York) . . . . .	136 "
Prisons. Class B . . . . .	134 "
Convicts at hard labour . . . . .	177 "
Atwater's standard . . . . .	125 "
Average from above physiological laboratory experiment	93 "

<sup>1</sup> Calculated per adult man.

Rowntree has adopted Atwater's standard and has concluded that 27 % of the population of the city of York are living in poverty partly on the ground that their protein diet falls below the Atwater standard. Although it cannot be claimed that the numbers obtained by us by urine analyses are strictly comparable to those given in the above table<sup>1</sup> (which refer to bought food), yet the differences are so great, and the actual nitrogen metabolism falls so far below the Atwater standard, that great care must be taken in drawing conclusions as to the sociological conditions from the amount of protein consumed. It is hoped that we shall be able to extend this investigation in other directions.

<sup>1</sup> It is also probable that the poorer classes take relatively larger quantities of vegetable protein, in which cases the loss of nitrogen in the faeces is greater than 10 %, the amount allowed for in our laboratory experiments.



# STUDIES IN THE CHEMICAL DYNAMICS OF ANIMAL NUTRITION

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## PART I

### INTRODUCTION. PROBLEMS RELATING TO THE FUNCTION OF ALBUMINOUS FOOD-STUFFS IN THE GENERAL ECONOMY OF NUTRITION

In spite of the fact that metabolism occupies a very important place in the literature of modern physiology, but little is known with certainty of the fate of the products of tryptic digestion of the albumens after leaving the alimentary tract. Various theories have been propounded to explain the rôle of the nitrogenous food-stuffs in the general economy of nutrition, of which that suggested originally by Liebig and modified by Pflüger has been, perhaps, the dominant. According to this theory the products of digestion of the albumens are incorporated into the protoplasm molecules of the living cells, and there in a readily oxidisable form, held available for the supply of the energy needs of the organism.

In quite recent years however, another, and almost diametrically opposed hypothesis, has been regarded with much favour, and has found an admirable exponent, amongst others, in the person of Speck.<sup>1</sup> According to this hypothesis, the nitrogen is rapidly eliminated from the products of digestion of the albumens, and excreted in the form of urea, whilst the carbohydrate part of the molecule forms, on oxidation, the main source of the energy supply of the organism. The tissues themselves undergo degradation only under exceptional circumstances, as, for example, when the food supply is insufficient for the energy needs, or when the conditions of metabolism are abnormal, as is the case during fever, or when the oxygen supply is insufficient, or after phosphorus poisoning. These abnormal conditions will be discussed again later. According to Speck, therefore, we must distinguish between the metabolism of energy and the metabolism of tissue, and as the main source of energy is derived from the oxidation of the non-nitrogenous part of the molecule, the albumens should play a comparatively subordinate rôle in the general economy of nutrition. Some support is given to this conception by the recent researches of Sivén<sup>2</sup> and of Chittenden,<sup>3</sup> both of whom find that equilibrium can be maintained on diets that contain very much less nitrogen than was supposed to be necessary by the earlier investigators.

An important new view of the matter has been advanced recently by Folin,<sup>4</sup> which does not differ very materially from that advocated by Speck. Folin has shown that the change from a nitrogen-rich to a nitrogen-poor diet is followed by marked changes in the composition of the urine. Not only does the urea output diminish in actual quantity; its relative quantity in comparison with the other nitrogenous constituents of the urine also diminishes. On the other hand, the creatinine output remains always constant, whether the diet be nitrogen-rich or nitrogen-poor. The neutral sulphur of the urine maintains a similar constancy. From these facts Folin concludes that metabolism can be considered under two heads, viz. :—as *endogenous* metabolism, which is due to a constant tissue waste (and is analogous, therefore, to the tissue metabolism of Speck), which is responsible for the constituents of the urine (creatinine and neutral sulphur) which do not

vary with the diet, and *exogenous* metabolism, which is responsible for the greater part of the urea, and other varying constituents. According to Folin then, the only nitrogenous matter necessary for the maintenance of equilibrium is that required to supply the waste represented by the endogenous metabolism. Any superfluous nitrogen is eliminated as rapidly as possible in the form of urea.

We have, therefore, widely different views as to the function of the nitrogenous constituents of the food-stuffs; on the one hand we have the view that the products of digestion are built up into the tissues, and that, relatively, large amounts of albumen are necessary to maintain the organism in full bodily vigour; on the other hand we have the view that only small quantities are necessary—just the amount that is required for the repair of the tissue waste which is continually taking place, and that the main part of the bodily energy is derivable from the non-nitrogenous constituents of foods.

Which of these views, if either, is correct? What is the true function of nitrogen in the economy of nutrition? With the object of obtaining an answer to these questions, the following researches were undertaken.

#### METHODS OF RESEARCH

Our ignorance is to be largely ascribed to the paucity of our methods of research. Most of the older theories are based on experiments which had for their object the determination of the balance between ingesta and egesta, either by the analysis of inspired and expired air (Zuntz, Speck, etc.), or by the estimation of nitrogen in food and excreta (Pflüger, Voit, etc.), or by the estimation of caloric values (Rubner, etc.), or by a combination of all these methods of experiment (Atwater).

The systematic determination of the distribution of nitrogen amongst the various nitrogenous constituents of urine by the method of Folin already referred to marks a distinct advance in the study of metabolism.

An attempt has been made in the following researches to throw some light on the mechanism of nutrition by an entirely different method. It was assumed that if the products of albumen degradation

be built up into the bioplasm, and held there in a form in which they could be readily eliminated for the supply of the energy needs of the organism, as demanded by the hypothesis of Pflüger, it should be possible to detect variations in the *immediate* post-mortem changes in the tissues, according to whether such tissues are derived from animals during digestion, or during a period of fasting. It was assumed that tissue of an animal with nitrogenous matter stored ready for elimination for the supply of energy needs, should show a more rapid post-mortem change than tissues derived from an animal during a period of fasting.

With the exception of the small intestine, which will be discussed in detail below, it was found, in the researches carried out in conjunction with Miss J. E. Lane-Clayton,<sup>5</sup> that but small change took place, as far as the nitrogen at any rate was concerned, during the first four hours of incubation with water; but that, in the case of the liver, after this so-called 'latent period,' a rapid degradation of the albumen set in (lasting generally from 4-6 hours), the rate of which depended on the state of nutrition of the animal at the time of death. This autolysis was more rapid in the case of the fasting than of the fed animal—the reverse of what might be expected if the hypothesis of Pflüger be correct. From the results obtained it appeared that the autolytic enzyme functioned by acting when the energy needs of metabolism were not satisfied by the food-stuff ingested. The results also furthered the hope that this method of research might be available as a general method for the study of metabolism. It was tested<sup>6</sup> by being applied to a special case of abnormal metabolism, viz., when animals are fed with thyroid glands, and results parallel in every respect with those got by other methods were obtained.

What is the mechanism now by means of which this autolytic enzyme acts? Does it exist in the tissue as a zymogen, from which the enzyme is liberated by the means of a kinase-like body, as in the case of trypsinogen, or is it always present in the tissue, but is prevented from acting during periods of full nutrition by the presence of inhibitory bodies derived from metabolites, which gradually disappear after ingestion of food?

The latter of these hypotheses seemed the more likely, and in the second paper on the subject of autolysis, it was suggested that the tissue stability of the liver was the resultant of the mass action of three sets of bodies, viz., the tissue itself, the metabolites, or bodies derived from metabolites, and the autolytic enzyme.

The following immediate questions then arose :—(i) In what way do the metabolites act? Do the products of tryptic digestion themselves inhibit the action of the autolytic enzyme, or is it the products derived therefrom which exert this inhibitory action? (ii) If such metabolites, or bodies derived therefrom, exert an action in the manner suggested, are they continually circulating in the blood-stream, or are they taken up in the tissues and acting intracellularly?

To obtain an answer to question (i) the influence of glucose, and of the products of tryptic digestion of caseine on the rate of autolysis of the liver were determined. For an answer to question (ii) the relative inhibitory action of sera from fed and fasting animals on autolysis was investigated. As a result of these experiments it was found that glucose exerted no action on the rate of autolysis, whereas the products of tryptic digestion exerted a marked inhibitory effect.

In the experiments on the action of sera, it was found that in one series no difference could be detected between the influence exerted by the serum of a fasting animal and that of a fed animal; in a second series, however, the inhibitory action of the serum of a fed animal was slightly greater. In this second series, the serum contained a larger amount of uncoagulable nitrogenous bodies. All experiments were carried out with sera diluted with a triple volume of normal saline.

As the result of the above-mentioned preliminary experiments, the question as to whether the products of tryptic digestion circulate in the organism acquires additional importance. That they inhibit autolysis there is little doubt: on the other hand experiments carried out with sera do not conclusively prove that the serum of a fed animal contains these products in quantity sufficient to shew a greater inhibitory power than the serum of a fasting animal.

A series of researches was, therefore, undertaken with the object of investigating the distribution of nitrogen in the tissues in the different stages of nutrition, and of determining the ratio of the nitrogen of the coagulable albumen to the total nitrogen. The difference between the total nitrogen and the nitrogen of the coagulable albumen has, throughout these researches, been termed 'residual nitrogen.' (Ger. Reststickstoff). This expression is more convenient than the older term 'extractives nitrogen,' for, as will be seen in the sequel, the latter, in part at least, appear to form an essential part of the tissue.

The problem of the residual nitrogen is no new one, and has been attacked by many investigators who have obtained varying results; their work will be referred to when discussing the experimental part of these researches. The contradictory results obtained are due, without doubt, to the faulty technique employed; for this reason a comparative investigation has been made of the value of two or three different methods: as a result a new method has been employed, which obviates the fallacies of the other methods. These researches are described in Part II.

Part III deals with the application of the method to the examination of serum, liver, and small intestine. As a result it was found that the tissues of fed animals do not contain more residual nitrogen than those of fasting animals. Other interesting results were obtained, the discussion of which is deferred to Part VI.

As it is obvious from the results obtained in Part III, that the products of tryptic digestion do not themselves directly act *in vivo* in inhibiting autolysis, a further search was made for agencies likely to bring about this result. These researches, with all the others bearing on autolysis, are described in Part V. It was found, finally, that acids cause autolysis of the liver without any previous latent period, whilst alkalis have a marked inhibitory action. Now it is known that ammonia is formed from the products of tryptic digestion in the alimentary tract, and stored up in the liver and other tissues. The quantitative relations of the amounts to the quantity of acid necessary to produce the maximum rate of autolysis have been

investigated. As a result of these researches, certain conclusions have been drawn as to the general chemical mechanism of nutrition, and as to the rôle played by the nitrogenous food-stuffs (Part VI).

Another problem, also bearing directly on the general chemical dynamics of nutrition has been investigated, and certain preliminary results obtained. Hofmeister has stated his belief that coagulable albumens can be synthesized from peptones or albumoses in the mucous membrane of the stomach. Glaessner, working in Hofmeister's laboratory, obtained experimental results tending to confirm this hypothesis. As, for reasons discussed in Part II, there are certain objections to the experimental method employed, and as, furthermore by the analytical processes used in this work, the coagulable albumen can be directly estimated, the researches of Hofmeister and Glaessner have been repeated. The experiments are described in Part IV. They do not confirm the results of Hofmeister and Glaessner.

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## PART II

## ON THE METHOD FOR ESTIMATING RESIDUAL NITROGEN IN SERUM AND TISSUES

Attention has often been called to the difficulty of precipitating albuminous bodies without at the same time removing from solution substances which are otherwise easily soluble in water. This subject has been investigated recently by Haslam, who has demonstrated certain fallacies in the methods devised for the separation of albumoses by fractional precipitation with salts. S. N. Pinkus (private communication) has obtained similar results.

In the previous work on autolysis in the papers already quoted, the nitrogen of the non-coagulable bodies has been estimated in the

filtrate after precipitating the albumen with trichloroacetic acid (added when the mixture is boiling). As the experiments have throughout been comparative, and carried out under as nearly as possible like conditions, the method has been satisfactory enough.

In the determination of the residual nitrogen in tissues, it was not always possible to carry out the experiments under perfectly identical conditions; it was necessary, furthermore, to determine the absolute quantities of residual nitrogen in each case. Preliminary experiments were therefore undertaken to test the reliability of older methods, and to determine how much the results varied under different conditions. For this purpose a mixture of a solution of Witte's peptone and serum was made, and this mixture was diluted with varying quantities of water; it was found that the amount of residual nitrogen obtained after precipitating with tannic acid (in the cold) or with trichloroacetic acid (boiling), varied quite appreciably with the strength of the solution in which precipitation took place; it was found that more residual nitrogen was obtained in the filtrate when the precipitants were added to a dilute than when added to a concentrated solution. Tannic acid precipitates of course a certain part of the peptone; trichloroacetic acid, on the other hand, precipitates only a minute quantity if added to a sufficiently dilute boiling solution. On the other hand, trichloroacetic acid, when added to the solution of the peptone-serum mixture above a certain dilution, produces a precipitate in such a finely divided state that filtration through filter paper is almost impossible. As a general result it was found that the trichloroacetic acid precipitation method gives fairly accurate results when the strength of the solution was maintained within certain definite limits of concentration.

It was important, therefore, to have a method available which would allow the coagulation to be carried out under perfectly constant conditions, and at the same time give the residual nitrogen in absolute numbers.

For such a method I am indebted to the suggestion of S. N. Pinkus. He has shown that if anhydrous sulphate of soda be added to solutions of albumens or to tissues, the water is abstracted from the same with a very slightly increase of temperature, and without any appreciable

alteration of the albumen. In this way the albumen solution or tissue can be dried (at a temperature not exceeding  $38^{\circ}$ ) and kept without alteration for a considerable time. The tissue dried in this way can be coagulated, and the nitrogen of the coagulum directly estimated. The difference between the number thus obtained and the number obtained by estimating the total nitrogen in the albumen solution or tissue represents the residual nitrogen.

The method has been carried out (in the case of serum) in the following way:—A measured quantity of serum is poured into a flat-bottomed, glazed, porcelain dish. An equal weight (roughly) of anhydrous sulphate of soda is then added. The mixture is then allowed to stand for some time. The anhydrous sulphate of soda is converted into the crystalline hydrated form, and the water is thus abstracted from the serum. The dried mass can be readily removed from the flat-bottomed dish by a knife (this is the reason for using a flat-bottomed dish instead of an evaporating basin) and powdered in a mortar. When finely powdered it is transferred to the Kjehldahl flask in which the incineration with sulphuric acid is carried out (a 700 c.c. round-bottomed Jena flask was always used for this purpose). Any small amount of the powder remaining in the porcelain dish or mortar can be removed by rubbing the vessels with small fresh quantities of anhydrous sulphate of soda, which is then added to the main quantity. The albumen is then coagulated by boiling the mixture in the Kjehldahl flask with absolute alcohol (100 c.c. alcohol for 15 c.c. serum, or 50 c.c. alcohol for 5 gr. tissue) for half-an-hour with reflux condensor. The alcohol is then decanted off on to a filter. Water is then added to the powder remaining in the flask (300-400 c.c.) and the mixture is then heated on a water bath. By this means the sulphate of soda and non-coagulable bodies are dissolved up and the coagulum remains suspended in the solution in the form of a fine powder. The water is decanted off through the same filter as that used for the alcohol, and the greater part of the coagulum remains behind in the Kjehldahl flask. This is again heated with water on a water bath, and the hot water is again decanted off through the filter. When the washings are free from sulphate of soda and have run through the

filter the filter paper containing small quantities of the coagulum is transferred to the Kjehldahl flask. Sulphuric acid is then added, the mixture incinerated and the nitrogen determined in the usual way.

In the experiments with serum described in Part III, 15 c.c. were always used for each experiment. The total nitrogen was estimated in this quantity by incinerating with 30 c.c. concentrated sulphuric acid; a filter paper was added to the mixture, so that the total coagulable albumen nitrogen was always determined under the same conditions. Another 15 c.c. were used for the determination of the coagulable nitrogen in the way just described.

For the examination of the tissues 5 grams of material were used for each determination. The finely divided tissue was weighed on a photographic balance (accurate to about 0.02 of a gram). It was then transferred to a flat-bottomed porcelain dish with a knife, and mixed up by means of the knife with a little more than an equal weight of the sulphate of soda. The glass pan of the balance was carefully cleansed by rubbing up with fresh quantities of sulphate of soda, and the mixture kept until wanted. The mixture was then powdered in a mortar, coagulated with alcohol, washed, and the nitrogen of the coagulum determined in the way already described for serum. The total nitrogen was determined in 5 grams of fresh tissue. Thirty c.c. concentrated sulphuric acid were employed for incineration in each case. A filter paper was added in the determination of the total nitrogen as in the case of the serum.

When working with solutions of albumens the same method can be employed. A little more sulphate of soda is added than the weight of water present.

The following series of experiments illustrate the foregoing remarks. In the last series the coagulable albumen is determined in a serum; the same serum is mixed with a concentrated solution of Witte's peptone, and the coagulable albumen determined in the mixture. These experiments were carried out very rapidly, and although the coagulum was washed only twice by decantation, it will be observed that only a minute quantity of the peptone nitrogen has been carried down. Far larger quantities of water-soluble bodies were present in this case than are ever met with in tissues.

## EXPERIMENTS

Determination of non-precipitable nitrogen in a mixture of sheep's serum and 10 per cent. solution of Witte's peptone after precipitating with tannic acid (Hedin's solution). The nitrogen estimated in an aliquot portion of the filtrate.

## Series I

	Serum c.c.	Water	Strength of serum solution %	Peptone solution c.c.	Hedin's solution c.c.	N in filtrate $\frac{N}{10} \text{H}^2\text{SO}^4$ required
<i>a</i>	25	25	50	5	25	20.1
<i>b</i>	25	75	25	5	25	20.2
<i>c</i>	25	175	$12\frac{1}{2}$	5	25	22.8
<i>d</i>	25	375	$6\frac{1}{4}$	5	25	24.8
<i>e</i>	25	75	25	5	25	21.1

In experiments *a*, *b*, *c*, *d*, the peptone solution was added to the diluted serum before adding the tannic acid. In (*e*) tannic acid was added to the serum before the peptone solution. These experiments shew that the amount of residual nitrogen obtained varies with the concentration of the solution in which the precipitation is carried out.

*Series II.* Same as I, with another serum, and another 10 per cent. peptone solution. The total N is represented by the following numbers :—(*a*) 24.0, (*b*) 26.0, (*c*) 27.9, (*d*) 28.6.

*Series III.* With trichloroacetic acid precipitation.

(*a*) 25 c.c. serum + 25 c.c. water + 5 c.c. peptone solution.

The mixture was then boiled. 25 c.c. of 10 per cent. trichloroacetic acid were then added to the mixture whilst still hot. Filtrate was washed with hot water; it was then removed from paper and washed again with hot water. Nitrogen estimated in total washings.

Result :

$$N = 49.0 \text{ c.c. } \frac{N}{10} \text{H}^2\text{SO}^4$$

(*b*) 25 c.c. serum + 75 c.c. water + 5 c.c. peptone solution.

Boiled. 25 c.c. trichloroacetic acid solution added.

Manipulation as in (*a*).

Result :

$$N = 51.8 \text{ c.c. } \frac{N}{10} \text{H}^2\text{SO}^4$$

Here again variation with dilution. Greater dilution impracticable owing to difficulty of filtration.

*Series IV.* Sodium sulphate method. Same serum and peptone solution as in *Series III*.

25 c.c. serum added to 5 c.c. peptone solution.

$$\text{Total N} = 490 \text{ c.c.} \frac{N}{10} \text{H}^2\text{SO}^4.$$

$$\text{Coagulable N, (Expt. I)} = 435.7 \frac{N}{10} \text{H}^2\text{SO}^4.$$

$$\text{(Expt. II)} = 434.7 \frac{N}{10} \text{H}^2\text{SO}^4.$$

$$\text{Residual N} = (490.0 - 435.2) \frac{N}{10} \text{H}^2\text{SO}^4 = 54.8 \frac{N}{10} \text{H}^2\text{SO}^4.$$

$$\text{N in peptone solution alone} = 47.4 \frac{N}{10} \text{H}^2\text{SO}^4.$$

Hence residual N in serum =  $7.1 \text{ c.c.} \frac{N}{10} \text{H}^2\text{SO}^4$ ; a number which subsequent experiments shows to be a trifle low.

*Series V.* Sodium sulphate method. Determination of N in coagulum.

$$15 \text{ c.c. of serum. Coagulable N} = 116.2 \frac{N}{10} \text{H}^2\text{SO}^4.$$

15 c.c. of serum + 10 c.c. 10 per cent. Witte's peptone.

$$\text{Coagulable N} = 118.5 \frac{N}{10} \text{H}^2\text{SO}^4.$$

Here, in spite of large amount of residual N from the peptone, very little is carried down with the coagulum.

The method gives, therefore, accurate results, and can be made independent of the concentration of a solution, as a dried powder is always used for coagulation.

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### PART III

#### THE DISTRIBUTION OF NITROGEN IN THE SERUM, LIVER, AND MUCOUS MEMBRANE OF THE SMALL INTESTINE DURING DIGESTION OF FOOD AND DURING A FAST

The method of research has been already described.

The liver was put through a fine mincing machine before weighing.

The intestine was cut out from below the pylorus to just above the ileo-coecal valve. It was opened up longitudinally and nailed down on a wooden board. The surface of the mucous membrane was carefully cleansed with cotton wool, and the mucous membrane was scraped off with the blunt edge of a knife. Scrapings were made alternately from the cardiac and the coecal end, and put into a tube and thoroughly mixed with a knife. The tube was stoppered until it was required for weighing out. The weighings were all made as soon as possible after killing the animals.

For the determination of total nitrogen the tissue was mixed with sulphuric acid in a Kjehldahl flask immediately after weighing. The portions of tissue required for estimation of the nitrogen of the coagulable albumen were mixed with sulphate of soda immediately after weighing. This mixture could be kept till wanted.

The animals used were always cats, except when otherwise stated. They were fully anaesthetized by A.C.E. mixture, and bled to death by opening the carotid artery when under the influence of the anaesthetic.

The blood was collected in a tall cylinder and allowed to clot. The serum was then poured off into a narrower cylinder, and the corpuscles, which were decanted off with the serum, allowed to settle. The required quantities of serum were pipetted directly, either into the Kjehldahl flask or into the flat-bottomed dish, according to whether the total N or the N of the coagulum was to be estimated.

Various morphological details were noted, such as weight, weight of liver, length of intestine, etc. In order to know the concentration of residual nitrogenous bodies, the total solids were estimated. In the case of serum this was done by heating about 2 c.c. in porcelain crucibles, first at about 60° and afterwards in a Victor Meyer bath jacketed with toluol at 107° C. The mucous membrane and liver were treated in the same way; instead of using a crucible, however, they were dried after spreading out as much as possible on a watch glass. In this case the ordinary paired watch glasses were used. Heating was continued to constant weight. The fed animals were killed about five hours after the last meal. The fasting animals were killed when the stomach was empty. The animals were killed in pairs, a fed and fasting animal

being killed on the same morning or the same afternoon. This was to obviate any difference that might be caused by differences of hydramic state of the tissues due to differences of temperature. The general results are given in the accompanying table (Table I). The following abbreviations are employed:—

F = animal fasting.

D = animal killed during active digestion.

N = total nitrogen, expressed in c.c.  $\frac{N}{10}$  H<sup>2</sup>SO<sup>4</sup> necessary for neutralization of NH<sup>3</sup>

R = residual nitrogen           "           "           "           "           "

N - R = nitrogen of coagulable albumen.

The results obtained are summarized below.

TABLE I\*

No.	Nutrition	Weight Kilos	SERUM			LIVER				SMALL INTESTINE					
			N	N-R	Solids %	Weight	N	N-R	Solids %	Length	Weight Mucous Membrane	N	N-R	Solids %	
1	F	1'95	127'0	115'0	8'7	...	...	...	...	...	...	...	...	...	...
2	F	2'85	126'0	116'0	8'3	...	...	...	...	...	...	...	...	...	...
3	D	2'60	131'6	122'0	9'0	..	...	...	...	...	...	...	...	...	...
4	D	3'10	125'5	112'2	8'8	...	...	...	...	...	...	...	...	...	...
5	F	3'20	130'0	118'0	8'4	...	...	...	...	...	...	...	...	...	...
6	F	3'15	124'0	116'0	8'3	...	...	...	...	...	...	...	...	...	...
7	D	3'50	149'0	135'0	9'9	...	...	...	...	...	...	...	...	...	...
8	D	3'45	108'0	99'0	7'4	...	...	...	...	...	...	...	...	...	...
9	F	2'85	135'6	124'0	9'2	45'9	144'0	128'2	31'6	...	...	...	...	...	...
10	F	3'30	136'5	119'4	9'0	54'1	145'5	127'7	33'3	...	...	...	...	...	...
11	D	2'90	121'7	109'0	8'4	63'6	130'0	118'2	30'3	...	...	...	...	...	...
12	D	2'25	125'5	117'2	8'8	61'3	122'5	107'8	29'1	...	...	...	...	...	...
13	F	3'25	130'4	117'7	9'3	54'8	126'7	107'5	31'0	...	...	...	...	...	...
14	F	3'60	131'3	127'0	9'6	58'4	131'0	114'5	30'2	...	...	...	...	...	...
15	D	4'35	125'6	114'0	8'8	98'6	97'5	79'8	37'7	...	...	...	...	...	...
16	D	4'40	143'6	135'2	9'8	109'8	103'5	94'9	34'3	...	...	...	...	...	...
17	F	2'60	131'7	113'3	9'1	109'2	99'0	82'0	47'6	4'6"	11'6	111'0	71'3	26'2	...
18	D	3'50	133'3	120'0	9'4	80'0	109'5	lost	33'4	4'0"	11'7	109'0	71'5	26'1	...
19	F	3'00	122'8	111'0	8'4	64'4	107'0	86'4	40'1	5'6"	11'2	117	lost	26'1	...
20	D	2'90	128'2	110'0	8'7	90'3	117'5	101'0(?)	31'2	6'0"	13'6	109'0	74'0	27'3	...
21	F	3'30	...	...	...	65'7	110'0	96'3	34'1	4'4"	16'0	112'0	80'5	25'9	...
22	D	3'30	...	...	...	84'3	102'7	87'2	27'7	4'10"	15'1	lost	68'5	23'9	...
23	F	2'85	...	...	...	56'7	124'0	104'2	30'3	...	14'6	111'0	72'0	23'9	...
24	D	29'0	...	...	...	79'3	123'1	107'3	28'9	5'0"	16'6	110'0	73'6	25'3	...

\* The nitrogen determinations refer to 15 c.c. of serum, and 5 grams of liver or of mucous membrane of intestine.

*Serum.* With reference to the influence of nutrition on the composition of the serum there are numerous researches, of which the most recent are those of von Bergmann and Langstein.<sup>1</sup> The question as to whether the products of tryptic digestion can be detected in the serum has, in spite of the number of works on this subject, been by no means definitely settled. Another question, which has given rise to considerable controversy, refers to the presence of albumoses and peptones in blood (bodies giving the biuret reaction). These have been claimed to be found by Emden and Knoop,<sup>2</sup> by Langstein,<sup>3</sup> and by Nolf.<sup>4</sup> Their existence, on the other hand, has been denied by Abderhalden and Oppenheimer,<sup>5</sup> and by Neumeister.<sup>6</sup> Now in Part II of these researches (see p. 129) it has been shown that when albumens are coagulated in concentrated solutions, the coagulum carries down with it a not inappreciable quantity of water soluble substances. On the other hand, when coagulation is carried out in dilute solutions the precipitate is so finely divided that satisfactory filtration is almost impossible. For these reasons it is not surprising that contradictory results have been obtained.

In nearly every case in these researches, etc., the filtrates obtained after coagulation, both alcoholic and aqueous, have been submitted to the biuret reaction. In no single case was a positive result obtained.

*Neither the liver nor the serum was found to contain a trace of albumose or peptone.* With regard to the residual nitrogen of the sera the results are evident from the following table :—

TABLE II

No.	Fasting animals			Animals killed during digestion			
	N	N-R	R	No.	N	N-R	R
1	127.0	115.0	12.0	3	131.6	122.0	9.9
2	126.0	116.0	10.0	4	125.5	112.2	13.3
5	130.0	118.0	12.0	7	149.0	135.0	14.0
6	124.3	116.0	8.0	8	108.0	99.9	9.0
9	135.6	124.0	11.6	11	121.7	109.0	12.7
10	136.5	119.4	17.1	12	125.5	117.2	8.3
13	130.4	117.7	12.7	15	125.6	114.0	11.6
14	131.3	127.0	4.3	16	143.6	135.2	8.3
17	131.7	113.3	18.4	18	133.3	120.0	13.2
19	122.8	111.0	11.8	20	128.2	110.0	18.2
Total	1295.3		117.9		1292.0		118.5
Ratio	$\frac{R}{N} = \frac{9.10}{100}$				$\frac{R}{N} = \frac{9.16}{100}$		

We see from these numbers, that both the total nitrogen and the residual nitrogen in 150 c.c. of serum, taken from ten different animals, are almost absolutely identical in quantity. We are bound, therefore, to conclude that there is *absolutely no evidence that the products of tryptic digestion as such, are circulating in the blood stream.*

The residual nitrogen, except in three or four cases, varies very little from a mean. The variations bear, however, no relation to the state of nutrition.

We can certainly conclude that it is not the products of tryptic digestion in the serum which exert any influence on the rate of autolysis of the liver.

The variations in the total nitrogen are influenced chiefly by the hydraemic condition of the blood. When the percentage of total solids is high, the total nitrogen is high, and *vice versa*.

### The Liver.

TABLE III

Fasting animals				Animals killed during digestion			
No.	N	N-R	R	No.	N	N-R	R
9	144.0	128.2	15.8	11	130.0	118.2	11.8
10	145.5	127.7	17.8	12	122.5	107.8	14.7
13	126.7	107.5	19.2	15	97.5	79.8	17.7
14	131.0	114.5	16.5	16	103.5	94.9	8.6
17	99.0	82.0	17.0	22	102.7	87.2	15.5
19	107.0	86.4	20.6	24	123.1	107.3	15.8
21	110.0	96.3	13.7	...	...	...	...
23	124.0	104.2	19.8	...	...	...	...
Total	987.2		140.4	Total	679.3		84.1
Average	123.4		17.5	Average	113.2		14.0

$$\text{Ratio } \frac{R}{N} = \frac{14.2}{100}$$

$$\text{Ratio } \frac{R}{N} = \frac{12.3}{100}$$

From this table, it is evident, that so far from the ratio of residual nitrogen to total nitrogen being larger in the case of animals during digestion than in fasting animals, it is smaller. The total nitrogen is also generally larger in fasting animals. There are two or three exceptions, as, for example, in the case of animals 17 and 19. It will be noticed, however, that in these two cases, the percentage of total

solids is very high (47.6 and 40.1 respectively). Furthermore, animal No. 17 weighs only 2.6 kilos, whereas the liver weighs 109.2 grams. These figures seem to indicate a very fatty liver, and probably this accounts for the abnormality. The numbers seem to indicate that in the fasting animals autolysis is already taking place, and that the tissue of the liver is degrading in order to supply the energy needs of the organism.

Here, again, there is no evidence that the products of tryptic digestion directly inhibit autolysis *in vivo*.

*The Mucous Membrane of the Small Intestine.*

TABLE IV

No.	Fasting animals			No.	Animals killed during digestion		
	N	N - R	R		N	N - R	R
17	111.0	71.3	38.7	18	109.0	71.5	37.5
21	112.0	80.5	31.5	20	109.0	74.0	35.0
23	110.0	72.0	39.0	21	110.0	73.6	36.4
Total	334.0		110.2	Total	328.0		108.9
	Ratio $\frac{R}{N} = \frac{33.0}{100}$			Ratio $\frac{R}{N} = \frac{33.2}{100}$			

It will be noticed in the above table that the ratio of residual to total nitrogen is a constant, and independent of the state of nutrition of the animal. The concordance of numbers obtained from different animals is so close, that in five out of the six cases the analyses would have been satisfactory if they had referred to a pure homogeneous product.

Another point of considerable interest is the fact, that the percentage of residual nitrogen is considerably higher than that found in any of the other tissues examined. As the mucous membrane of the small intestine plays an important part in the general nitrogenous metabolism, this point is of great significance, and as will be shown below, the ratio is higher in the carnivora, in which the mucous membrane of the intestine has been examined, than in the herbivora. It appears to be a constant for each species of animal.

Only a part of this residual nitrogen is eliminated on simple coagulation by boiling the fresh tissue; if, however, the tissue be

incubated with water, a gradual elimination takes place in four hours; the degradation changes then cease. This phenomenon has been already described in the first paper (*Journ. Physiol.* 32, p. 159); it is to be distinguished from ordinary autolysis.

The physical constants determined show that very little change takes place on coagulating the moist fresh tissue; no more, in fact than takes place in the case of the liver, which does not contain a large quantity of residual nitrogen, and which shews very small change during the first four hours of incubation with water. All these facts justify the conclusion that the greater part, at least, of the residual nitrogen of the small intestine is not mere 'extractive' nitrogen, but represents a part of the protoplasm molecule which is held in loose combination, and which is intimately connected with the processes of nitrogenous metabolism, as is evidenced from the fact that it is greater in quantity in the case of carnivorous animals than in that of herbivora. This point will be referred to again in the summary, (part VI).

*Comparison of the ratio residual nitrogen : total nitrogen in different animals.*

Dog—No. 1.	Fasting	...	41.2	} 38.8 (average).
„ „ 2.	Fed	...	36.5	
Pig	...	...	...	32.3
Rabbit—No. 1...	...	...	29.7	} 28.8
„ „ 2...	...	...	28.2	
Sheep	...	...	...	27.6
Ox	...	...	...	19.2

In the case of the ox, the sample for analysis was not taken from the whole intestine.

In the rabbit, only small quantities could be obtained for each analysis; these were accurately weighed on a fine balance.

#### SOME PHYSICAL CONSTANTS OF TISSUE EXTRACTS

The following determinations of physical constants were made. The results throw but little light on the method of combination of the residual nitrogen with the main part of the bioplasm. It might be

expected from the generalisations made below that changes other than those affecting the nitrogen take place in the tissues ; the results obtained show no parallelism between the physical and the nitrogenous changes.

Five grams of tissue were allowed to stand for a short time with 50 c.c. of cold water, made just acid with one drop of acetic acid. A part of the extract was, in each case, heated rapidly to 100°, in a closed vessel (so that no evaporation took place), and maintained at this temperature in a boiling water bath for 15 minutes. The determinations were made with boiled and unboiled extracts.

*Liver. Electrolytic conductivity*

Unboiled extract	R = 495.2, c = 651.670, k = 1317
Boiled extract	R = 443.5, c = 651.670, k = 1469
	<hr/>
Difference	... 152

*Small Intestine. Electrolytic conductivity*

Unboiled extract	R = 320.9, c = 651.670, k = 2031
Boiled extract	R = 290.6, c = 651.670, k = 2242
	<hr/>
Difference	... 211

*Freezing point*

Unboiled extract	... Δ = 0.08°
Boiled extract	... Δ = 0.09°

Another experiment was made. Five Grams of tissue were boiled for half hour with 50 c.c. alcohol. The latter was decanted off, and the precipitate washed several times with hot water. The washings, both alcoholic and aqueous, were then evaporated to a small bulk in *vacuô*, and dissolved in water. This solution was made up to 32 c.c. The freezing point of this solution was then compared with the freezing point of an extract of 5 grs. tissue in 30 c.c. cold water. Result :

Δ For tissue extract	... = 0.17°
Δ For extractives solution	= 0.17°

Attention has been called in an earlier paper to the fact that a considerable degradation of the small intestine takes place in the first four hours of incubation with water, which is followed by a period of small change. This phenomenon is discussed in detail in Part VI. The following experiment shows that the changes of electrical conductivity are not parallel to this change; there is no sudden increase in the first four hours, followed by a period of no change.

The experiment was carried out with a suspension of 5 grs. tissue in 50 c.c. water; temperature 40°. The conductivity of the boiled extract was also determined.

Time	Resistance
11'10	... Incubation commenced.
11'18	... 267'6
11'33	... 257'1
11'48	... 252'0
12'15	... 242'5
12'30	... 236'0
1'27	... 199'0
2'5	... 170'5
2'40	... 145'1
3'13	... 126'0
4'20	... 94'4
5'0	... 82'5
Boiled extract.	Resistance 171'8

These results show a continuous decrease, which is due probably to changes other than nitrogenous degradation.

#### REFERENCES

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4. Nolf, *Extraits des archives de biologie*, vol. 20, 1903.
5. Abderhalden and Oppenheimer, *Hoppe-Seyler's Zeitschrift*, 42, p. 155, 1904.
6. Neumeister, *Zeitschrift für Biologie*, 24, p. 272, 1888.

References to analyses of small intestine during absorption of food :

Neumeister, *loc. cit.*

Zunz, *Hofmeister's Beiträge*, 3, p. 339, 1902-3.

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## PART IV

THE QUESTION OF THE SYNTHESIS OF ALBUMENS IN THE MUCOUS  
MEMBRANE OF THE STOMACH

This question has already been referred to in the introduction.

The latest experiments on the subject, viz., those of Glaessner, conducted in Hofmeister's laboratory, were carried out in the following manner:—The mucous membrane of the stomach was stripped off and divided into two symmetrical halves. The one half was coagulated, and the amount of peptones and albumoses determined in the filtrate of the coagulum by the method of Pick. The other half was incubated in a moist chamber at 40° for three hours, and then coagulated, and the albumoses and peptones determined in the same way as in the unincubated half. Glaessner showed that there was a diminution of these bodies which reached a maximum when the animal was killed five hours after a meal.

The method employed in this research was similar; instead however, of using Pick's method, the coagulable albumen was determined directly by the procedure described in Part II.

## Results :

Dog A. Killed four hours after a heavy meat meal.

Non-incubated half.	Total N.	103·0	N - R	94·0	Ratio	$\frac{N-R}{N} \times 100$	91·2
Incubated half.	„	105·0	„	92·2	„	„	87·7

Dog B. Killed five hours after a meal.

Non-incubated half.	Total N.	108·5	N - R	96·8	Ratio	$\frac{N-R}{N} \times 100$	91·2
Incubated half.	„	113·0	„	100·7	„	„	89·1

The residual nitrogen was also determined in a fasting dog and found to be higher than in digesting dogs, *i.e.* as 23 : 97 (total N).

The changes taking place in a tissue during secretory activity are now forming the subject of another research. It is hoped to deal with these in a subsequent paper.

The above experiments give no evidence of a synthesis of albumens.

## PART V

FURTHER RESEARCHES ON AUTOLYSIS: THE RELATION OF  
AUTOLYSIS TO NUTRITION*Section A. Autolysis of the Liver*

The method employed in the following researches is essentially that described in the first paper<sup>1</sup> (Lane-Clayton and Schryver, *loc. cit.*)

One or two minor modifications were made. After precipitating with trichloroacetic acid, the mixture was allowed to cool. It was filtered when cold, after standing for several hours; the filtrate was washed with hot water and, in the event of the bulk not being too great, directly evaporated down with sulphuric acid in the Kjehldahl flask.

Except when otherwise stated, the numbers given under the heading 'autolysis' refer to the number of c.c.  $\frac{N}{16}$  H<sub>2</sub>SO<sub>4</sub> necessary for neutralisation of NH<sub>3</sub> obtained in the Kjehldahl analysis. Four grams of tissue were used for each experiment.

*Series I. Effect of Serum.*—In all the experiments with serum, a portion was incubated with saline as a control (same volume as liquid in actual experiment). After incubation, serum in same dilution (also incubated for the same time) was added to the control; this mixture was then rapidly heated to boiling, and trichloroacetic acid in definite quantity added. The same amount of saline as was used for the control was added to the portion incubated with serum. The mixture was then boiled, and the same amount of trichloroacetic acid added as was used in the control. By this means coagulation in the actual experiment and the control were carried out under absolutely identical conditions.

*Sheep's Serum on Cat's Liver.*—20 c.c. liquid to 4 grams liver.

Time of Autolysis	Serum %	Autolysis *	Control *	Difference
7 hours	100	18·8	31·2	12·4
7 "	50	15·5	26·0	10·5
9½ "	100	21·2	36·0	14·8
9½ "	25	17·7	28·5	10·8

A star indicates total N in filtrate from coagulum (includes residual N, N from products of tryptic digestion, etc.)

From these numbers it is evident that, whereas serum (even 100 per cent.) strongly inhibits the rate of autolysis, it does not entirely stop it.

*Series II.—Effect of sera of fed and fasting animals (cats) on autolysis of liver of a fasting animal.*

*Series (a).* Twenty c.c. of 25 per cent. solution of serum to 4 grams liver. Same liver used for experiment with fed as with fasting animal.

Time	Serum of fed animal			Serum of fasting animal		
	With serum*	Control*	Difference	With serum*	Control*	Difference
7 hours	16.3	20.0	3.7	16.5	16.2	0
9 hours	19.3	27.8	8.5	21.0	24.0	3
		Total 12.2			Total 3.0	

Here the serum of fed animal exerts an inhibitory action. Note, however, that the numbers under the heading 'control' are higher. This indicates that the serum of the fed animal contains a larger quantity of extractives.

*Series (b).*—Same as *Series (a)*. Liver and sera from different animals.

Time	Serum of fed animal			Serum of fasting animal		
	With serum*	Control*	Difference	With serum*	Control*	Difference
6½ hours	18.2	24.0	5.8	19.5	26.6	7.1
9¾ hours	19.5	25.2	5.7	22.0	25.0	3.0
		Total 11.5			Total 10.1	

Here the same difference of the inhibitory action is not observed as in *Series II (a)*. From experiments on the composition of serum in Part III, one would not expect to find any very definite relationship between the inhibitory action of the serum and the state of the nutrition of the animal from which it has been obtained. The factors influencing autolysis will be demonstrated later.

*Series III.—Effect of the action of the products of tryptic digestion of caseine on autolysis.*

*Series III (a).*—Caseine in with twenty times its weight of water was digested in alkaline solution with trypsin. The mixture was then boiled and made just acid with acetic acid. Ammonia was then added until the reaction was plainly alkaline, and the whole was then concentrated by gently warming on a water bath.

The control was made by incubating with saline, and then adding the same quantity of products of tryptic digestion as was used in the actual experiment. The portion incubated with the products of digestion was diluted before coagulation with the same amount of saline as was used for the control. The filtrate in which N was estimated includes, of course, the products of digestion as well as those of autolysis.

5 c.c. trypsin solution =  $36\cdot0$  c.c.  $\frac{N}{10}$  H<sub>2</sub>SO<sub>4</sub> (Kjehldahl)  
20 c.c. saline + 5 c.c. to 4 grams of liver; control in 25 c.c. saline.

Time	With products of digestion	Control	Difference
7 hours	48.0	58.0	10.0
8 "	57.7	65.0	7.3
9 "	62.7	66.3	3.9

*Series III (b).*—To test the effect of the quantity of the products of digestion on autolysis rate. The control in this case represents the N of the filtrate obtained by boiling the tissue with the products of tryptic digestion, adding trichloroacetic acid, etc., without previous autolysis.

T.D. = products of tryptic digestion. (5 c.c. = 36 c.c.  $\frac{N}{10}$  H<sub>2</sub>SO<sub>4</sub>)  
S = Saline

Solution used	Time	* N in filtrate	Autolysis
20 c.c. T.D. No saline.	0 hours (control)	147.5	...
	7 hours	153.2	5.7
	9 hours	157.5	10.0
10 c.c. T.D. + 10 c.c. S.	0 hours (control)	77.2	...
	7 hours	87.0	9.8
	9 hours	89.4	12.2
5 c.c. T.D. + 15 c.c. S.	0 hours (control)	38.7	...
	7 hours	53.3	14.6
	9 hours	57.2	18.5
5 c.c. T.D. + 35 c.c. S.	0 hours (control)	40.4	...
	7 hours	54.3	13.9
	9 hours	57.2	16.8

These figures indicate that the rate of autolysis is influenced by the quantity of the products of tryptic digestion, but little by the dilution.

*Series III (d).*—With another sample of the products of digestion of caseine the following results were obtained :—

Products of digestion alone  $N = 111.7 \text{ c.c.} \frac{N}{10} \text{H}_2\text{SO}_4$  (20 c.c.)

Time	With T.D. *	Control *	Difference
8 hours	128.5	138.0	9.5
24 ,,	140.0	135.2	4.8

Experiments were also made to determine whether previous treatment of the product of tryptic digestion with mucous membrane of the small intestine exerted any influence on the inhibitory power. No difference could, however, be detected.

*Series III (e).*—It will be shown below that acids accelerate autolysis, whereas alkalis inhibit it ; it was, therefore, of interest to determine whether the products of tryptic digestion inhibit autolysis in distinctly acid solutions, especially as it is somewhat difficult to be sure of the reaction to litmus of the products of digestion.

The sample of digestion products used had been prepared by digesting an alkaline solution (5 per cent.) of caseine for three months with *Rhenania trypsin*.

Amount of acid used for 4 grams liver = 20 c.c.  $\frac{N}{10}$  acetic acid

To this was added 10 c.c. T.D. solution = 95.5  $\frac{N}{10}$   $\text{H}_2\text{SO}_4$

Time	With T.D. *	Control A *	Control B *
2 hours	106.0	116.3	115.5
4 ,,	115.5	129.8	131.0
6 ,,	123.3	144.5	144.5

Control A was made by boiling the mixture incubated with water before adding the T.D. solution ; then adding trichloroacetic acid. In control B the T.D. was added first ; then the mixture boiled and trichloroacetic acid added. These results clearly indicate the marked inhibitory action of the products of tryptic digestion.

*Series III (f).*—*Attempts to reverse autolysis.*

4 grams of liver in each case were incubated with 20 c.c.  $\frac{N}{10}$  lactic acid. After  $2\frac{1}{4}$  and  $3\frac{1}{4}$  hours respectively, the following mixtures were added :—

- (a) 20 c.c. T.D. (of which N = about 190 c.c.  $\frac{N}{10}$   $H_2SO_4$ )  
 (b) 20 c.c. T.D. + 20 c.c.  $\frac{N}{10}$  NaOH, so as to neutralize the lactic acid added.  
 (c) 20 c.c. T.D. + 25 c.c.  $\frac{N}{10}$  NaOH, so as to have a distinctly alkaline solution.

The effects of these solutions on the autolysed tissue, after acting for one and for two hours in the incubator, were investigated. In each case, instead of estimating the N in the filtrate, the N of the coagulable albumen was determined (by the method given in Part II), sulphate of soda being added in sufficient quantity to absorb all the water (*i.e.*, somewhat more than the weight of the whole mixture).

N of coagulum after autolysis with acid for  $2\frac{1}{4}$  hours = 66 c.c.  $\frac{N}{10}$   $H_2SO_4$

Product of $2\frac{1}{4}$ hours autolysis + T. D.				N of coagulum	
				after 1 hour	65.5
"	$2\frac{1}{4}$	"	+ T. D. + 25 c.c. NaOH	" 1 "	64.2
"	$2\frac{1}{4}$	"	+ T. D.	" 2 hours	65.0
"	$2\frac{1}{4}$	"	+ T. D. + 20 c.c. NaOH	" 2 "	65.2
"	$3\frac{1}{4}$	"	+ T. D.	" 1 hour	63.0
"	$3\frac{1}{4}$	"	+ T. D. + 25 c.c. NaOH	" 1 "	63.0
"	$3\frac{1}{4}$	"	+ T. D.	" 2 hours	54.5
"	$3\frac{1}{4}$	"	+ T. D. + 25 c.c. NaOH	" 2 "	56.0

N of coagulum after autolysing with acid for  $4\frac{1}{4}$  hours = 57.5  $\frac{N}{10}$   $H_2SO_4$

Although these results do not give any evidence of the reversal of the autolytic process, such an action is by no means improbable; there is no doubt of the powerful inhibitory action, which indicates that the reaction is probably reversible.

*Series IV.—Effects of products of peptic digestion.* In the following experiments, the autolysis was investigated in 20 c.c. saline to which were added 5 c.c. of a carefully neutralized 10 per cent. solution of Witte's peptone.

Time	With peptone *	Control *
0	67.3	67.3
2 hours	72.3	72.8
4 "	74.0	76.3
6 "	86.5	86.0
8 "	92.3	94.0
24 "	103.0	113.2

The peptone exerts, therefore, an inhibitory action only in the later stages.

*Series V.—The effects of acids and alkalis on autolysis.* When the main facts in the following series had been already discovered, a paper by Hugo Wiener appeared in the *Physiologische Centralblatt* (August, 1905) in which he suggested that the latent period of autolysis described in the first paper, was due to the fact that the tissues were still alkaline, and that the autolytic enzyme did not act until the tissues became acid (Cf. Hedin).

*Series V (a).*

ANIMAL I			ANIMAL II		
Time	With acid		Time	With acid	
	H <sup>2</sup> SO <sup>4</sup> $\frac{N}{40}$ 20 c.c.	Control in Saline 20 c.c.		H <sup>2</sup> SO <sup>4</sup> $\frac{N}{40}$ 20 c.c.	Control in Saline 20 c.c.
0	8.7	8.7	0	11.7	11.7
4 hours	23.3	11.5	4 hours	25.4	15.6
6 "	40.0	22.2	6 "	33.5	21.1
8 "	44.7	29.2	8 "	41.0	31.7
10 "	50.7	37.0	10 "	45.8	38.5
24 "	61.8	55.8	24 "	52.5	49.5

*Series V (b).*

Time	Effect of NaOH $\frac{N}{40}$ 20 c.c.	
	With alkali	Control
0	7.7	7.7
4 hours	9.1	10.5
6 "	9.7	12.0
8 "	13.8	18.3
10 "	15.4	25.2
24 "	26.3	34.2

*Series V (c).*

Time	Effect of Na <sup>2</sup> CO <sup>3</sup> $\frac{N}{40}$ 20 c.c.	
	With alkali	Control
0	10.6	10.6
4 hours	12.0	14.2
6 "	13.7	19.0
8 "	16.4	27.5
10 "	22.1	34.6
24 "	41.4	50.8

## Series V (d).

Effect of  $\text{NaHCO}_3 \frac{\text{N}}{40}$  20 c.c.

Time	With bicarbonate	Control
0	9.8	9.8
4 hours	11.2	10.9
6 "	11.8	14.5
8 "	19.5	22.5
10 "	32.0	31.0
24 "	43.0	50.7

As the result of these experiments it will be observed that acid causes a very marked acceleration of autolysis, whereas alkalis have a strong inhibitory action. Bicarbonate of soda acts as a weak alkali in this respect. The curves representing Series V (a) and V (c) are given in the accompanying diagram.

Series V (e).—The effects of sulphuric acid with varying solutions or varying quantities on the rate of autolysis.

In the following experiment 20 c.c. of  $\text{H}_2\text{SO}_4$  solution were used in every case:—

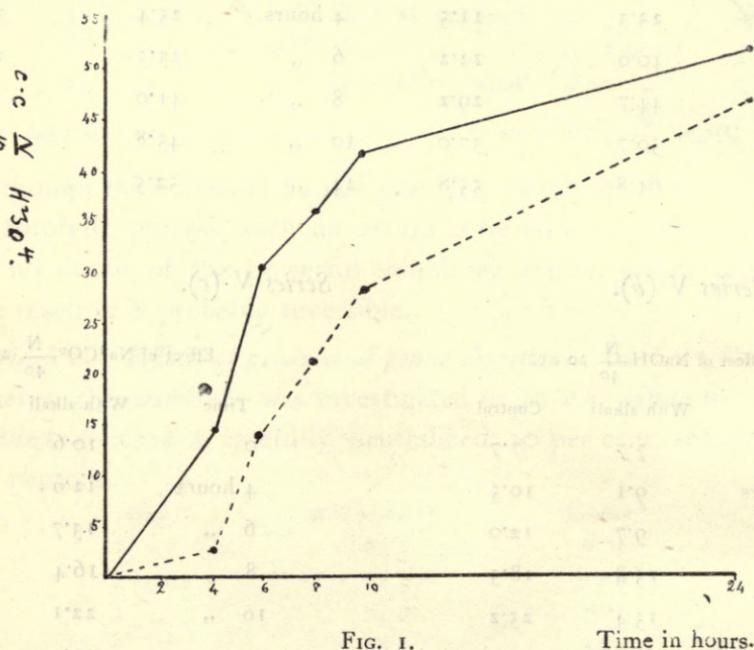


FIG. 1.

Time in hours.

Broken line: control in saline.

Effect of 20 c.c.  $\frac{\text{N}}{40}$   $\text{H}_2\text{SO}_4$  solution on autolysis of 4 grams of liver tissue.

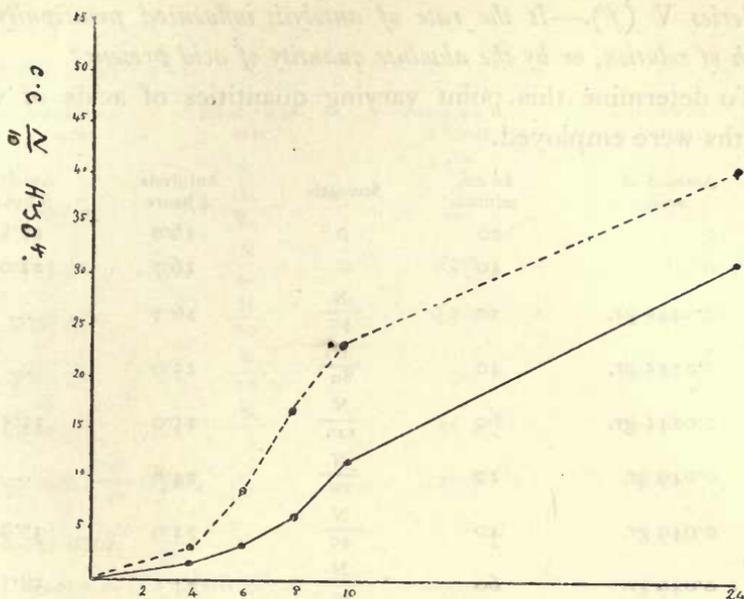


FIG. 2.

Time in hours.

Broken line : control in saline.

Effect of 20 c.c.  $\frac{N}{40}$   $\text{Na}_2\text{CO}_3$  on autolysis of 4 grams liver tissue.

Strength of solution	Amount of acid in 20 c.c.	Fasting animal		Fed animal	
		Autolysis 4 hours	Autolysis 6 hours	Autolysis 4 hours	Autolysis 6 hours
0	0.0	10.0	22.0	10.5	12.3
$\frac{N}{320}$	0.0030 gr.	9.7	22.3	11.8	12.8
$\frac{N}{160}$	0.0061 gr.	13.4	25.4	13.3	16.2
$\frac{N}{80}$	0.01225 gr.	19.5	33.3	17.5	26.2
$\frac{N}{40}$	0.0245 gr.	lost	38.5	22.7	32.5
$\frac{N}{20}$	0.049 gr.	26.4	40.7	24.8	29.5
$\frac{N}{10}$	0.098 gr.	22.8	31.0	21.5	27.2

We see from the above table a progressing rate of autolysis with an increasing strength of acid up to the concentration of  $\frac{N}{20}$ . Concentrations above this strength appear to be destructive to the enzyme.

1. By autolysis in this and the succeeding experiments, up to Series V (g), is meant the N in the filtrate after coagulation.

*Series V (f).*—Is the rate of autolysis influenced principally by the strength of solution, or by the absolute quantity of acid present?

To determinè this point varying quantities of acids of varying strengths were employed.

Amount of acid	In c.c. solution	Strength	Autolysis 4 hours	Autolysis 6 hours
0	20	0	16.0	17.8
0	40	0	16.7	21.0
0.0245 gr.	20	$\frac{N}{40}$	26.3	...
0.0245 gr.	40	$\frac{N^*}{80}$	25.0	...
0.0245 gr.	60	$\frac{N}{120}$	25.0	35.3
0.049 gr.	20	$\frac{N}{20}$	24.8	...
0.049 gr.	40	$\frac{N}{40}$	24.9	37.9
0.049 gr.	60	$\frac{N}{60}$	24.5	38.3
0.098 gr.	20	$\frac{N}{10}$	20.0	35.0
0.098 gr.	40	$\frac{N}{20}$	22.0	39.0
0.098 gr.	60	$\frac{N}{30}$	25.3	42.2

These results indicate that provided sufficient acid is present (and it has been already shown in *Series V (e)* that 20 c.c. of  $\frac{N}{40}$  acid produces nearly the maximum result), the dilution in the case of a strong acid, like sulphuric acid, has but little effect. The action of the acid is akin, therefore, to the neutralization of a base, or the setting free of a weak acid from a salt, rather than to a phenomenon of hydrolysis. It is the amount of acid, therefore, that exerts the influence on the rate of autolysis rather than the concentration. There is, however, an inhibition with sulphuric acid when the concentration reaches  $\frac{N}{10}$ . This inhibition is due, as will be seen in the above table, entirely to the concentration and not to the quantity. It is probably, as already mentioned, due to hydrolysis of the enzyme.

*Series V (g).*—The influence of organic acids on autolysis.

In the experiments with acetic and lactic acids, the rate of autolysis with varying strengths and quantities has been compared with the rate in 20 c.c.  $\frac{N}{10}$  sulphuric acid.

The following experiments have been carried out with 20 c.c. liquid.

*Acetic acid.*

Amount of acid	Strength of acid	Autolysis in 4 hours	Autolysis in 6 hours
0	0	11.7	13.5
0.015 gr.	$\frac{N}{80}$	...	28.6 (?)
0.03 gr.	$\frac{N}{40}$	25.6	27.5
0.06 gr.	$\frac{N}{20}$	28.2	33.6
0.12 gr.	$\frac{N}{10}$	...	36.0
0.24 gr.	$\frac{N}{5}$	38.2	41.6
20 c.c. $\frac{N}{40}$ H <sub>2</sub> SO <sub>4</sub>		27.8	28.2

*Lactic acid.*

Amount of acid	Strength of acid	Autolysis in 4 hours	Autolysis in 6 hours
0	0	15.7	23.3
0.0225 gr.	$\frac{N}{80}$	31.2	37.0
0.045 gr.	$\frac{N}{40}$	...	47.8
0.09 gr.	$\frac{N}{20}$	39.6	51.0
0.18 gr.	$\frac{N}{10}$	...	58.2
0.36 gr.	$\frac{N}{5}$	44.7	55.0
20 c.c. $\frac{N}{40}$ H <sub>2</sub> SO <sub>4</sub>		27.3	41.0

We see from these figures that both acetic and lactic acid exerts a very powerful accelerating influence on the rate of autolysis, especially the latter acid. Lactic acid accelerates the autolysis more than does sulphuric acid in the corresponding strength of solution. This is important in view of the fact that lactic acid is known to be formed at times within the organism.

*Series V (h).—Influence of ammonia and trimethylamine on autolysis.*

In the following experiments the liver was incubated with the alkaline solutions in corked flasks. The autolysis was determined by estimating the nitrogen in the filtrate from coagulum in a portion before

incubation and a corresponding portion after incubation. Trichloroacetic acid was added before boiling to avoid loss of volatile alkali. The following numbers represent the amount of autolysis in  $7\frac{1}{2}$  hours :—

Solution used	Amount of Alkali	Autolysis
Without $\text{NH}_3$	0.	15.4
20 c.c. $\frac{\text{N}}{40} \text{NH}_3$	.0085 gr. $\text{NH}_3$	8.3
20 c.c. $\frac{\text{N}}{20} \text{NH}_3$	.017 " "	6.5
20 c.c. $\frac{\text{N}}{10} \text{NH}_3$	.034 " "	5.4
20 c.c. $\frac{\text{N}}{5} \text{NH}_3$	.068 " "	3.4
20 c.c. $\frac{17}{10} \times \frac{\text{N}}{10} \text{N}(\text{CH}_3)_3$	.2008 gr. $\text{N}(\text{CH}_3)_3$	3.9

Here again we have a strong inhibition due to the alkali. This, as will be shown in the sequel, has an important bearing on the mechanism of nutrition.

#### *Influence of acids on the production of acid products of autolysis.*

Magnus-Levy has shown that when autolysis is carried out without antiseptics under conditions assuring asepsis, as far as can be determined by cultures, a considerable evolution of gas takes place (both  $\text{CO}_2$  and hydrogen), and certain quantities of fatty acids (amongst which is lactic acid) are produced. It has been noticed in the experiments recorded above that when acids (either sulphuric or an organic acid) are present, although nitrogenous degradation products are formed very rapidly, there is no evolution of gas, and no smell of fatty acids, even after 24 hours incubation. It would seem, therefore, that those conditions which favour the formation of nitrogenous bodies inhibit the formation of acid. It was also noticed that when the liver of a well-fed dog was autolysed in the presence of acid, the mixture at the end of 24 hours was still quite turbid with glycogen. The corresponding mixture in the case of a fasting dog was perfectly clear. This subject requires further research.

#### *Physiological action of the products of autolysis.*

Attempts were made to prepare a cytotoxic serum by the injection of cats' liver into the peritoneum of a rabbit. If the fresh liver be

injected in the form of an emulsion comparatively large quantities and repeated injections could be tolerated by the rabbits, without evincing any symptoms. On the other hand, when the liver had been incubated for only four hours (*i.e.*, till incipient autolysis), the animals invariably died, even after the interperitoneal injection of very dilute solutions. In one case an animal died within two or three hours of an injection.

These products when injected into the external jugular vein of an anaesthetized animal, of which the carotid artery was connected with a manometer, showed no change in the blood pressure.

The products of autolysis or the protoplasm of tissue in its unstable condition are evidently highly toxic. This subject is of considerable interest in connection with the toxæmic symptoms observed in atrophy of the liver. It is proposed to investigate this subject more completely.

*Section B. The autolysis of some tissues other than the liver.*

*Series VI.—Effect of acids on autolysis of kidney and muscular tissue.*

*A. Muscular tissue.* (Quadriceps extensor of cat).

Solution used	Water 20 c.c.	H <sub>2</sub> SO <sub>4</sub> $\frac{N}{40}$ 20 c.c.	Acetic acid $\frac{N}{5}$ 20 c.c.	Lactic acid $\frac{N}{5}$ 20 c.c.
4 hours	17.2	21.2	24.0	28.0
6 hours	16.8	21.2	24.0	27.0 (slight loss)

*B Kidney*

4 hours	16.7	43.7	55.0	53.4
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These results indicate that acid excites the autolytic enzyme in the case of the kidney. The influence of the acid in the case of the muscle is slight; as there is no degradation between 4 and 6 hours the action appears to be a solvent one, rather than one exciting the enzyme.

*Series VII.—The influence of muscular activity on autolysis.*

In this experiment, the sciatic nerves of a cat were divided on each side as near the crural sciatic plexus as possible. The sciatic nerve on one side was tetanized for 25 minutes, and the animal was killed during tetanization (anaesthetic A.C.E.). The autolysis of the corresponding resting and acting muscles from the two legs was then determined.

Time	Resting muscle	Tetanized muscle
0	12.6	12.2
2 hours	13.2	12.7
4 "	15.8	14.1
6 "	16.3	...
8 "	17.4	18.0
10 "	22.4	23.4
24 "	34.2	32.8

The rate is practically identical for the two sides. The rate of autolysis does not appear, therefore, to be influenced by activity.

Experiments were also made on the rate of autolysis of fresh and frozen meat. No difference could be detected.

In the case of frogs' muscles, tetanization appeared to exert an inhibitory influence on autolysis. The difference was, however, but slight. No precautions were taken in these cases for asepsis, so that no definite conclusions could be drawn.

*Series IX. The comparative rate of autolysis in different tissues.*

The blood does not autolyse to any appreciable extent in 24 hours, either when incubated with ten times its volume of water, or in 0.5 per cent. ammonium oxalate solution. The thyroid gland also autolyses but to a very slight extent in 24 hours.

In the following experiments, the relative rate of autolysis of 4 grams from each organ in 24 hours was determined. Toluol was employed as an antiseptic.

Organs taken from two different dogs, given in table under columns I and II. In animal I, autolysis lasted 24 hours; in animal II, 20 hours.

Organ	Total N in 4 grams.		N <sup>I</sup>		N <sup>II</sup>		N <sup>II</sup> - N <sup>I</sup>		$\frac{N^{II} - N^I}{\text{Total N}} \times 100$	
	Animal I 24 hrs.	II 20 hrs.	Nitrogen in filtrate before autolysis		Nitrogen in filtrate after autolysis		I.	II	I	II
			I	II	I	II				
Spleen	82.4	89.5	14.0	13.5	49.7	38.5	35.7	25.0	43.3	28.0
Liver	97.6	...	8.7	...	47.8	...	39.1	...	40.0	...
Kidney	76.5	...	14.5	...	32.8	...	18.3	...	23.9	...
Striated Muscle }	95.3	99.5	9.8	12.0	25.0	26.2	15.2	14.2	15.9	14.3
Heart	80.0	87.4	10.2	11.2	27.6	33.7	17.4	12.5	21.7	14.3

It will be observed from this table that the maximum amount of autolysis occurs in the liver and spleen, the two organs which lose the greatest amount of weight during starvation. This affords a striking confirmation of the suggestion made in the first paper, viz., that the autolytic enzyme has the function of protecting the organism from starvation.

## PART VI

### A THEORY OF NITROGENOUS METABOLISM, DEDUCED FROM THE RESULTS OF THE FOREGOING RESEARCHES

Attention has already been called to the controversy as to whether the products of tryptic digestion of albumens can be detected in the blood; it has been claimed, furthermore, that the methods hitherto employed for investigating this problem have not been entirely satisfactory. As it was imperative to arrive at a decisive settlement of this question, if possible, in order to gain a clear view as to the function of the albumens in nutrition, researches were undertaken with the object of obtaining a reliable method of chemical analysis, by means of which the nitrogen of the coagulable albumen could be accurately estimated; such a method is described in Part II. The difference between the total nitrogen and the nitrogen of the coagulable albumen, designated throughout as 'residual' nitrogen, should, if the products of tryptic digestion circulate in the blood-stream, be greater in the case of animals killed during active digestion than it is in that of fasting animals. The method of research was also applied to a similar investigation of the liver and the mucous membrane of the small intestine. A large number of animals were used, and it was found then in no case was the average amount of residual nitrogen greater in the case of fed animal than in that of fasting animals.

The general conclusions drawn from the researches on residual nitrogen may be summarized under the following heads:—

I. The residual nitrogen, as deduced from the analyses of ten sera of fasting animals was found to be 9.10 per cent. of the total nitrogen; corresponding analyses of the sera of ten fed animals gave the number 9.16. These figures are so close that they

may be regarded as practically identical ; the difference lies well within the limits of experimental error. The individual sera give numbers which do not differ very greatly from this mean.

Furthermore—*In no case was any substance yielding the biuret reaction detected in the filtrate from the coagulum.*

II. In the liver, the percentage of residual nitrogen is larger (14.2) in the case of the fasting than of the fed animals (12.3). This result is probably due to the fact that autolysis was already taking place in the livers of the fasting animals at the time of death.

*In no case again were any bodies yielding the biuret reaction to be found in the filtrates from the coagulum.*

III. In the case of the mucous membrane of the small intestine, the following results were arrived at :

(a) The residual nitrogen varies within very narrow limits, if indeed, at all. The analysis made of six samples taken from six different animals gave in five cases numbers that agree so closely that had they referred to a single homogeneous body they could hardly have been more satisfactory. From this we may conclude that the percentage of residual nitrogen in the mucous membrane of the intestine is a constant for a particular species of animal.

(b) It is not influenced by the state of nutrition.

(c) It varies with different animals. It was found to be 38.8 in the case of the dog (two samples), 33.2 in the case of the cat (six samples), 32.3 in the case of a pig, 28.8 in a rabbit (two samples), 27.6 for a sheep, and 19.2 for an ox. It will be noticed therefore, that the percentage is higher in the case of carnivora than of herbivora.

(d) It is also higher in the case of the mucous membrane of the intestine than in any of the other tissues investigated.

(e) In addition, the mucous membrane shews certain other peculiarities. Attention has already been called to the fact that, unlike other tissues, when incubated with water it *immediately* commences to undergo disintegration.

This disintegration proceeds for three or four hours, after which in most cases it entirely ceases, at any rate for some time (Lane-Clayton and Schryver, *Journ. Physiol.* 31, pp. 173-4). In the researches just quoted, the residual nitrogen was determined in the filtrate from the coagulum, after precipitating with trichloroacetic acid in boiling solution. The preliminary researches of Part II shew that when this method is used, nearly all the residual nitrogen passes into the filtrate, at any rate in fairly dilute solutions. Now the amount found by this method of analysis in the mucous membrane of intestine of five animals was equivalent to 17.8 c.c.  $\frac{N}{10}$   $H_2SO_4$  for 5 grams of tissue if the residual nitrogen be determined before incubation. After incubation for 4 hours, however (that is to say after the first rapid disintegration had come to a standstill), this number rose to about 32 c.c.  $\frac{N}{10}$   $H_2SO_4$ . This is very nearly the same amount as that found by coagulating the tissue, when dried by sodium sulphate according to the method described in Part II. This seems to indicate that part, at least, of the bodies represented by the residual nitrogen is in some form of chemical combination with the bioplasm.

What conclusions are to be drawn now from the above results ?

In the first place, there is no evidence that the products of tryptic digestion as such can circulate in indefinite quantities in the blood-stream. How, then, are we to explain their fate after leaving the alimentary tract ? What light do the above results throw on this subject ?

Four sets of facts are of special significance in connection with these questions ; they are :—

- (i) The percentage of residual nitrogen is very high in the mucous membrane of the intestine, a tissue most intimately connected with the nitrogenous metabolism.
- (ii) In the limited number of cases examined, it is higher in carnivora than in the herbivora.

- (iii) It is independent of the state of nutrition, and is the same in the fasting as in the fed animal.
- (iv) There is a certain amount of evidence that the bodies represented by the residual nitrogen are in a state of loose chemical combination with the bioplasm.

With these facts to guide us, the following explanation of the mechanism does not seem unreasonable :---

The bodies represented by the residual nitrogen may be regarded as in the same kind of chemical combination, such as exists between an enzyme and its substrate (or, perhaps, between a toxin and antitoxin) ; that in this state they undergo certain chemical changes like hydrolysis or oxidation, such as would take place through the action of an enzyme ; the products of the change would be eliminated and carried in the blood-stream to other parts of the organism ; after chemical change and elimination they would be replaced by other similar side chains, which would, in their turn, undergo the same kind of changes. The more rapid the blood-stream through the organ, the more rapid will the changes be.

According to this conception, then, the passage of the products of tryptic digestion through the mucous membrane is analogous to a continuous chemical process. The bioplasm acts as an enzyme or collection of enzymes, to specific points of which side chains are anchored ; it keeps, furthermore, always saturated with side-chains, as is shown by the fact that the residual nitrogen is the same during digestion as during a fast.

The theory is analogous to that suggested by Verworn<sup>1</sup> to account for the utilisation of carbohydrates.

The question now arises : how does the bioplasm maintain its saturation with the side-chains in the absence of ingested food-stuffs ?

To obtain an answer to this question, reference must be made to the researches on autolysis and on the products of metabolism carried from the digestive tract to the portal vein. The following sets of facts are known :—

- (i) The autolytic enzyme acts more rapidly in the liver of a fasting animal than in that of an animal during active digestion.

- (ii) It is inhibited by the action of ammonia and other alkalis, but accelerated by the presence of acids, especially lactic acid.
- (iii) This acceleration of acids is a function, in the case, at any rate, of sulphuric acid, depending on the *absolute* quantity of acid present, but not, except to a very limited extent on the concentration (Part V, Experiment Series V (*f*) p. 152).
- (iv) According to the researches of Nencki, Pawlow, and Zaleski<sup>2</sup>, ammonia is formed as a product of nitrogenous metabolism in the alimentary tract; there is more ammonia in the portal vein than in any other part of the vascular system. The liver, furthermore, eliminates ammonia on treatment with weak alkalis, and more of this body is obtainable from the organ of a well fed animal than from that of a fasting animal. The numbers are lower in the case of herbivora than in the carnivora.

These facts offer a ready explanation as to the way in which the supply of products of albumen degradation can be maintained for the saturation of the bioplasm with nitrogenous side-chains in the mucous membrane of the intestine and other organs. Nencki, Pawlow, and Zaleski, show that the liver of a dog on treatment with weak alkalis at 40°C, yields on an average 26 mg. of ammonia per 100 gm. of tissue if the animal be killed shortly after a heavy meat meal, about 7.6 mg. if fed on bread and milk only, and about 7.3 mg. if killed during a fast.

The numbers are equivalent to 1.0 and 0.3 mg.  $\text{NH}_3$  for 4 grams of tissue, quantities which require 0.0028 and 0.0008 gm. sulphuric acid for neutralization.

Now, on reference to Experiment Series V (*e*), Part V, (p. 150) it will be seen that in the case of cats, 20 c.c.  $\frac{\text{N}}{320} \text{H}_2\text{SO}_4$  containing 0.0030 gm. acid, a quantity only just sufficient to neutralize 1.0 mg. ammonia, exerts but little influence on the rate of autolysis, whereas the same volume  $\frac{\text{N}}{160}$  acid produces a marked acceleration. (There will, of course, be small quantities of sodium carbonate and other

alkalis in the tissues\*). Furthermore, it has been shown, that in the case of sulphuric acid, it is the absolute quantity rather than the dilution which increases the rate of autolysis.

From these results we arrive at the following conclusion as to the function of nitrogenous foodstuffs in nutrition :—

*In order to maintain nitrogenous equilibrium, nitrogenous food stuffs must be ingested in such quantities, and in such form that the ammonia produced therefrom in the digestive tract is sufficient to maintain the intracellular alkalinity of the liver and probably other tissues.*

In order to fully understand this mechanism it is of importance here to consider the products other than nitrogenous bodies which are formed during autolysis. Magnus-Levy<sup>3</sup> has shown that the liver on autolysis under the most stringent precautions for asepsis yields carbonic and other organic acids, such as lactic acid ; furthermore, the degradation products of fats and carbohydrates are of acidic nature—all would on hydrolysis or oxidation produce acids ; it matters not, however, for the purposes of the present argument whether the acid products are produced from the stored-up food-stuffs, or from the bioplasm itself.

We have, therefore, two classes of products producible, viz., non-nitrogenous acidic bodies and nitrogenous bodies ; the production of the latter class, it has been shown, is stimulated by the presence of the former. In well-nourished animals there is, however, always an excess of ammonia present, hence in this case the tissues will not become acid *in vivo*. The excess disappears gradually, however, if the animal is deprived of food. A certain stage will then be reached, when the production of acid exceeds the amount of ammonia available for neutralization ; the autolytic enzyme then comes into play, liberates amino-acids, etc., which in their turn pass the alimentary tract, and by means of the metabolic processes there taking place liberate ammonia, which again inhibits the production of nitrogenous degradation products. Degradation of tissue should proceed, therefore, at a definite uniform rate. From the preliminary experiments carried out it seems that the production of acids is inhibited by the presence of acids ; we have,

\* Ammonia is also responsible for the production of sodium carbonate  $(\text{NH}_4)_2 \text{CO}_3 + 2 \text{NaCl} = \text{Na}_2\text{CO}_3 + 2 \text{NH}_4\text{Cl}$ .

therefore, a mechanism which tends to prevent excessive acidity or alkalinity of the liver ; in the presence of acid nitrogenous degradation products are formed, from which ammonia is produced in the digestive tract ; it is possible also that the presence of alkali stimulates the production of the non-nitrogenous acid bodies. Further research is needed on this point.

The production of nitrogenous degradation products proceeds also at such a rate that there is never a dearth of nitrogenous bodies in the blood stream, and the bioplasm of the tissues, especially the mucous membrane of the small intestine, can remain saturated with side chains, for we have seen that the liver of a fasting animal contains more residual nitrogen than does that of a well-fed animal.

From these researches, it follows, that while the animal derives most of its energy from the oxidation of carbohydrates, and rapidly eliminates nitrogen from albumens, the latter, for all that, play an important rôle in nutrition, for the degradation products are needed, not only to satisfy, as Folin has suggested, the needs of endogenous metabolism of the organism representing the wear and tear of the tissues, but also to supply sufficient ammonia to maintain a certain amount of general intracellular alkalinity. In the absence of this amount, nitrogenous equilibrium ceases to be maintained.

It remains, lastly, to consider the bearing of these conclusions in certain abnormal cases, resulting in what Speck has called tissue metabolism (*Nahrungstoffwechsel* as contrasted with *Kraftstoffwechsel*).

Four instances are cited by Speck, in which tissue degradation sets in, viz. : (i) lack of oxygen ; (ii) poisoning with phosphorus, arsenic, etc. ; (iii) withdrawal of water ; (iv) in fever.

The factors regulating the animal heat of the body are too little known to render it profitable to discuss the course of metabolism in fever ; whether high temperature is due to increased oxidation or not, is doubtful, as the results obtained by the investigation of the respiratory quotients are open to doubt. Little is known also of the course of metabolism in the absence of water.

The metabolism with lack of oxygen, and in phosphorus poisoning, have formed the subject of numerous researches, and several facts

have been discovered, which are in accord with the theory of metabolism just propounded.

The cases of deficiency of oxygen have been investigated by Fränkel and Geppert<sup>4</sup> and v. Terray<sup>5</sup>. The latter has shown that there is not only an increased nitrogenous output, but also an elimination of acids, such as lactic acid, in the urine. The cause of the increased nitrogenous output is here clear. Owing to lack of oxygen, the oxidation of carbohydrate does not proceed to the formation of the final product, carbonic acid, which is rapidly eliminated by the lungs; intermediary bodies, such as lactic acid, are formed, in the presence of which, the autolytic enzyme is brought into play, and so tissue degradation takes place. It is of interest to note, that increased nitrogenous output does not take place directly after a deficiency of oxygen; it generally follows in the succeeding days. This delay corresponds to the latent period of autolysis, and there is sufficient ammonia present to neutralize the lactic acid when first formed.

A similar explanation can be applied to the case of phosphorus poisoning, for Bauer<sup>6</sup> has shown that administration of phosphorus causes a diminished output of carbonic acid and a decreased oxygen consumption; it acts as a toxin to the oxydases.

We see from the above researches that the autolytic enzyme functions, by setting up tissue degradation when the food supply is insufficient for the energy needs of the organism; in this case, these needs are supplied by the utilization of degradation products of the tissue. *In this case autolysis is a strictly physiological process.*

There are, however, cases in which the process is pathological, as for example, in atrophy of the liver, in phosphorus poisoning, etc. Such conditions can result, as the above researches indicate, from a variety of causes, such, for example, as insufficient respiration, local stasis, etc. The difference between physiological and pathological autolysis is probably quantitative rather than qualitative; in the former case, it always proceeds at such a rate, that the tissue, by the mechanism already demonstrated, never becomes strongly acid; in the latter case, it is possible that the acid is produced at such a rate, that

the liver tissue becomes strongly acid, and degrades so rapidly that its functions are thrown out of gear, as in the case of an Eck fistula, and that instead of taking up ammonia after it is formed in digestion, and converting it into urea, it allows this body to circulate in the blood-stream and be eliminated in the urine. It is in these cases accompanied by lactic acid; the concurrent production of these two bodies is, in this case, no protective mechanism; it is a process which is pathological in the strictest sense of the word.

In such cases, the above researches would seem to indicate as a remedy the intravenous injection of carbonate of soda.

Another point of interest is the cause of toxæmia in such cases. Preliminary experiments have shown that the liver in an incipient stage of autolysis is intensely toxic. It remains to be determined whether this toxicity is due to the products of autolysis. Further researches will be undertaken for the elucidation of this point.

It remains also to apply the methods and principles already enunciated to be investigated of pathological cases; much light might be thrown on the pathogenesis of various diseases of the liver by the investigation of the autolysis, under various conditions, of organs from the *post-mortem* room. Such a field of research must be left to pathologists.

There are also various other problems which await solution. What is the mechanism, *e.g.*, of restitution of tissue after autolysis? Preliminary researches [Part V, Series III (*f*)] show that the products of tryptic digestion of caseine exert a powerful inhibitory effect on autolysis; it is possible, as already suggested, that the process may be made reversible.

Light might also be thrown on various problems of metabolism by the production of anti-sera to autolytic enzymes, and the investigation of the physiological properties of these sera.

Lastly, much remains to be ascertained as to the functions of the residual nitrogen; the investigation of the chemical differences of acting and secreting glands should throw some light on this subject.

It is proposed to continue research in these directions.

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**OBSERVATIONS ON HUMAN CHYLE.** BY J. MOLYNEUX  
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THE difficulty of establishing and keeping permanent a chylous fistula in animals has been a formidable barrier to the experimental investigation of the chyle flow. Further, the small amount of chyle which can be obtained from animals and the difficulty of collecting it place additional obstacles in the way of accurate work. For these reasons the rare cases of chylous fistula or, if I may suggest the term, chylorrhœa, which have been described from time to time as occurring in man, afford a favourable opportunity both for the examination and analysis of the chyle and for the solution of problems relating to the absorption and utilisation of ingested fats and allied substances.

Accounts of the chyle of man varying in degree and detail have been given by Johannes Müller<sup>(24)</sup>, Marchand and Colberg<sup>(21)</sup>, Hensen<sup>(18)</sup>, Gubler and Quevenne<sup>(13)</sup>, and Munk and Rosenstein<sup>(26)</sup>. The chyle in these cases was obtained from a fistulous opening, the work of the last two investigators being the best and most exhaustive of any which has yet appeared upon the subject. There are also accounts of chylous ascites by Quincke<sup>(30)</sup>, Whitla<sup>(38)</sup>, I. Strauss<sup>(37)</sup>, Hasebroek<sup>(19)</sup>, and Minkowski<sup>(23)</sup>, and of chylous urine by Erben<sup>(8)</sup>, Chabrie<sup>(6)</sup>, Brieger<sup>(3)</sup>, Grimm<sup>(9)</sup>, and Carter<sup>(5)</sup>. Cases in which chyle, obtained from the cut thoracic duct, has been investigated are described by Rees<sup>(31)</sup>, Paton<sup>(29)</sup>, Carlier<sup>(4)</sup>, and H. Strauss<sup>(36)</sup>.

The observations of the above workers have shown that the general characters of human chyle are the same as those of animals but their observations are incomplete in many points and it was desirable to take any opportunity that offered of making a detailed examination under varying conditions. Such an opportunity was afforded to me by the kindness of Mr Barker who had under his care in University College Hospital a patient with a chylous fistula.

The patient was a well developed man, aged 20, weight 140 lbs. Except for the abnormality immediately to be described he enjoyed

fairly good health and applied for treatment only on account of the inconvenience which the condition occasioned.

His lungs and heart were normal, and no enlarged lymphatic glands could anywhere be detected. Over the lower part of the abdomen below the umbilicus and to some extent over the right buttock was a soft œdematous swelling. The right thigh and leg were also involved but here the œdema was much firmer in character than in the abdominal region. The scrotum was but little affected. The skin in the region of the right groin was covered with a large number of vesicles varying in size from a pea to a walnut. On the summit of one of these vesicles situated in the region of the saphenous opening was a small hole from which chyle issued.

The abnormality was first noticed as a swelling in the groin when the patient was ten years old. Four years later it became septic and was diagnosed as a psoas abscess. An operation was performed and a large amount of watery fluid escaped, the wound closing up for the time being. Eventually, however, it re-opened and allowed of the intermittent escape of a thin watery fluid. Septic trouble supervened from time to time, the swelling gradually extending, the fluid discharge becoming milky and increasing in quantity until the condition already described was attained.

The lymphatic fistula with its accompanying œdema is probably due to congenital obstruction of the thoracic duct.

*The Chyle flow.* From the open mouth of the fistulous opening in the upper part of the thigh made by the patient himself and maintained by him as a means of relief a milky fluid constantly trickled away. The flow could easily be checked by a firmly adjusted bandage but as the fluid, which was constantly being formed, accumulated in the subcutaneous lacunæ the tension increased to such an extent as to become insupportable and had to be relieved by allowing some of the chyle to escape. At these times, when the controlling power of the bandage was removed the pent-up chyle spurted forth in a jet of considerable force. In this way comfort could be obtained if about 100 c.c. of fluid were drawn off each day. As we shall see later on, the daily production of chyle is enormously greater than this; it follows, therefore, that the blocking of the duct is by no means complete and that when the pad and bandage are in position the greater part of the chyle is absorbed, probably through dilated collateral channels, its passage being aided by the increase of pressure consequent upon the continual accumulation of chyle behind the obstruction.

## I. THE CHARACTERS OF THE CHYLE.

The chyle as it flowed from the fistula was an opaque milky fluid; when examined in bulk, however, the colour of samples was found to vary slightly from time to time. Thus, sometimes the chyle was bluish-white, at others it had a yellowish tinge; the yellowish samples were always found to have the highest fat content. In no case, even after meals rich in fat, did the fat in the chyle separate out as a definite layer like cream on milk; after standing for 24 hours the chyle remained homogeneous throughout. Munk and Rosenstein<sup>(26)</sup> observed a separation into layers after their patient had been fed on mutton fat; these observers also noticed that the chyle lost its milky appearance and became a translucent opalescent fluid after at least twelve hours abstinence from food. I was never able to obtain the chyle in this condition owing to the patient's objecting to remain without food for the necessary period. The chyle collected before breakfast had a low fat content which gave it a characteristic bluish-white tint, this, however, was the nearest approach I obtained to a fat-free chyle.

The chyle was without odour, it had a saltish taste and was alkaline to litmus paper. On standing for a few minutes it clotted, forming a bulky loose coagulum which, after a few hours, contracted down to a small shreddy mass floating in the main bulk of fluid. Clotting could be prevented if the chyle were received into a little potassium oxalate solution as it left the body. If calcium chloride were then added in excess of that required to remove the oxalate from the solution, the chyle clotted in a few minutes. It has been stated by Müller that chyle on standing in air acquired a reddish colour; this has been denied by Owen Rees<sup>(30)</sup>, who says that the appearance was due to the contamination of the chyle with red blood corpuscles. Munk also, in the chyle which he collected, failed to observe this alleged development of colour. My own observations are in entire agreement with these two writers, in no sample of chyle which I examined did a pink colour ever develop on exposure of the chyle to the air.

On microscopical examination of the chyle the fat appeared in the form of exceedingly minute particles floating in the surrounding fluid; a few larger droplets of fat were also present but these were never so numerous nor so large as those which occur in milk. A few leucocytes and, after the examination of several fields, an occasional

isolated red blood corpuscle could be seen. If 100 c.c. of oxalated chyle were centrifuged a small sediment separated out at the bottom of the vessel. This sediment was faintly pink in colour and on microscopical examination was found to consist chiefly of leucocytes with a few red blood corpuscles and epithelial cells amongst them. The sediment obtained from the chyle collected during the period when the tissue surrounding the fistula was in a state of inflammation was slightly larger in amount and was much pinker owing to the presence of more red corpuscles. The number of red corpuscles in the chyle was of course a measure of the degree of contamination of the chyle with blood from the capillaries in the walls of the spaces in which the chyle was contained. It is thus evident that the chyle obtained when the patient's temperature was normal was of a high degree of purity and exceedingly free from foreign elements derived from the structures with which it came into contact.

*The rate of the Chyle flow.* In order to obtain some idea of the amount of chyle which could be produced in a day the bandage was removed from the fistulous opening and the chyle was allowed to trickle away into a vessel placed to receive it. In this way over 4 litres of chyle were collected in twelve hours. The experiment was then discontinued at the patient's request owing to the great exhaustion which it produced. Noel Paton<sup>(29)</sup> estimated the flow in a case under his observation at about 4 litres per day.

*The composition of the Chyle.* At midday as much chyle as possible was allowed to escape from the fistula which was then firmly bandaged. At 10 p.m. of the same day the bandage was removed and the chyle which had formed during the interval was collected. The bandage was then replaced and at midday of the following day the chyle formed during this second interval was also collected. The two samples thus obtained were then mixed and used for the following determinations:—

*The Specific gravity* was 1·007. During this research three other measurements were made, none of which differed materially from the number given.

*The total Solids* estimated in 4·03 grams of chyle amounted to 3·87%. The ash from the same chyle was ·83% of the whole.

*The fat.* 50 c.c. of chyle were mixed with sand and evaporated to dryness on a water-bath. The residue was extracted in a Soxhlet with dry ether for 12 hours. The ether was then evaporated off from the extract and the fatty residue weighed; ·672 gms. of fat were obtained. or 1·344 gms. per 100 c.c. of chyle.

The estimation of the total nitrogen was made by Kjeldahl's method. 5 c.c. of the chyle was found to contain .0182 gms. of nitrogen or .364 gms. of nitrogen per 100 c.c. of chyle.

*Nitrogen in the filtrate after treatment with trichloroacetic acid.* 100 c.c. of a 10% solution of trichloroacetic acid in water were added to 100 c.c. of the chyle and the precipitate which formed was filtered off. The nitrogen in 25 c.c. of the filtrate was determined by Kjeldahl's method and was found to be .0014 gms., or .0112 gms. of nitrogen per 100 c.c. of chyle. Another portion of the filtrate was acidified with hydrochloric acid and treated with phosphotungstic acid. No precipitate occurred, showing the absence of such nitrogenous substances as peptones and diamino-acids. Thus by far the greatest part of the nitrogenous constituents of the chyle are precipitable by trichloroacetic acid. The very small amount of nitrogen not so precipitated is probably due to traces of some simple amide such as urea, since a slight evolution of gas occurred when the filtrate from the trichloroacetic acid precipitate was treated with a solution of sodium hypobromite.

*For the estimation of Lecithin* two samples of chyle collected during a period of 24 hours were mixed together. The amount of phosphorus in the ether extract obtained from this chyle was taken as a measure of the quantity of lecithin present. The method employed for estimating the phosphorus was a modification by Bayliss and Plimmer<sup>(1)</sup> of Neumann's original method<sup>(28)</sup>. I found that the oxidation of the fat proceeded more rapidly when the nitric acid was added frequently and in small quantities as the mixture darkened in colour than when larger quantities at longer intervals were used. Two determinations gave the following results:—

(1) 3.460 gms. of ether extract required  $10.2 \text{ c.c. } \frac{\text{N}}{2} \text{ NaOH}$ .

(2) 2.163 gms. of ether extract required  $6.5 \text{ c.c. } \frac{\text{N}}{2} \text{ NaOH}$ .

which is equivalent respectively to:

(1) 12.93 mgs. of  $\text{P}_2\text{O}_5 = .370 \text{ gms. } \text{P}_2\text{O}_5$  per 100 gms. of ether extract.

(2) 8.24 mgs. of  $\text{P}_2\text{O}_5 = .371 \text{ gms. } \text{P}_2\text{O}_5$  per 100 gms. of ether extract,

or, 4.204 gms. of lecithin per 100 gms. of ether extract.

*Cholesterin.* 4.320 gms. of ether extract were taken and saponified by heating with alcoholic potash. The mass was evaporated to dryness

on a water-bath and the residue extracted with ether. The ethereal extract was then evaporated and the residue consisting of crystals of cholesterin was weighed. From 4.320 gms. of ether extract .225 gms. of cholesterin were obtained, *i.e.* 5.2 gms. of cholesterin per 100 gms. of ether extract.

No reducing sugar could be detected in the chyle; Fehling's solution showed no reduction when heated with the chyle itself or with the filtrate obtained from it after the addition of trichloroacetic acid.

*Enzymes.* Grohé<sup>(10)</sup>, and later Hensen<sup>(15)</sup> have demonstrated the presence of amylase in the chyle; Munk and Rosenstein<sup>(26)</sup>, in the case which they examined have confirmed these authors observations. The method I employed was as follows:—The chyle was filtered under pressure through a clay filter, the operation was a slow one owing to the readiness with which the fat in the chyle blocked the pores of the filter. Eventually, however, a slightly opalescent filtrate was obtained. Two test-tubes, each containing 1 c.c. of a boiled starch solution, were taken; 3 c.c. of chyle filtrate were added to one and 3 c.c. of boiled chyle filtrate to the other. Both tubes were kept at 37° C. with toluol for 15 hours. At the end of this time the boiled tube gave no reduction when heated with Fehling's solution whereas the unboiled tube gave a heavy precipitate of cuprous oxide.

The presence of lipase in the filtrate was proved by two different methods:—(1) Two test-tubes each containing 3 c.c. of the filtered chyle were taken; one of these was boiled and served as a control. 1 c.c. of neutral olive oil was then added to each tube and both were kept in the presence of toluol for 15 hours. At the end of this time the boiled tube was found to be still neutral whereas the unboiled tube required 1 c.c. of  $\frac{N}{10}$  NaOH to neutralise it. The second method employed depends upon the fact that amyl salicylate is split by a lipase into amyl alcohol and salicylic acid, which latter gives a violet coloration with a drop of ferric chloride. Amyl salicylate is for this purpose superior to other esters since it is very stable and does not easily hydrolyse in the presence of water alone. Two test-tubes each containing 1 c.c. of amyl salicylate were taken; 3 c.c. of the filtered chyle was added to one tube and 3 c.c. of the boiled filtered chyle was added to the other as a control. The tubes were then kept at 37°C. for 15 hours in the presence of toluol. At the end of this time a drop of ferric chloride was added to each of the tubes. No alteration was observed in the boiled tube but in the other a deep violet coloration was produced.

## II. THE DIURNAL VARIATION IN THE FAT CONTENT OF THE CHYLE.

In estimating the diurnal variation in the fat content of the chyle the patient received his usual daily diet. For breakfast he had fish or eggs with tea, bread and butter at 8 a.m. Dinner at noon consisted usually of meat, vegetables, and pudding. Tea, with bread and butter, was supplied at 4 p.m. and, in addition, at 7 p.m. the patient received a supper consisting of bread and meat or cheese. He complained of always being hungry and ate his meals with relish; he, however, disliked fat and ate as little of it as possible.

The chyle was collected in the following manner:—As much as possible was allowed to flow away at 10 p.m. on the day before the experiment. At 8.30 a.m. on the following day the first sample was collected, this represented the chyle which had accumulated during the night. The next sample was collected at 10.30 a.m. and so on at two-hourly intervals throughout the day. The last sample was taken at 8.30 p.m. The fat in 50 c.c. of each of these samples was then estimated by the method already described with the following result:—

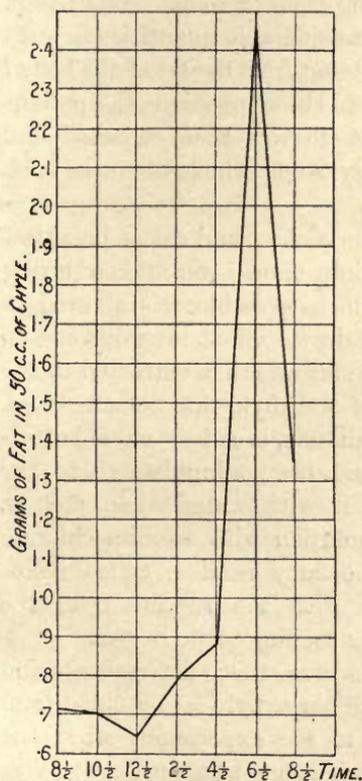


Fig. 1.

Time at which sample was taken	Gms. of fat in (a) 50 c.c. of chyle or, in (b) 100 c.c. of chyle	
	(a)	(b)
8.30 a.m.	·710	1·420
10.30 a.m.	·697	1·394
12.30 p.m.	·645	1·290
2.30 p.m.	·788	1·576
4.30 p.m.	·882	1·764
6.30 p.m.	2·455	4·910
8.30 p.m.	1·357	2·714

Thus with the ordinary diet as given above the fat content of the chyle rises rapidly to a maximum about 6 or 7 hours after the chief meal of the day. This of course corresponds with the time at which the food reaches the small intestine and at which fat-digestion is at its full height.

### III. THE RELATIONSHIP BETWEEN THE CHYLE-FAT AND THE FAT OF THE FOOD.

The question as to whether the fat in the chyle varies in nature according to the kind of fat taken in the food has been exhaustively investigated for the higher fats by Munk and Rosenstein<sup>(26)</sup> and others. With regard, however, to the fats of the glycerides of the lower fatty acids such as butyric acid we know nothing with certainty.

Analyses of human fat show amounts of the lower fatty acids small out of all proportion to the quantities taken in with the food. Thus Lebedeff<sup>(18)</sup> found that human fat contained only  $\cdot 02$  to  $\cdot 2\%$  of volatile fatty acids and Jaekle<sup>(16)</sup> from an analysis of several samples of fat obtained a Reichert-Meissl number varying from  $\cdot 2$  to  $\cdot 3$ . What then is the fate of these lower fats present in considerable quantities in such foods as butter and milk and yet almost absent from the fat of the body? In order, if possible, to obtain an answer to these questions the patient was fed on a diet rich in butter, the chyle was then collected and examined for the presence of lower fatty acids which occur in such large quantities in butter.

The patient was given as much butter as he could eat at breakfast and again at dinner; in all, more than eight ounces were taken during the day. Before breakfast as much chyle as possible was allowed to drain away and after this the chyle was drawn off at intervals of two hours throughout the day till 10 p.m. The fat was then extracted in the way already mentioned from the whole of the chyle thus collected and, in the investigation to be described immediately, is called "chyle butter-fat." A sample of the butter which had been administered to the patient was then purified by washing it with water when melted, dissolving it in ether, drying the ether solution with calcium chloride and then evaporating off the ether; the fatty residue, called below "pure butter-fat," was now comparable with the residue from the ether extract of the chyle obtained after feeding with butter.

The iodine value of these two fats was then determined and compared with the value obtained for ordinary chyle-fat obtained from the chyle collected some days previous to the experiment when the patient was on normal diet. The determinations were made by Wij's modification of the original Hubl method. As Lewkowitsch<sup>(19)</sup> has shown this method gives extremely accurate results and is much easier to use than the older process. The results obtained for the three fats were as follows:—

Ordinary chyle-fat	66.2
Butter chyle-fat	38.0
Purified butter-fat	31.0

Their Reichert-Meissl values were also determined :—

Ordinary chyle-fat	1.9
Butter chyle-fat	3.0
Purified butter-fat	27.5

From these results we see that as far as the higher fats, *e.g.* olein, are concerned the chyle comes to resemble very closely the composition of the butter-fat taken in the food. Very different, however, is the behaviour of the lower fats. Quite an insignificant amount of volatile fatty acids appear in the chyle as compared with the quantity present in the butter ingested. We see then that although the lower fats appear to be absorbed and to pass into the chyle they disappear there with surprising rapidity. Cohnstein and Michaelis<sup>(7)</sup> have shown that blood exercises a lipolytic action on fats causing them to disappear with the production of substances insoluble in ether but soluble in alcohol. The nature of these products is as yet doubtful. Mayer<sup>(22)</sup> has found that the sodium salts even of the lower fatty acids exert a toxic effect when injected into the blood stream, the toxicity increasing as we ascend the series. Munk<sup>(25)</sup> has shown that sodium oleate when injected is markedly toxic; on the other hand oleic acid itself produces no ill effects. In this connection, however, the nature of the products formed need not detain us; that lipolysis is the mechanism by which the disappearance of the fats is brought about seems to be established<sup>(20)</sup> and, we can account for the rapid disappearance of the lower fats on the assumption that lipolysis proceeds more rapidly and energetically in the case of the lower than the higher esters of a series. Support is lent to this view by the results obtained by Hanriot<sup>(12)</sup> in his investigations on the synthetic action of lipase. He found that in the case of the higher fatty acids the formation of esters took place more easily and to a greater extent than in the case of the lower members of the series and, conversely, the lower members underwent hydrolysis more easily and more completely than did the higher members of the series or, in other words, equilibrium is attained in the case of the higher members of a series only when relatively large quantities of ester are present and in the case of the lower members only when the amount of ester present is sufficiently small. The small amount of lower fatty esters in human fat really represents the equilibrium point conditioned and maintained by the supply of lower fatty acids in the food. In this connection the

experiments of Siegert<sup>(32)</sup> and Jaeckle<sup>(16)</sup> are of some interest. These observers found that the fat of quite young infants possessed a much lower iodine value and contained a much higher percentage of lower fatty acids than fat obtained from adults. Clearly, the large quantity of lower fatty acids in the food and therefore the correspondingly larger amount in the chyle has altered the equilibrium point and allowed of the storage of greater quantities in the tissues.

*The absorption of lecithin.* The fate of lecithin taken by the mouth has long been a matter of dispute. Bokay<sup>(2)</sup>, Nesbitt<sup>(27)</sup>, Kutscher and Lohmann<sup>(17)</sup>, and Paul Mayer<sup>(22)</sup> are of the opinion that it decomposes in the alimentary canal into cholin, fatty acids and glycerophosphoric acid. On the other hand Slowtsoff<sup>(35)</sup>, and Stassano and Billon<sup>(34)</sup> are disposed to believe that lecithin passes, at any rate to some extent, into the chyle and the last two writers<sup>(33)</sup> deny that pancreatic juice has any destructive action on lecithin.

The following experiment was made on this question:—The patient was given  $\frac{1}{2}$  oz. (14 gms.) of lecithin at 7 p.m., previous to which as much chyle as possible was drawn off (*A*). Chyle was then collected on the following day at 9 a.m. (*B*), 12 noon (*C*), and 6 p.m. (*D*). The fat was extracted in the usual way from these separate portions and the amount of phosphorus in portions of each sample determined by the method already described.

The results were as follows:—

<i>A</i>	=	·291	gms. of $P_2O_5$	per 100	gms. of ether extract.
<i>B</i>	=	·567	"	"	"
<i>C</i>	=	·487	"	"	"
<i>D</i>	=	·039	"	"	"

This single experiment shows a rise followed by a gradual fall in the amount of ether-soluble phosphorus in the chyle after a meal containing a large quantity of lecithin and goes to support the view that lecithin, at any rate in part, is capable of being absorbed and of passing into the chyle. It was unfortunately not possible to make a more extended series of observations on this subject owing to the objections of the patient to a continuance of the experiments.

*The effect of feeding on paraffin.* Since petroleum in the form of an emulsion has a certain vogue in the treatment of wasting diseases and since its efficacy in this respect is very doubtful this case seemed to afford a good opportunity of observing whether or not petroleum administered internally is absorbed and carried by the chyle into the general circulation. For this purpose two ounces (56 gms.) of liquid paraffin (*B.P.*) were thoroughly emulsified with tragacanth and were

given to the patient immediately after the midday meal. The chyle was then collected at two-hour intervals for ten hours after the meal. About 800 c.c. of chyle were thus obtained. The chyle was then saponified by boiling with caustic soda under a reflux condenser and finally the excess of water was distilled away. No paraffin came over in the distillate. The pasty residue was then extracted several times with ether. The ether extract was dried with calcium chloride and filtered; the filtrate was then distilled to get rid of the ether, nearly one gram of a crystalline residue, cholesterin, being left behind. No trace of paraffin was found in the residue after the removal of the ether. It is thus evident that paraffin does not pass through the walls of the small intestine into the chyle. Henricques and Hausen<sup>(14)</sup> in experiments conducted with the object of ascertaining whether fats were absorbed as such or in the form of soaps observed that paraffin given to animals by the mouth can be completely recovered from the fæces. I have therefore been able to confirm, in man, the result which they obtained by a different method, in the case of animals.

#### SUMMARY.

1. Human chyle varies in colour from a bluish-white to a yellowish tinge as its fat content increases. Its reaction is alkaline and it clots rapidly when removed from the body. Oxalates prevent its clotting. Microscopically, very fine particles of fat, leucocytes and occasionally a few red corpuscles are visible. As much as four litres of chyle have been obtained in 12 hours.

2. An average sample of chyle had the following composition:—

Total solids	= 3·87 %.
Ash	= ·83 %.
Fat	= 1·344 gms. per 100 c.c. of chyle (very variable).
Total Nitrogen	= ·364 gms. " " "
Extractive Nitrogen	= ·0112 gms. " " "
Lecithin	= 4·204 gms. per 100 gms. of ether extract.
Cholesterin	= 5·2 gms. " " " "

The presence of lipase and amylase was also proved.

3. The fat content of the chyle varied considerably during the day being greatest six hours after the principal meal.

4. When a mixture of lower and higher fats are given in the food the ratio of the higher to the lower fats is greater in the chyle than in the food.

5. Lecithin when given by the mouth produces a rise in the ether-soluble phosphorus of the chyle.

6. Petroleum (Paraffin liquidum *B.P.*) when given by the mouth cannot be recovered from the chyle.

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THE CONTRACTILE MECHANISM OF THE GALL-BLADDER AND ITS EXTRINSIC NERVOUS CONTROL<sup>1</sup>. BY F. A. BAINBRIDGE AND H. H. DALE.  
(Eighteen Figures in Text.)

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*Introduction.* The experiments here recorded were undertaken as a preliminary to a general consideration of the causes which lead to the pouring of bile into the duodenum at the stage of digestion when its presence is required. The investigation has proved more complicated than was anticipated; and it seems better to communicate at once our observations on the contractile mechanism of the gall-bladder, reserving for future discussion experiments on the actual process of secretion.

*Methods.* All the experiments were made on dogs, fully anaesthetised with the A. C. E. mixture, after a preliminary dose of  $\frac{1}{2}$ —1 gr. of morphine. Direct observation of the contractions of the gall-bladder was found to be impossible, the movements being too slow to be accurately followed. Various methods of mechanical recording were, therefore, given a trial. To the obvious method of inserting a cannula into the common duct and connecting with a water-manometer there are two fatal objections: it is impossible to be certain that nerves coursing up the duct to the bladder are not injured; and the presence of valves in the common duct makes it impossible to refill the bladder when once it has emptied itself, so that a relaxation cannot be recorded. The record so obtained, in fact, is merely that of the maximum pressure exerted by the gall-bladder during the experiment. An attempt was made to avoid these difficulties by passing a small gum elastic catheter up the common duct into the gall-bladder: but, in this case, the viscosity of the bile and the necessarily narrow bore of the catheter formed a combination fatal to an accurate record.

<sup>1</sup> The expenses of this research were in part defrayed by grants from the Government Grants Committee of the Royal Society, and the Scientific Grants Committee of the British Medical Association.

The following method which we ultimately adopted, and found in most ways quite satisfactory, is that devised by Doyon<sup>1</sup>, with modification in details. A thin balloon of india-rubber is tied over the end of a gum elastic catheter, about 1 inch of the catheter being inside the balloon. The catheter is connected by a length of small-bore india-rubber tubing with a wide glass reservoir half filled with water, and usually at a height of about 15 cm. above that of the gall-bladder. The upper end of this reservoir is connected by rubber-tubing with a small Hürthle piston-recorder. The balloon is inserted in the deflated condition through a small cut made in the fundus of the gall-bladder, tied in, and then filled with warm water from the reservoir.

In our earlier experiments the abdominal wound was closed. Under such conditions several evident sources of error presented themselves. Variations of external pressure to which the gall-bladder yields passively are registered by the recorder and may be quite indistinguishable from the effects of active contraction of the muscular wall of the bladder itself. Such variations may be caused by:—

- (1) Varying contraction of the abdominal walls and diaphragm.
- (2) Activity of contiguous contractile viscera.
- (3) Changes in volume and turgescence of the liver substance, with which the gall-bladder is frequently in contact over the greater part of its surface.

These difficulties were met as follows:—

(1) The animal was either fully curarised, or the chest was opened, the phrenic nerves cut and the diaphragm divided according to a method to be described later. The abdominal walls were widely opened by a crucial incision and the flaps held back by weighted hooks, the gall-bladder being protected from cold and evaporation by frequently renewed flannels soaked in warm saline, or by immersing the animal in a warm saline bath. If insufficient attention be given to this protection against cooling it is very easy, when the sympathetic nerve-supply is stimulated, and especially when adrenalin is the mode of stimulation, to produce an effect of simple hyperæmia of the gall-bladder muscle, which will be described later.

(2) Care was taken to prevent contact with contractile viscera by drawing these aside with hooks and screening the gall-bladder with sponges and flannels. These measures, however, were merely precautionary, since the danger of disturbance from this cause is but slight

<sup>1</sup> Doyon, *Archives de Physiol.* xxv. p. 678. 1893.

when the abdominal walls are freely opened. We have, in fact, deliberately allowed contact between a vigorously contracting stomach and the gall-bladder, under such conditions, without being able to detect any indication of the contractions upon the gall-bladder record.

(3) Upon the effect of changes in the volume of the liver we intend to lay some stress, as it has been completely left out of consideration in earlier investigations. We found it essential to eliminate this influence either by separating the gall-bladder from the liver, or by a dissection which gave the liver perfect freedom of expansion.

Another precaution taken was the registration of the blood-pressure simultaneously with the contractions of the gall-bladder. This is omitted in the tracings unless there is special reason for giving it.

#### I. SPONTANEOUS CONTRACTIONS.

A record of the volume of the gall-bladder, obtained as above described, shows spontaneous variations, which occur rhythmically at the rate of 1 to 3 per minute (Fig. 1); though noticeable even at the beginning of an experiment, this rhythm is usually more obvious after section of the splanchnic nerves, and still more so after the injection of

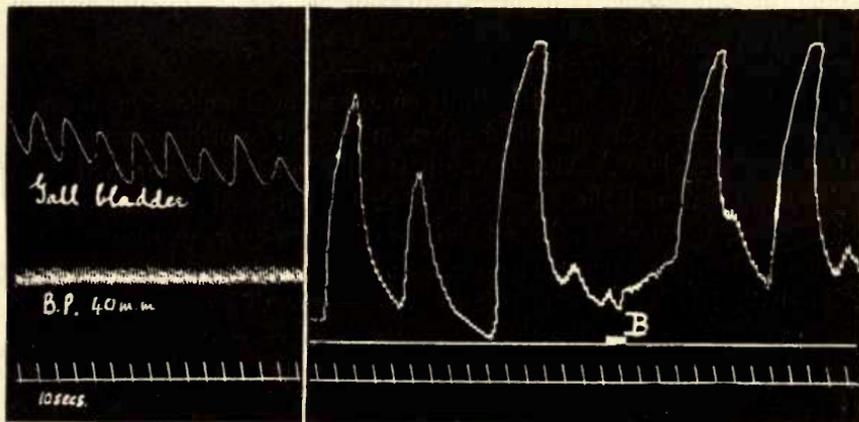


Fig. 1.

Fig. 2.

- Fig. 1.  $\times \frac{1}{17}$ . To show the intrinsic rhythm of the gall-bladder. The animal was under curare, the gall-bladder separated from the liver. Vagi and splanchnics cut.
- Fig. 2.  $\times \frac{1}{17}$ . Curare. Vagi and left splanchnic cut. Pressure on balloon in gall-bladder raised to 50 cm. of water, the exaggerated rhythm resulting. Right splanchnic cut at B, section causing momentary inhibition of rhythm. The small undulations in this and succeeding tracings were produced by the artificial respiration.

chrysotoxin. It is as well developed when the gall-bladder has been separated from the liver as when it is left in its natural relations, and it is clear that the undulations in question represent a genuine rhythmic contraction and relaxation of the gall-bladder muscle itself. On this point our experience is merely confirmatory of that of Doyon<sup>1</sup>, who further detected a similar rhythmic activity in the gall-bladder even after its complete removal from the body.

Occasionally, but not always, we found that raising the water reservoir, and thereby subjecting the gall-bladder to an excessive distending force, produced great exaggeration of this rhythm, so that the record gave the picture of a series of violent contractions alternating with deep relaxations (Fig. 2). Diminution of the pressure restored the normal slight rhythm.

## II. SYMPATHETIC NERVOUS SUPPLY.

Heidenhain<sup>2</sup> found that stimulation of the splanchnic nerves caused an initial increase in the flow of bile, followed by a diminution. He attributed the increase to contraction of the gall-bladder and bile passages.

Langley<sup>3</sup> found, on the other hand, that intravenous injection of supra-renal extract caused an increased flow of bile, which was preceded by a brief phase of slowing if the gall-bladder was not excluded by a clip. He suggested that the extract probably caused a relaxation of the gall-bladder tone.

The only direct observations made until recently, of the effect on the gall-bladder of stimulating the splanchnic nerves, are those of Doyon<sup>4</sup>. Using the balloon method of recording, with the apparatus arranged for recording changes of pressure rather than of capacity, he described a prolonged, slow contraction of the gall-bladder as the result of stimulating either splanchnic nerve. With adrenalin he subsequently obtained a similar effect.

A paper recently published by Freese<sup>5</sup> also gives an account of the nervous control of the gall-bladder. He appears to have confined his attention to the splanchnics, and obtained varying results—motor,

<sup>1</sup> Doyon, *Arch. de Physiol.* xxv. pp. 678, 710. 1893.

<sup>2</sup> Heidenhain, *Stud. d. Phys. Instit. z. Breslau.* Parts II and IV. p. 227. 1861—1868.

<sup>3</sup> Langley. *This Journal*, xxvii. p. 237. 1901.

<sup>4</sup> Doyon, *loc. cit.*, and *Arch. de Physiol.* xxvi. p. 19. 1894.

<sup>5</sup> Freese, *Bulletin*, Johns Hopkins Hospital. June, 1905.

inhibitory, or polyphasic, with a preponderance of motor effects—very similar to those described below as obtained in our own earlier experiments. He concludes that the splanchnics contain both motor and inhibitory fibres for the gall-bladder, deciding that the motor effect is genuine on the ground that it can be obtained after bleeding the animal to death.

Our own earlier experiments on the effect of stimulating the splanchnic nerves and of intravenous administration of adrenalin led to conflicting results. In some cases we obtained a result apparently identical with that of Doyon: in others we observed pure relaxation: in others again we found mixed effects, di- or even triphasic curves being

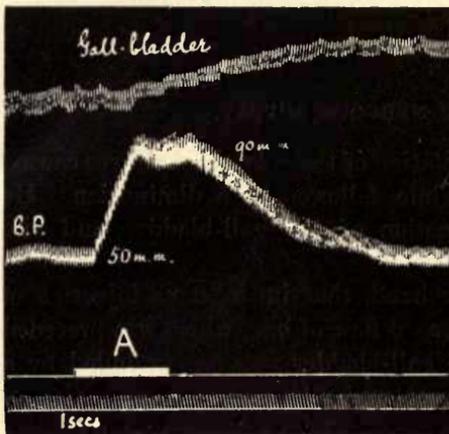


Fig. 3.

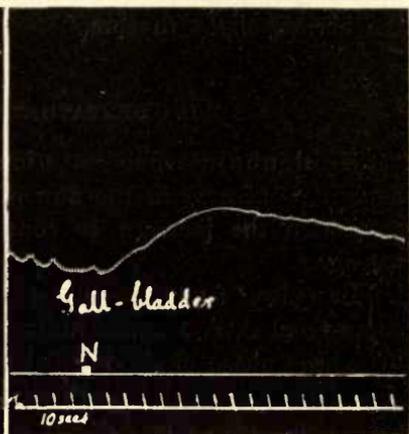


Fig. 4.

Fig. 3.  $\times \frac{1}{18}$ . Curare. Gall-bladder not separated from liver. Right splanchnic cut, the peripheral end being placed on Ludwig electrodes. Blood-pressure from carotid.

At A stimulation of right splanchnic, coil at 3000 (Berne graduation).

Fig. 4.  $\times \frac{1}{18}$ . Curare. Gall-bladder not separated from liver. At N 4 c.c. 1 in 50,000 adrenalin chloride intravenously. Usual rise of blood-pressure.

produced. Figs. 3 and 4 show the apparently motor response to sympathetic stimulation often obtained when the gall-bladder is left *in situ*.

Thinking that this variety of result was due to the presence of at least two conflicting effects we attempted to determine which, if any, of these were due to extraneous causes, and which to intrinsic movements of the gall-bladder. We inserted between two adjacent lobes of the liver a balloon exactly similar to that in the gall-bladder and similarly con-

nected to a piston-recorder. This led to the discovery that the effects of apparent contraction observed in our tracings, and described by Doyon as the true and sole effect of stimulation of the sympathetic nerve-supply, were all produced equally well in the record taken from the control balloon. Whether the splanchnic nerves were faradised or adrenalin given intravenously this "artificial gall-bladder" always simulated contraction, even when the tracing from the gall-bladder itself indicated pure relaxation.

It was clear, therefore, that one of the effects complicating our records was the swelling of the liver which is caused by stimulation of the splanchnic nerves, and still more by the injection of adrenalin. The effect of stimulating the sympathetic nerve-supply was accordingly reinvestigated after removal of this complication as far as possible.

This was effected successfully by two methods. The first was the simple separation of the gall-bladder from the liver, down to the commencement of the cystic duct. This is easily performed by snipping through the peritoneal investment, where it passes from the liver on to the gall-bladder, inserting a finger into the opening thus made and gently tearing the gall-bladder away from its attachment. Although a small amount of liver substance always remains adherent to the gall-bladder, the bleeding is negligible. This method satisfactorily eliminates the effect of alteration in the liver volume, but irregular contractions of the diaphragm still complicate the tracing, and it is therefore necessary to give curare.

The second method obviated the use of curare and the handling of the gall-bladder in separating it from its attachments. Its principle was to allow free expansion of the liver by destruction of the dome of diaphragm normally enclosing it and resisting its outward enlargement. The chest was opened by longitudinal division of the sternum, the tendon of the diaphragm slit down to the vena cava, and the diaphragmatic muscle paralysed by section of the phrenic nerves. The chest was held open by weighted hooks so that the halves of the diaphragm gaped widely apart, the liver fell back into the cavity of the thorax, and the gall-bladder was brought uppermost. The liver was thus given perfect freedom of expansion, and its swelling merely raised the gall-bladder as a whole to a slight degree.

By both these methods we obtained uniform results. Under normal conditions—that is, unless both the blood-pressure and the tone of the gall-bladder had fallen very low, as at the end of a long experiment—relaxation was the invariable result of faradising the right splanchnic

nerve or intravenous injection of adrenalin. Fig. 5 shows the effect of stimulating the right splanchnic after opening the chest wall and slitting the diaphragm. Stimulation of the left splanchnic under such conditions, if it produces any effect at all on the gall-bladder, merely causes a small gradual increase in tone and rhythmic activity, such as accompanies a rise of general blood-pressure from any cause (Fig. 6). In Figs. 7 and 8 is shown the effect of adrenalin with the chest wall and diaphragm intact, the difference produced by separating the gall-bladder from the liver being clearly exhibited. It will be seen that the relaxation, which in Fig. 7 appears as a mere preliminary dip in a curve giving the impression of a predominantly motor effect, is shown in Fig. 8 to be the essential feature of the response to adrenalin. The pressure exerted on the gall-bladder by the swelling of the liver is further illustrated in both figures by the record obtained from the "artificial gall-bladder," which simulates a contraction. Another feature of Figs. 5 and 8 has yet

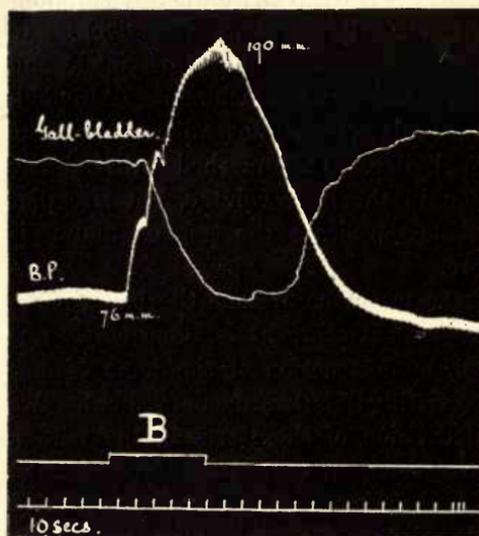


Fig. 5.

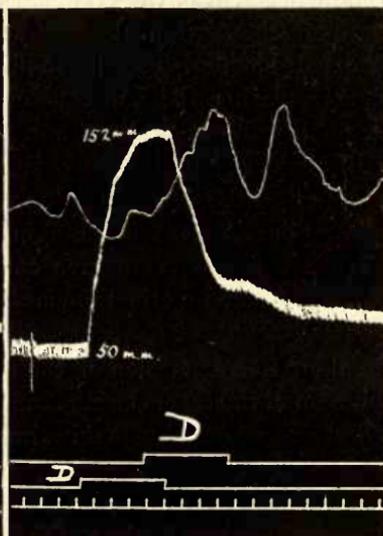


Fig. 6.

Fig. 5.  $\times \frac{1}{2}$ . Chest opened, diaphragm divided, phrenics cut. No curare. Right splanchnic dissected from the front, ligatured and cut above ligature. At B peripheral end of right splanchnic stimulated with coil at 10 cm.

Fig. 6.  $\times \frac{1}{2}$ . Conditions as in Fig. 5. Left splanchnic dissected from the front: at D the peripheral cut end of it stimulated with coil at 10 cm. The rise of b.-p. is accompanied by increase in tone of gall-bladder. (The point of the piston-lever not being opposite that of the manometer float, there are two signal lines: the upper for the gall-bladder, the lower for the blood-pressure.)

to be discussed. In both it can be seen that the record of the gall-bladder response to sympathetic stimulation shows, apart from the false effect of extraneous pressure, a true rise of tone, succeeding the inhibitory fall, and persisting for some time. It is probable that the improvement of blood-supply, caused by the greater permanence of the general than of the local effect of the sympathetic stimulation, is mainly responsible

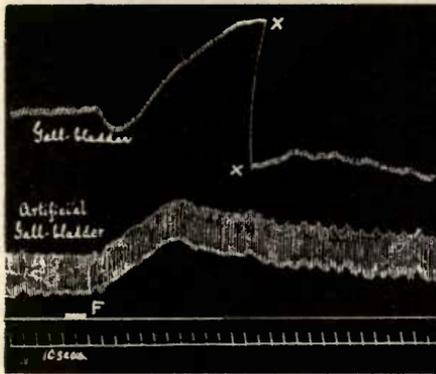


Fig. 7.

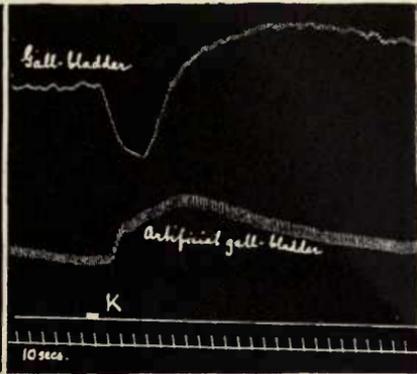


Fig. 8.

Fig. 7.  $\times \frac{11}{3}$ . Curare. Gall-bladder not separated from liver. "Artificial gall-bladder," between lobes of liver. Vagi and splanchnics cut. At *F* 4 c.c. 1 in 30,000 adrenalin chloride intravenously. Usual rise of blood-pressure. Between *X*—*X*, the lever of recorder having reached its limit, air was let out of recorder.

Fig. 8.  $\times \frac{11}{3}$ . From same experiment as Fig. 7, after separation of gall-bladder from liver. At *K* 6 c.c. of 1 in 30,000 adrenalin chloride intravenously. The usual rise of blood-pressure occurred.

for this. In harmony with this view is the greater prominence of this after-tone with adrenalin than with stimulation of the right splanchnic. The hyperæmia of the abdominal viscera, as the effect of an adrenalin injection wanes, is a well-known phenomenon. We have already mentioned the fact that stimulation of the left splanchnic, which we hold to be without direct effect upon the gall-bladder, produces a rise of its muscular tone, which we regard as an effect of the kind under discussion, due to the rise of general blood-pressure.

When the blood-pressure and the tone of the gall-bladder have both sunk to a low level, as at the end of a long experiment, we have several times observed a true increase of the tone of the gall-bladder in response to an injection of adrenalin. Fig. 9 shows such an effect. In this case the injection of adrenalin caused a permanent improvement in the blood-pressure, and to this we attribute at least the greater part of the

permanent improvement in the tone of the gall-bladder. A succeeding dose of adrenalin caused in this case a further rise of blood-pressure and a further increase in the tone of the gall-bladder. On the other hand, under conditions which allow the sympathetic stimulation to occur

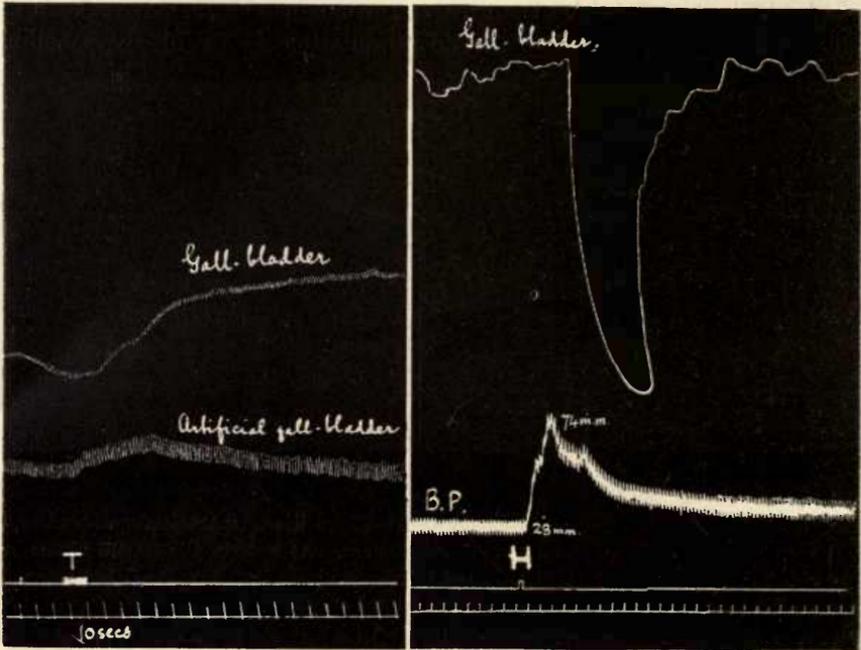


Fig. 9.

Fig. 10

Fig. 9.  $\times \frac{11}{8}$ . Curare. Gall-bladder separated from liver. From the end of a long experiment. Blood-pressure had become very low (20 mm.); so had the tone of gall-bladder. At T 5 c.c. of 1 in 50,000 adrenalin intravenously.

Fig. 10.  $\times \frac{11}{8}$ . Chest opened, diaphragm divided, phrenics cut. No curare. Vagi and splanchnics cut. Blood-pressure from carotid. At H 1 c.c. 1 in 10,000 adrenalin intravenously.

without a large simultaneous rise of blood-pressure, the gall-bladder responds by pure relaxation without any after-tone. For example, the effect of adrenalin on the blood-pressure is greatly reduced by leaving the vagi intact, or when, as is often the case, the heart is weakened by the exposure entailed in opening the chest. Under either of these conditions we have obtained pure relaxation of the gall-bladder in response to adrenalin, without after-tone (Fig. 10). Again, when, by the administration of chrysotoxin<sup>1</sup>, the endings of motor sympathetic fibres are

<sup>1</sup> Cf. Dale. *Proc. Physiol. Soc.* p. lviii. 1905. (*This Journal*, xxxii.)

paralysed, adrenalin produces practically no effect on the blood-pressure. Under these conditions the inhibition of the gall-bladder is very prolonged and quite uncomplicated by after-tone<sup>1</sup> (Fig. 11).

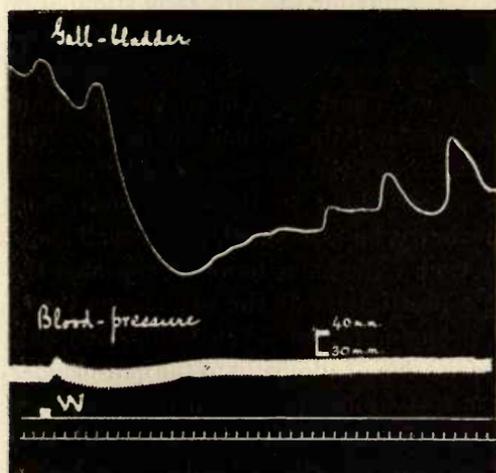


Fig. 11.

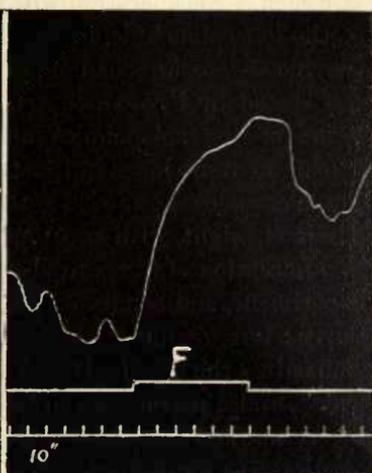


Fig. 12.

Fig. 11.  $\times \frac{1}{2}$ . From same experiment as Fig. 9, after intravenous injections of sodium chrysotoxin amounting to 0.2 gm. in all. At *W* 1 c.c. of 1 in 10,000 adrenalin intravenously.

Fig. 12.  $\times \frac{1}{2}$ . Conditions as in Fig. 5, but towards the end of an experiment, when animal was moribund, and blood-pressure very low (20 mm.). At *F* peripheral end of rt. splanchnic stimulated with coil at 8 cm. Blood-pressure rose to 40 mm.

When, however, due allowance has been made for the undoubtedly large part played by blood-pressure changes in producing these motor effects, and when, further, the simulation of motor effects by changes in liver-volume, which complicates the experiments of Doyon and of Freese, has been definitely excluded, there remains a small residuum of phenomena not satisfactorily accounted for. The difficulty, in fact, is not dissimilar to that met with by various observers in dealing with the effect of stimulating the splanchnics on the movements of the small intestine<sup>2</sup>.

When the tone of the gall-bladder and the blood-pressure have both become low we have occasionally provoked a decided contraction by

<sup>1</sup> Here, however, the possibility must not be neglected of an admixture of motor fibres in the sympathetic supply to the gall-bladder. Chrysotoxin would paralyse these.

<sup>2</sup> Cf. Bunch. *This Journal*, xxii. p. 357, 1898, who gives the literature of the subject: also Bayliss and Starling. *This Journal*, xxiv. p. 99. 1899.

stimulating the right splanchnic nerve (Fig. 12). The possible effect of the rise of blood-pressure must be borne in mind, though in one such case exposure and cooling of the intestines had rendered this very small: but such a consideration will not account for the fact that stimulation of the left splanchnic, in the same experiment, while more effective on the blood-pressure, had no such effect on the gall-bladder. Again, it was stated by Freese that this motor effect was obtainable by stimulation of the splanchnics after bleeding the animal to death. The tracing he reproduces in illustration shows the effect of stimulating with the electrodes applied to the cystic duct; so that the contraction obtained might, with great probability, be attributed, as we shall show, to stimulation of fibres from the vagi. We have, however, repeated the experiment, and confirmed the result, stimulating the splanchnics themselves: but we must add that, in our experience, the right splanchnic alone gives the result. Direct application of adrenalin to the gall-bladder, which, under normal conditions of tone, causes inhibition, has sometimes a very marked motor effect after bleeding to death (Fig. 13). This contraction was, in one experiment, of sufficient vigour for detection by the

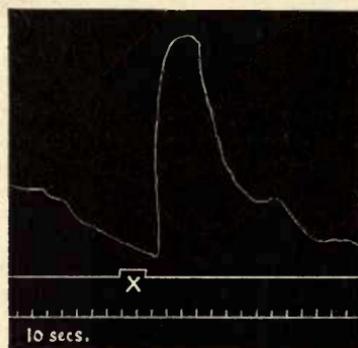


Fig. 13.  $\times \frac{1}{2}$ . Effect of directly applying adrenalin to gall-bladder of an animal bled to death. The solution used was 1 in 1000 pure adrenalin chloride, without preservative.

naked eye, and had the appearance of being limited to about one-fifth of the gall-bladder; the part affected being that adjoining the cystic duct. To this point we hope to give further investigation, and it will suffice now to point out that the conditions under which the result is obtained—complete anæmia, leading to intense inhibition of the fundus of the gall-bladder—are just those which would enable a localised motor effect to be recorded by the balloon or similar mechanical contrivance for registration. Whether the distribution be thus localised or not, we cannot

doubt that there is some admixture of motor fibres in the sympathetic nerve-supply to the gall-bladder. This, however, requires special conditions for its detection, and when the tone is maintained by an unimpaired circulation we find that inhibition is the invariable effect of exciting the sympathetic nerve-supply, and are disposed to attribute most of the motor effects described by previous observers to extraneous pressure. We find, further, that the sympathetic fibres to the gall-bladder run in the right splanchnic nerve only.

### III. EFFECT OF THE VAGI.

Doyon<sup>1</sup> regarded the vagus as carrying only afferent impulses from the gall-bladder. On stimulating the central end of either vagus he observed inhibition of the gall-bladder tone. Presumably both the splanchnics were left intact. He obtained a similar result by stimulating the central end of either splanchnic when the other was intact.

Courtade and Guyon<sup>2</sup>, accepting Doyon's account of the motor function of the splanchnics, examined the vagi for efferent fibres to the gall-bladder. Stimulating these nerves in the thorax they observed a motor response from the gall-bladder. They state that these motor fibres run in the gastric branches of the vagus, and claim to have traced them along the lesser curvature of the stomach to the cystic duct.

Our own experiments have been few in number, and have been confined to the confirmation of the motor effect of the vagus, without tracing the course of the fibres beyond the thorax. The effect is, in our experience, quite a small one and by no means easy to demonstrate. Stimulating the vagi peripherally in the neck, after a dose of atropine sufficient to abolish cardiac inhibition, we only once succeeded in producing an effect on the gall-bladder, and that merely a slight, though distinct and regularly produced augmentation of existent rhythmic contractions. Stimulation of the vagi intra-thoracically, even after section of the splanchnic nerves on both sides, gave no clear and unmistakable result unless chrysotoxin had been previously administered. It has been shown, in other connexions, that this drug, while not affecting sympathetic inhibitory nerve-endings to such an extent as to abolish the effect of adrenalin or of electrical stimulation, so far alters them as to block the normal tonic inhibitory impulses from the central nervous system or the ganglionic plexus, allowing stimulation of the cranial

<sup>1</sup> Doyon. *loc. cit.*

<sup>2</sup> Courtade and Guyon. *C. R. Soc. de Biol.* lvi. pp. 313 and 874. 1904.

autonomic supply to produce its full effect. The intestine, for example, after a large dose of chrysotoxin, responds more vigorously to stimulation of the vagus than it does after section of the splanchnic nerves only. We have found the same effect in the case of the gall-bladder. After a large dose of chrysotoxin (0.2 gram or more for a medium-sized dog),

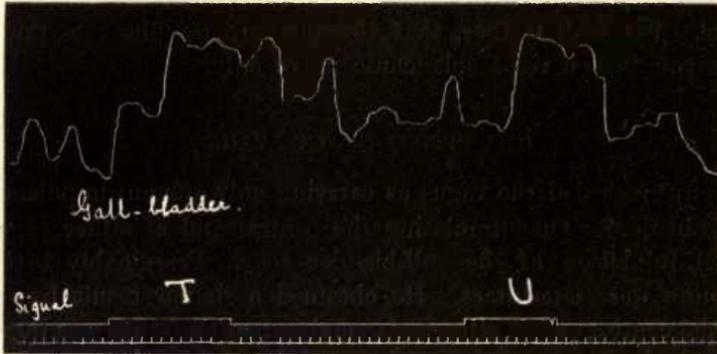


Fig. 14.  $\times \frac{1}{2}$ . From same experiment as Figs. 10 and 11. After 0.2 gm. chrysotoxin. At *T* and *U* peripheral end of left vagus stimulated in the thorax with coil at 6 and 5 cm. Blood-pressure unaffected.

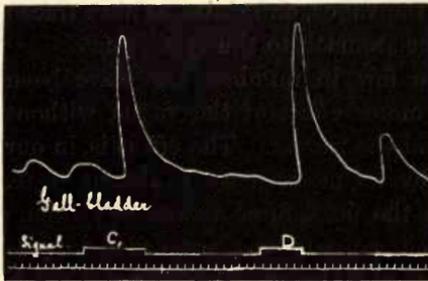


Fig. 15.

Fig. 15.  $\times \frac{1}{17}$ . Conditions as in Fig. 14. At *C* and *D* the left vagus stimulated peripherally in the thorax, coil at 4 cm. Blood-pressure unaffected.

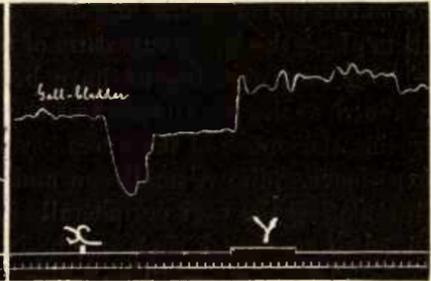


Fig. 16.

Fig. 16.  $\times \frac{1}{17}$ . Conditions as in Figs. 14 and 15, with less sensitive recorder. 0.2 gm. chrysotoxin given. At *X* 1 c.c. 1 in 10,000 adrenalin intravenously. At *Y* left vagus stimulated peripherally in the thorax: coil at 3 cm. The effects of sympathetic and vagus stimulation are contrasted.

stimulation of either vagus nerve in the thorax always causes a distinct augmentor effect on the gall-bladder, in which either increase of tone or exaggeration of rhythm may be the predominant feature, whilst in other cases, again, both may be simultaneously affected. In our experiments

the left vagus has always given a more marked effect than the right. (Figs. 14, 15, 16.)

#### IV. EFFECT OF VARIATION IN THE BLOOD-SUPPLY.

The stimulant effect on the gall-bladder muscle of hyperæmia has already been described in analysing the effects of injecting adrenalin into the circulation. As might be expected, the opposite holds good; anæmia produced by occlusion of the thoracic aorta causing a very marked inhibition, which affects the tone rather than the rhythmic activity of the muscle. The genuine nature of the effect is proved by obtaining it after separation of the gall-bladder from the liver (Fig. 15). The effect of anæmia corresponds therefore, in this as in other cases, to

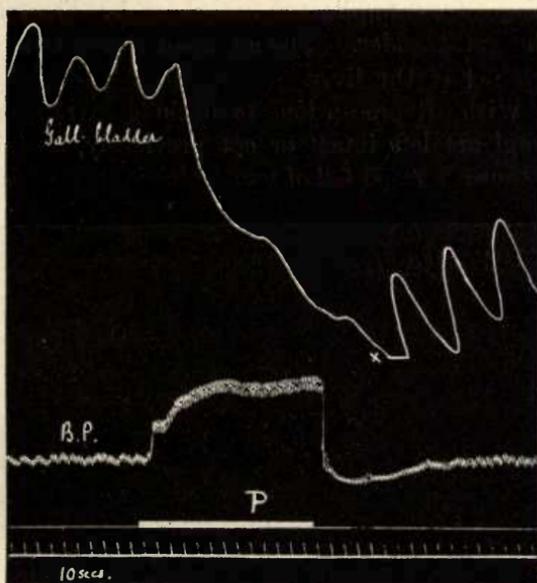


Fig. 17.  $\times \frac{1}{2}$ . Curare. Gall-bladder separated from liver. Chest opened and loop put round thoracic aorta. Blood-pressure from carotid. At P thoracic aorta occluded. At X lever of recorder reached its lower limit.

that of stimulating the sympathetic nerve-supply<sup>1</sup>. But here, as elsewhere, the inhibitory effect of sympathetic stimulation is shown to be independent of the associated local anæmia by the fact that it is easily obtained when vaso-constriction has been excluded by the action of chrysotoxin.

<sup>1</sup> Cf. Elliott. *This Journal*, xxxi. p. 164. 1904.

## V. THE ACTION OF CERTAIN DRUGS.

*Nicotine.* The effect of intravenous injection of 2—4 mgms. of nicotine is indistinguishable from that of adrenalin, the stimulant effect on sympathetic ganglion cells entirely overpowering the concomitant excitation of ganglion cells on the course of the vagus fibres which must be supposed to take place.

*Bile-salt.* The muscle of the gall-bladder is affected like all other plain muscle by intravenous injection of bile-salt, its activity being depressed, and relaxation being the result.

*Pilocarpine.* When the gall-bladder is left in its natural relation to the liver pilocarpine causes an apparent increase of tone when injected intravenously. We have not been able to produce this effect under conditions giving an uncomplicated record, or by direct application of the drug to the gall-bladder. The apparent rise of tone was therefore due to the swelling of the liver.

*Atropine.* With all precautions to exclude extraneous effect, and whether the vagi are left intact or cut previously, atropine (5 mgms. intravenously) causes a rapid fall of tone which is only gradually regained

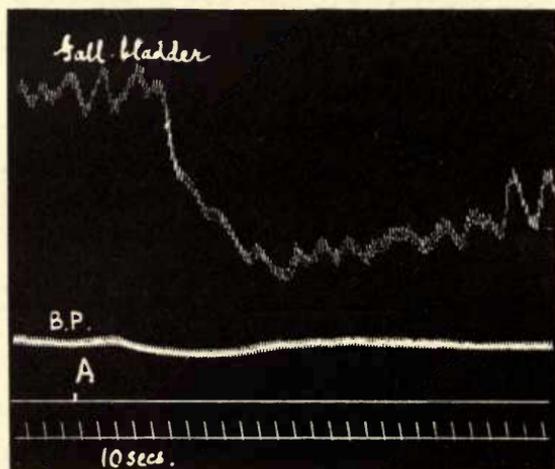


Fig. 18.  $\times \frac{1}{2}$ . Curare. Gall-bladder separated from liver. Blood-pressure from carotid. At A 5 mgm. atropine sulphate intravenously.

(Fig. 18). When atropine has thus been given, stimulation of either vagus produces no effect even when chrysothoxin has been given as well. The endings of the vagus in the gall-bladder would appear, therefore, to be more sensitive to atropine than those in the small intestine.

*Amyl nitrite* causes relaxation of the gall-bladder as of other plain muscular structures.

*Peptone*. Ellinger<sup>1</sup> states that intravenous injection of peptone causes contraction of the gall-bladder. We have found that the apparent contraction does, indeed, occur when the gall-bladder and liver are left in their natural relations. When arrangements are made to eliminate the effect of swelling of the liver the apparent contraction completely disappears, so that there can be no doubt that the increase of liver volume caused by injection of peptone entirely accounts for the phenomenon.

#### VI. THE QUESTION OF REFLEX ACTION THROUGH THE CENTRAL NERVOUS SYSTEM.

Heidenhain<sup>2</sup> attributed the first gush of bile, which he observed at a certain interval after a meal, to the passage of acid chyme over the biliary papilla causing a reflex stimulation of the gall-bladder.

Bruno<sup>3</sup>, with a Pawlow fistula, did not observe this preliminary gush: but it is evident that his experiment does not invalidate the conclusion of Heidenhain, since the method of operation excludes contact of chyme with the papilla.

The discovery of secretin and its effect on secretion of bile suggested another possible explanation of the effect described by Heidenhain. The first effect of a rapid secretion of bile might cause a distension of the gall-bladder, which, upon the analogy of the intestine, might react by a sudden contraction. We have attempted to produce such an effect by sudden distension of the balloon in the gall-bladder with water. We observed no trace whatever of such a contractile reaction.

On the other hand we do not find, under the conditions of our experiments, any reflex effect on the gall-bladder contractions when acid (0.4% HCl) is injected into the duodenum or applied directly to the papilla, or when the papilla is electrically stimulated. It is impossible, however, to attach much significance to the failure to elicit such a reflex in an anæsthetised animal. Further, it is not improbable that a reaction to distension exists, which, like that of the urinary bladder, is dependent on a reflex through the central nervous system and abolished by anæsthetics. Our experiments, therefore, do not give any definite information

<sup>1</sup> Ellinger. *Hofmeister's Beiträg. z. chem. Phys. u. Path.* 1902.

<sup>2</sup> Heidenhain. *loc. cit.*

<sup>3</sup> Bruno. *St. Petersburg Arch. des Soc. Biol.* p. 87. 1899.

as to the method whereby the entry of gastric contents into the duodenum causes contraction of the gall-bladder—if it do so indeed. To settle this question would probably necessitate experiments on an animal with a permanent fistula of the gall-bladder.

#### SUMMARY AND CONCLUSIONS.

1. The gall-bladder shows rhythmic variation in volume: the rhythm is increased in extent after the removal of tonic inhibitory impulses either by section of the splanchnic nerves, or the intravenous injection of chrysotoxin. Spontaneous contractions of the gall-bladder had been previously observed by Doyon.

2. The normal effect of stimulation of the sympathetic nerve-supply to the muscular coat of the gall-bladder, whether by electrical excitation of the right splanchnic nerve or by intravenous injection of adrenalin, is relaxation.

The apparently motor effects first described by Doyon are in all probability due to extraneous causes, namely, the mechanical pressure on the gall-bladder caused by swelling of the liver, and the increased tone of the muscle due to hyperæmia.

We find, however, that the right splanchnic contains an admixture of motor fibres, the presence of which can be detected when the tone of the gall-bladder is lowered by enfeeblement or stoppage of the circulation.

3. We have confirmed the observations of Courtade and Guyon that the vagus contains motor fibres for the gall-bladder. Definite motor effects are obtained most readily by stimulating the vagus in the thorax after the administration of chrysotoxin. Both the tone and the rhythm of the muscular coat are augmented, though not necessarily to the same degree. The left vagus is the more effective of the two. The effect is abolished by atropine. Stimulation of the central end of the vagi has no influence on the gall-bladder.

4. We found it impossible under the conditions of our experiments to elicit a reflex contraction of the gall-bladder either by applying acid or the products of gastric digestion to the duodenal mucous membrane or the biliary papilla, or by electrical excitation of the papilla, or by rapid distension of the gall-bladder. We draw no conclusion, however, with regard to the existence of such reflexes in the unanæsthetised animal.

5. Bile-salt and amyl nitrite injected intravenously cause relaxation of the muscular wall of the gall-bladder; a similar effect is produced by atropine.

Pilocarpine and peptone cause an apparent contraction of the gall-bladder, but this is solely due to the mechanical effect of swelling of the liver. Neither of these drugs appears to have any effect on the gall-bladder itself.

6. Anæmia, caused by compression of the thoracic aorta, produces a rapid fall in the tone of the muscle of the gall-bladder.



**THE FACTORS OF THE URETER PRESSURE.** BY  
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SEVERAL observers in the course of their studies on the mechanism of the secretion of the urine have connected the ureter with a manometer and have thus caused the kidney to secrete against a pressure created by its own activity. Cushny and others by the production of such a pressure have slowed the urinary production in the one kidney as compared with the other and have studied the changes in chemical constitution of the urine that the altered conditions produced. Gottlieb and Magnus have devoted one of the papers in their series on the action of the kidney to a study of this condition. Some experiments instituted with a somewhat different end in view seemed to point to the need of a more critical study of this phenomenon if it is to be pressed into service to throw light upon the function of the kidney. The results of this study are contained in this paper.

The methods employed were familiar ones. One ureter was connected by a cannula to a mercury manometer whose style wrote upon smoked paper. A side-tube closed by a clip allowed the pressure to be removed at any time, and the secretory activity of the kidney to be noted. Only when a high pressure was maintained for a prolonged time (such did not occur in the course of the experiments described), and simultaneously a marked hydræmic plethora was produced, did a markedly œdematous condition of the kidney, with the formation of blebs under the capsule, occur. Such a condition seemed to be much more readily produced in rabbits than in dogs. In some cases the cannula was inserted high up into the ureter (practially into the kidney pelvis), but the results were essentially the same as when it was inserted lower down. A blood-pressure tracing was taken throughout from either the carotid or the femoral artery. In several experiments the kidney in connection with the manometer was placed in a plethysmo-

graph. The changes in volume of its contents were recorded with a Hürthle piston-recorder. The flow of urine from the other kidney was usually simultaneously recorded by allowing the drops from a cannula inserted in its ureter to fall upon one of a pair of tambours. Rabbits were anaesthetized with urethane and A. C. E., dogs with morphia and A. C. E.

The factors which at once were seen to play a more or less essential part will be considered in order, but first a word or two about the contractions of the ureter musculature and the pressures produced by them. Correctly these alone should be spoken of as the ureter-pressures. These contractions seem normally to occur at very varying rates and often not with a quite regular rhythm. They sometimes occur in roughly rhythmical groups. They seem to increase in rate with the rate of urine flow, *e.g.*

Exp. XIII.	Dog.	Rate of contractions.	4 :	of urine	$\frac{3}{4}$	drops,	per minute.
	Later	" "	6 :	" "	1	" "	" "
	Again	" "	10 :	" "	4	" "	" "
Exp. XV.	Dog.	" "	13 :	" "	6	" "	" "
	Later	" "	19 :	" "	8	" "	" "
	Again	" "	28 :	" "	13-18	" "	" "

Similar observations were made in rabbits. The increase in rate of flow was produced by saline injections or caffeine. They often become slightly more frequent as the pressure rises. They disappear in the dog with a pressure of from 26—32 mm. Hg, in the rabbit at from 8—16 mm. Hg. A slight artificial rise in pressure often serves to excite them or increase their rate when present. Stimulation of the splanchnic as shown by Protopopow or of the hypogastric, as shown by Fagge also, will often produce contractions in a ureter seemingly paralysed by pressure. Adrenalin has the same effect. The slow return to a maximum of the ureter-pressure after splanchnic stimulation in Figs. 3 and 4 is due to their presence. When vigorous they may cause considerable variations in pressure, *e.g.* in exp. XIV. Dog, with a ureter-pressure of 6 mm. Hg, several contractions occurred, each of which produced a rise of 36 mm. Hg, rate 4—5 per minute. In a rabbit the greatest change noticed was 12 mm. Hg. Irregularities due to these contractions were in some cases avoided by allowing paralysis of the musculature due to over-distension to supervene before beginning a series of observations.

The phenomenon usually described as ureter-pressure has nothing to do with the ureter but depends entirely upon the kidney. The first factor in its production is what may best, perhaps, be termed the onco-

metric effect. The renal tubules and vessels lie pressed together within a capsule which is but slightly elastic, hence such a sudden rise in blood-pressure as in Fig. 1, due to the injection of a 20% solution of sodium sulphate, caused at once a rise in ureter-pressure. And as further, there may occur, as pointed out by Loewi, a dilatation of the vessels of the kidneys, which may be entirely out of proportion to the dilatation



Fig. 1.

Fig. 1. Dog. Exp. V. May 12th. Upper line, blood-pressure; 2nd, ureter; 3rd, outflow other ureter; 4th, clock, 10 second intervals.



Fig. 2.

Fig. 2. Exp. XXVII. Dog. As in Fig. 4, except that the signal is the ureter base line. Decapsulated kidney. Stimulation of vagus. Plethysmograph is set to left of blood and ureter manometers.

of the entire kidney, this alone may cause a sudden rise owing to the forcing of urine out of the tubules due to their compression by the vessels. The ureter-pressure therefore depends very largely upon the local blood-pressure in the kidney. The extent to which this is true may be seen in Figs. 2, 3 and 4. It may be noted that these curves show that a rise in general blood-pressure may be accompanied by a fall in ureter-pressure due to constriction of the kidney vessels (splanchnic

stimulation), or the blood-pressure remaining constant a local shrinkage will cause a like fall (stimulation of renal nerves), or the further possible case, fall in blood-pressure with accompanying fall in ureter-pressure. Decapsulation only to a certain extent does away with this effect (Fig. 2). In consequence of this lack of complete elasticity on the part of the capsule and of the varying volume that the vessels may fill within it, no absolute measure of the ureter-pressure at any time can be made unless the ureter manometer connections have been opened and the kidney freed from pressure since the last change in local vascular conditions. A slower fall in blood-pressure caused by a slow general vaso-dilatation

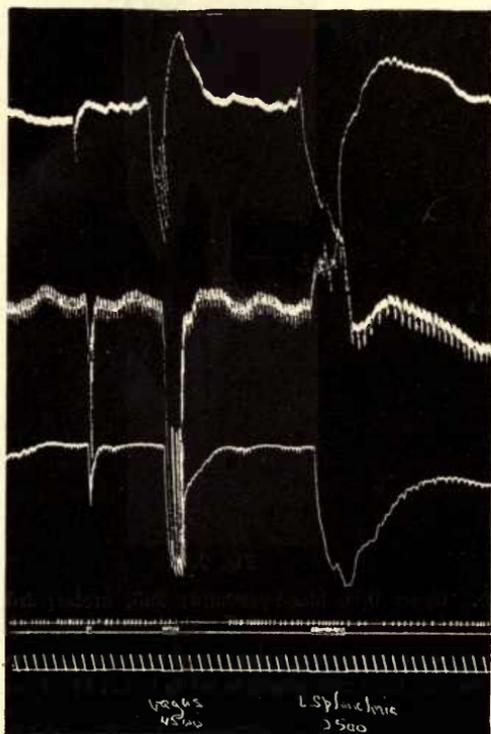


Fig. 3.

Fig. 3. Exp. XXV. Dog. Upper line, oncometer; 2nd, blood-pressure; 3rd, ureter of oncometer side; 4th, secretion from right kidney; 5th, signal; 6th, base for both manometers, time in 10 second markings. Stimulation of vagus and of left splanchnic, *i.e.* of oncometer side.  $\times \frac{1}{4}$ .



Fig. 4.

Fig. 4. Exp. XXVI. Dog. Upper line, plethysmograph of left kidney; 2nd, blood-pressure; 3rd, ureter-pressure; 4th, signal; 5th, base for both manometers, time in 10 second intervals. Stimulation of renal nerves.  $\times \frac{1}{4}$ .

or other condition will bring about a similar fall in ureter-pressure, only that here the exact parallelism may be altered in two directions, firstly reabsorption of fluid out of the tubules more rapidly than produced by the kidney at the lowered blood-pressure, or secondly and oppositely the kidney blood vessels remaining widely dilated and the kidney capillary pressure proportionately high, the pressure in the ureter would be insufficient to dilate the tubules and hence to allow a fall proportional to the fall in the general blood-pressure. I have no doubt that this is the only possible explanation of the remarkable result reported by Gottlieb and Magnus as they seem to have fulfilled all the necessary theoretical conditions. In their experiment a rabbit received a continuous injection intravenously of normal saline, the blood and ureter-pressures were noted while a fall in general blood-pressure was brought about by injections of chloral. Reabsorption from the tubules would here be slight if not indeed quite absent, and the renal vessels would be widely dilated, hence it is not surprising that in the rabbit's last minute the ureter-pressure was read at 14 mm. Hg, that of the blood in the carotid only at 13 mm. The ureter-pressure was insufficient to press the fluid in the connections back into the kidney. Though I have worked in several experiments with very similar conditions I have never seen such a result, and I think that this is due to the care taken to avoid error by opening the ureter manometer connections on inducing changes in the circulatory conditions, which Gottlieb and Magnus did not do. Consequently, too, no absolute value should be placed on measurements of ureter-pressure as it is in large part but a function of the blood-pressure in the kidney, and the blood-pressure should always be given when the ureter-pressure is used in experimentation.

The second factor is the constitution of the plasma. Starling measured the osmotic pressure value of the proteid constituents of the plasma and found it to be about 25—30 mm. Hg. He pointed out that with a normally constituted plasma one did not find a secretion of urine with a lower blood-pressure than about 35 mm. Hg, nor under like conditions could one obtain a ureter-pressure which was not lower than the blood-pressure by at least this amount. Without diuretics in rabbits the minimum difference between blood and ureter-pressure in my experiments was 66 mm. Hg, with caffeine 46—50, hypertonic salts 45; in dogs 60—70 mm., with diuretin or caffeine 55—65, with hypertonic salts (10 c.c. of a 25% solution of sodium sulphate) 47 mm. Hg. As my experiments were performed in warm weather it was very hard to obtain maximal activity in response to diuretics, and hence in part the

high values found. In none of these cases, however, could the osmotic value of the proteid present have been greatly reduced. By bleeding and replacing the blood with isotonic saline it readily could be. The following experiment is typical.

Exp. IX. Dog 9 kg. Usual connections made. Between each alteration in circulatory conditions, *i.e.* before bleeding or injecting saline, etc., the ureter manometer connections were opened. Tabular protocol in brief. See also Fig. 5.

Time	Experimental changes	Blood-pressure	Ureter-pressure	Diff.	Flow from other kidney in drops per min.	P.c. of hæmoglobin*	P.c. of total solids
11.50	Normal	102	40	62	app. 0.8	100	100
12	Diuretin 5 c.c. 10 % solution						
12.2	Fig. 5 A	116	58	58	app. 2.0		
12.4	10 c.c. 20 % Na <sub>2</sub> SO <sub>4</sub>						
12.6		130	71	59	4		
12.15	100 c.c. saline 0.9 %						
12.30	Bled 100 c.c.						
12.40	10 c.c. sulphate						
12.44		96	59	37	17	89	76
12.50	300 c.c. saline						
1.9	Bled 200 c.c.						
1.20		64	37	27	12	51	39
1.32	250 c.c. saline						
1.35	Fig. B	70	39	21	16	47	—
1.37	Bled 125 c.c.						
1.50	Fig. C	70	34	37	5	45	—
2.1	200 c.c. saline						
2.4	Fig. D	72	54	18	20	38	28
2.7	Bled 150 c.c.						
2.11	300 c.c. saline						
2.18	Fig. E	52	35	17	13	22	26
2.20	Bled 200 c.c.						
2.23	300 c.c. saline						
2.25	Fig. F	49	38	11	11	14	23
2.27	Bled 150 c.c.						
	300 c.c. saline						
2.40	Bled 100 c.c.						
	600 c.c. saline						
2.50		58	48	10	16	9	19

\* Hæmoglobin percentage was obtained by a tintometer, the first sample serving as normal (100 %). Total solids were obtained by drying and are given as percentage of first sample as 100. Results as indicating the proteids are naturally too high in final stages by the latter method.

As may be seen, the flow here was quite rapid when there was a minimum difference of but 10 mm. Hg. In no case was a lower difference obtained. In exp. V. abundant secretion occurred with a blood-pressure of but 32 mm. Hg; the percentage of hæmoglobin was less than

10. In no case was secretion in any quantity observed with a lower blood-pressure, as asphyxia owing to the blood-dilution intervened. I do not doubt however that it might occur under more fortunate conditions. Indeed Gottlieb and Magnus report a case in which in a rabbit under continuous intravenous injection of saline, and a fall of blood-pressure due to chloral, 0.3 c.c. of urine was secreted in 5 min. with a blood-pressure of less than 30 mm. Hg. As the plasma must have been greatly diluted this exceedingly small secretion does not appear impossible though very exceptional. As pointed out above, the measured urinary pressure (the minimum difference was but 6 mm.) was probably far too high. The further secretion of one drop of urine with a blood-pressure 16—13 mm. Hg, difference of 3 mm., has of course little significance, as such at times appears before death under such conditions and probably is due to an emptying of kidney pelvis and ureters.

This experiment, with the other examples given in brief in the addenda, points clearly it seems to the constitution of the plasma and especially of its proteid constituent having a marked influence on the relation which subsists between ureter-pressure and blood-pressure, and goes to justify the explanation given by Starling of the lack of secretion with low blood-pressure, *i.e.* less than 30 mm., and of the occurrence of a minimum difference of about the same amount between blood and ureter-pressure when the plasma is normal in its proteid content.

A third factor which definitely affects the ureter-pressure, and that in two distinct ways, is the rate of production of urine. The first effect of a more rapid production of urine is a more rapid rise of the ureter-pressure. This may be distinctly seen in Fig. 6 A and B. This is of course principally due to the fluid necessary to cause the rise being more rapidly supplied and to a less extent to the fact that dilated kidney vessels will occupy more of the space within the capsule and allow but a small dilation of the tubules. In exp. XVI., Fig. 6, Rabbit 2.6 kg., the more rapid flow of urine was produced by an injection of 2 c.c. of 10% solution of caffeine sodium salicylate. A mercury-valve connected with one carotid prevented the blood-pressure rising above its previous maximum. The ureter manometer connections were not closed until the blood-pressure and urinary flow had become constant. Curve B in exp. IX., Fig. 5, was obtained soon after an injection of normal saline. The ureter manometer connections were then broken, the animal bled 125 c.c., the connections again closed and curve C obtained. It is to be noted that the blood-pressure is approximately the same, and the constitution of the plasma has altered but little, hæmoglobin indices 47%

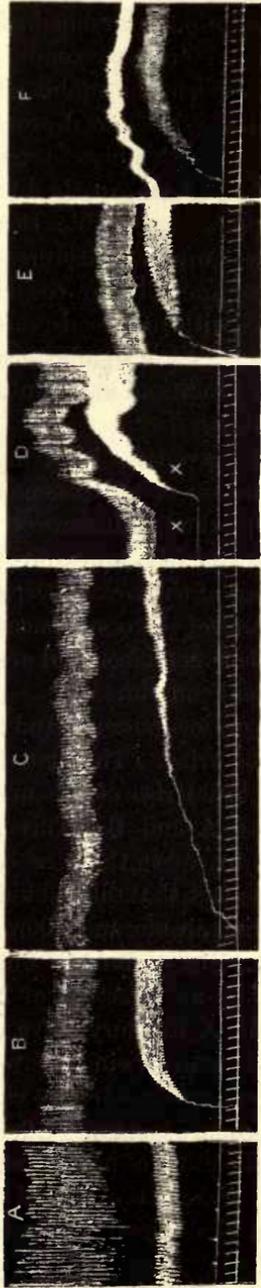


Fig. 5. Exp. IX. Upper line, blood-pressure; 2nd, ureter-pressure; 3rd, drops of urine from other ureter; 4th, base of blood and ureter manometers, except in A. Time in 10 second intervals. Reduced nearly  $\frac{1}{3}$ .

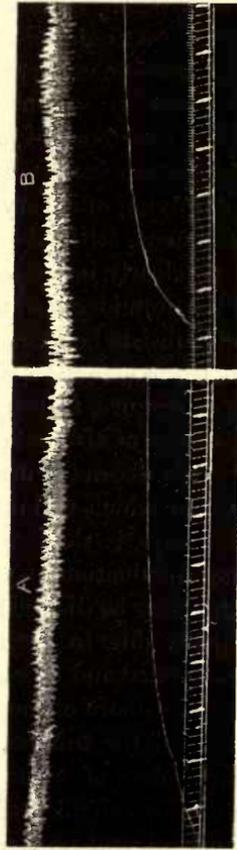


Fig. 6. Exp. XVI. Rabbit. As before. Interval of 9 min. between A and B. Ureter manometer connection opened and 2 c.c. 10% sol. caffeine sodium salicylate given. Reduced about  $\frac{1}{3}$ .

and 45 % as may be seen in the protocol given above. The difference in the rate of urinary flow is undoubtedly due to a decreased flow of blood through the kidney.

But further than this somewhat unimportant difference in the rate of rise of the ureter-pressure to its maximum is the lessening of the minimum difference between blood and ureter-pressure which the more rapid flow produces. As Heidenhain quite correctly pointed out, the ureter-pressure represents not a secretion pressure but the pressure at which secretion (or filtration) and reabsorption balance each other. The theoretical maximum ureter-pressure, or minimal difference between that and the blood-pressure, required to show the existence of a filtration pressure which is needed to overcome the proteid osmotic pressure of Starling, can only be obtained when the rate of absorption is negligible when compared with the rate of urine production. That the point of balance is not reached by a slowing of the production process (absorption not being admitted to occur) seems to be pretty well established by the studies of Cushny on the salt distribution in urine of two kidneys, one secreting against pressure, and the similar experiments of Rüdell on the urinary reaction. Fresh evidence, also, in favour of an absorption occurring will be offered in this paper. It is to be noted that Fig. 6 B represents a minimum difference of 64 mm., rate of outflow 13 drops per min.; A a difference of 73 mm., rate of flow 2. That in my experiments I did not more closely approach Starling's minimum difference is probably due to the experiments having been performed in hot weather when the animals were poor in water and consequently unable to react to diuretics with an abundant secretion. My experimentation has led me to feel strongly that many confusions and difficulties in previous studies of the kidney secretion are largely due to overlooking the important factors of the water content and of the salt content of the animal as a whole. A subsequent paper will be devoted to their study. That a certain rapidity of flow is necessary to produce the minimum difference between blood-pressure and ureter-pressure with any given constitution of the plasma suggests that absorption occurs as a normal act and that the minimum difference is but the product of these two factors balancing against each other.

The need of a better demonstration of the possibility of reabsorption from the tubules led to a further series of experiments. Other observers have been more or less successful in injecting the tubules from the ureter, but as a review of their methods and results is contained in a recent paper by Lindemann it seems unnecessary to discuss them.

The experiments were carried out before Lindemann's paper came into my hands; otherwise hints contained in it might, I believe, have led to a greater success. The following variations upon the method employed by other observers were made use of. In dogs under morphia and A.C.E. the kidney and ureter were exposed, and freed from the surrounding tissues; especial care was taken to carefully clean the artery, vein, and ureter, at the hilus of the kidney, so that all lymphatic connections were broken. Cannulæ were inserted in the ureters and the carotid artery and the jugular vein. An injection of a concentrated solution of dextrose (approx. 35 g. dextrose in 50 c.c. water) intravenously was given so as to, firstly, make the animal as poor in water as possible, and, secondly, lower as much as possible the percentage of electrolytes in the urine. During the diuresis records of the possible ureter-pressures were taken and during its subsidence the ureter cannula was connected by a tube with a small reservoir; connecting tube and reservoir were filled with a solution of indigo-carmin. The following protocol is given in full.

Exp. XVII. Dog 8 kg. Operation as above.

Time	Blood-pressure	Ureter-pressure	Outflow. Drops per min.	Ureter connections
10.20	140	—	$\frac{1}{2}$	Ureter connections
50 c.c. 50% dextrose	144	90	20	opened after each
10.50	140	84	6	observation
10.55	Ureter-pressure allowed to rise, and then ureter cannula connected with pressure-vessel.			
	Pressure 125 cm. H <sub>2</sub> O = approximately 92 mm. Hg (2 mm. only above the pressure observed above).			
11.35	Slight blue tinge in urine of other kidney; fluid-level in reservoir had fallen approximately 6 mm. = 8 c.c. H <sub>2</sub> O.			
12	Urine distinctly blue; fall of fluid-level 10 mm. = approximately 13 c.c. H <sub>2</sub> O. Blood-pressure 138 mm.			

Right ureter ligatured, vessels ditto. Kidney removed. Washed. Tissue beneath the capsule showed areas, especially towards the ends of the kidney, of mottled bluing. Kidney bisected longitudinally; washed lightly with running water. Narrow wedge-shaped stripes are to be seen, especially towards the ends of the kidney, which radiate from pelvis to cortex, in which they are not to be made out or in which they are less distinctly marked and wider but reach to the capsule. Parallel sections on either side show similar pictures. Thin slices hardened in abs. alc. imbedded in paraffin and stained with alcohol eosin.

Histological examination of these blued areas showed abundant precipitates (crystalline or granular) of indigo-carmin in the collecting tubules, and in the distal convoluted tubules of the cortex, in many cases directly underlying the capsule, and in the tubules of the ascending limb of Henle's loop. Deposits were also found but were not common in the descending loop of Henle. In many of the convoluted tubules and in those of Henle's

loop pigment could be seen within the cells. Only very rarely was the pigment to be found in interstitial tissue about the vessels, except within the capsule.

Similar results were obtained in other cases, a synopsis of two of which are given in the addenda.

The following points especially are significant. That there was an absorption of fluid and of pigment. That this did not take place from the pelvis alone, as in spite of the low diffusibility of the pigment it was to be found high up in some tubules. That there is no evidence of a tear into interstitial tissue and that the distribution of the pigment speaks against its being reabsorbed from the interstitial spaces and secreted by the cells of the tubules. That the pressure hardly surpassed a previously measured ureter-pressure. In exp. XVII it did not reach this point and we can hardly suppose, in view of the pigment-distribution in the other cases that, in this the absorption was not by the tubules even though not found far up in them.

The macroscopic appearances of these kidneys seems to have closely resembled those obtained by Lindemann and led to a careful search for masses of pigment within the interstitial tissue along the vessels. In several of his experiments the pigment did pass up the tubules to a greater or less extent. In other experiments the pigment or oil was almost solely to be found in the interstitial tissue. The results so at variance with mine I find hard to explain, largely perhaps because so little information as to the prevailing conditions is given. No measurements of blood or ureter-pressures seem to have been taken. The injection pressures appear to have been selected arbitrarily. In several of Lindemann's experiments, whether on the extirpated or intact kidney, certain tubules were more or less completely injected. The occurrence and the irregularity of the distribution of these injected tubules Lindemann explains as being due to such tubules having been empty at the time the injection was undertaken. This explanation will I think hardly hold good in my experiments where all the tubules must have been functioning. I rather hold that the high pressure needed to turn the balance in favour of absorption to such an extent as to more than enable it to cope with the secretion and of sufficient duration to allow of the pigment fluid entering the tubules, leads to the closing of the opening of many tubules into the pelvis or obstructing them elsewhere to a greater or less extent, and that in consequence only those tubules which are fortunately placed and well filled with urine will be injected. As noted also by Lindemann the best injected tubules occur towards the end of the kidney and do not enter through the central

papillæ. Like Lindemann I have also noted that one of the first effects of pressure was flattening of the papillæ and hence probably closure of the tubules opening there.

This fresh evidence, like the work of Cushny, Loewi, Sollmann, Rüdell, Hüber and others, furnishes a new support to the theory of the recurrence of absorption. The close relationship which in the earlier part of the paper was shown to exist between the blood-pressure and the blood-constitution on the one hand and the ureter-pressure on the other and the fallacies, incorrect pressure measurements, shown to exist in the work which was supposed to disprove this relationship point more clearly to the correctness of the filtration-reabsorption hypothesis than to that of specific secretion.

It gives me much pleasure to be able to thank Prof. Starling for permitting me to carry out the above experiments in his laboratory and for much kind advice and criticism received from him.

#### CONCLUSIONS.

1. The ureter-pressure depends, in the first instance, on and varies with the blood-pressure and is not a secretion-pressure properly so-called.

2. The minimum difference between ureter-pressure and blood-pressure will vary firstly with the rate of urinary production, and secondly with the proteid constitution of the plasma, and that hence the explanation given by Starling firstly of the dimension of the observed minimal difference between blood and ureter-pressures, and secondly of the absence of urinary flow with low blood-pressures and a normally constituted plasma, is probably correct.

3. Reabsorption of water and of slightly diffusible substances may take place from the tubules.

SELECTED PROTOCOLS IN BRIEF.

Exp. III. Rabbit 2.6 kg.

Time	Blood-pressure	Ureter-pressure	Difference	Outflow in c.c. in 10 min.	
10.50	120	30	90	1	Caffeine 2 c.c. 10 % sol.
11	120	38	82	5.7	
11.48	Blood I.				Hæmoglobin 100 %
11.50	124	49	75	13.9	
11.55—12.2	Bled 35 c.c., injected 45 c.c. saline intravenously				
12.18	90	40	50	6	Caffeine given
12.30	78	48	30	6	Hæmoglobin 74 %
12.28—12.32	Bled 40 c.c., injected 50 c.c. normal saline				
12.40	90	44	46	3.8	Caffeine
12.42	58	42	26	3	Hæmoglobin 53 %

Exp. V. Dog 13 kg.

Time	Blood-pressure	Ureter-pressure	Difference	Flow in drops per min.	
2.35	100	50	50	2	Hæmoglobin 100 %
3.20	40 c.c. 20 % Na <sub>2</sub> SO <sub>4</sub> solution intravenously				
3.28	114	67	47	16	
3.37	Bled 100 c.c., 200 c.c. saline intravenously				
3.46	106	64	42	21	„ 92
4—4.6	Bled 100 c.c., injected 200 c.c.				
4.34	100	64	36	31	„ 79
4.50—5.6	Bled 100 c.c., injected 250 c.c.				
5.5	96	61	35	38	„ 72
5.12—18	Bled 100 c.c., injected 250 c.c.				
5.28	82	55	27	42	„ 66
5.43—6.8	Bled 100 c.c., injected 800 c.c.				
6.13	40	30	10	39	„ 15

Exp. VII. Dog 7 kg.

11.20	140	76	64	4—5	„ 100
11.30	10 c.c. 20 % Na <sub>2</sub> SO <sub>4</sub> solution				
11.35	160	110	50	26	
11.37	Bled 100 c.c. 11.46 20 c.c. sulphate				
11.48	130	76	54	20	
11.55	200 c.c. saline				
12.2	130	80	50	25	„ 85
12.16	Bled 100 c.c. 12.30 Injected 100 c.c.				
12.36	110	64	46	32	„ 71
12.45	Bled 100 c.c. 1.55 200 c.c. saline				
1.30	118	79	39	50	„ 63
1.35	Bled 100 c.c. to 1.25 250 c.c. saline				
2.7	72	50	22	40	„ 50
	At 2.12, 2.32, 2.45, 2.55 bled 100 c.c.				
	At 2.20, 2.38, 2.43, 3.0 injected 200 c.c. saline				
3.4	40	29	11	—	„ 10

Exp. XII. Dog 11 kg. Prepared as described above, exp. XVII. Maximum ureter-pressure 80 mm. Hg, blood-pressure 128. Injection-pressure of indigo-carmin 91 mm. Hg.  $1\frac{1}{2}$  hrs. later, blue-tinged urine secreted from other kidney.  $2\frac{1}{4}$  hrs. later, killed. Irregular patchy bluing of the kidney capsule. On section definite radiating wedge-shaped blue areas extending from pelvis to the capsule. Histological examination showed pigment deposits, most abundant in the collecting tubules and in the convoluted tubules. Two or three glomeruli also contained small deposits. In some areas where much pigment occurred within the tubules and in their cells pigment also was present interstitially in small quantities in lymphatic spaces and within blood vessels.

Exp. XVII. Dog 10 kg. Prepared as above. Blood-pressure 120. Ureter-pressure 30 mm. Hg. Injection-pressure 40 cm. water = approximately 30 mm. Hg. In  $1\frac{1}{4}$  hrs. a trace of blue in urine of right kidney. After  $2\frac{1}{2}$  hrs. killed. Slight bluing of patches of capsule. On section one or two faint blue streaks extending out to the capsule. Histologically indigo-carmin in collecting tubules and in a few convoluted tubules only.

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**Zur Lehre**  
**von der Bildung des Kammerwassers**  
**und seinen quantitativen Verhältnissen.**

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vorgelegt von

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**Herrn Prof. Dr. Pflügers**

in Dankbarkeit gewidmet.



Das Kammerwasser wird als eine Lymphe betrachtet, welche einige Sonderheiten besitzt, die sie befähigen, nicht nur ihre Ernährungsfunktionen zu versehen, sondern auch ihren spezifisch-optischen Aufgaben zu genügen: die Transparenz der vorderen Kammer und der Hornhaut aufrecht zu erhalten. Es ist vorauszusehen, dass das Spezialstudium der Bildung und Absorption des Humor aqueus einen analogen Verlauf genommen haben und nehmen werde, wie das Studium der Bildung und Absorption der Lymphe im allgemeinen. Es wird daher nicht unfruchtbar sein, erst einen Blick in die physiologischen Arbeiten über die Lymphe zu werfen.

### **I. Produktion und Absorption der Lymphe.**

Bis zum Jahre 1890 galten unbeanstandet die Lehren über die Lymphbildung von Ludwig und seiner Schule, welche dieselbe abhängig machte von zwei Faktoren:

1. von der Differenz des Kapillardruckes und des Druckes der Gewebsflüssigkeit, welche einen Austritt von Flüssigkeit durch die Kapillarwand verursacht;

2. von der chemischen Verschiedenheit der beiden Fluida, welche Osmose und Diffusion durch die Kapillarwand hervorruft.

Nachdem schon Cohnheim und Tigerstedt Einwände gegen die reine Filtrationstheorie erhoben hatten, wies Heidenhain (1891) der Filtration bei der Lymphbildung nur eine bescheidene Rolle zu. Nur der Flüssigkeitsaustritt aus den Kapillaren bei voller venöser Stauung wird auf sie zurückgeführt. Die Kapillarwände spielen nicht die Rolle passiver Filter, sondern ihre Endothelien sind einer sekretorischen Tätigkeit fähig, welche durch bestimmte Reize, wie die Anwesenheit der Lymphagogen erster und zweiter Ordnung erhöht werden kann.

Starling hat zum Teil in Gemeinschaft mit Bayliss 1894 in zwei Arbeiten die Einwände Heidenhains gegen die Filtrationslehre als nur scheinbare zurückgewiesen.

Er zeigt, dass der massgebende Kapillardruck allerdings nicht direkt gemessen, wohl aber durch gleichzeitige Messung des Arteriendruckes und des Venendruckes indirekt geschätzt werden kann. Starling hat in seiner ersten Arbeit die Veränderungen des Kapillardruckes bei verschiedenen Eingriffen notiert, in seiner zweiten Arbeit die Wirkung derselben Eingriffe auf die Lymphbildung bestimmt.

Durch die Vergleichung der Resultate kommt er zur Überzeugung, dass der Intrakapillardruck der Hauptfaktor in der Lymphbildung ist.

Nachdem die Einwände Heidenhains gegen die Filtrationstheorie und auch die von Hamburger in seinen Studien am Halslymphstrang des Pferdes erhobenen, weil experimentell nicht einwurfsfrei, zurückgewiesen waren, handelte es sich darum, den Diffusionsvorgängen im Organismus nahe zu treten, speziell den Fragen über die Resorption durch die Blutkapillaren, über die Durchlässigkeit der Kapillarwände und der zelligen Elemente der Organe, über die Faktoren in der Zusammensetzung des Blutes, der Lymphe und der Zellen, welche den Flüssigkeitsaustausch regeln. Das Studium der Resorption aus den Gewebsspalten und mehr noch das der analogen Resorption aus den serösen Höhlen förderte die Erkenntnis. Die bezüglichen Studien verschiedener Forscher (Orlow, Hamburger, Leathes, Starling, Roth und O. Cohnheim) führten zu übereinstimmenden Resultaten. Die Resorption hypertotonischer und hypotonischer Salzlösungen aus der Peritonealhöhle des Kaninchens bieten dem Verständnis keine Schwierigkeiten. Die Resorption isotonischer Lösungen führten Starling und Cohnstein auf das osmotische Äquivalent des im Blutserum enthaltenen Eiweisses zurück. Ob bloss die Lymphgefäße oder auch die Blutkapillaren sich an der Resorption der Proteide beteiligen, konnte nicht entschieden werden. Die von Orlow und Hamburger postulierte aktive Beteiligung der Zellen bei der Resorption kann auch nicht als erwiesen betrachtet werden. Die Durchlässigkeit der Kapillarwand für isotonische Lösungen verschiedener Stoffe ist von Roth für Harnstoff, Kochsalz und Traubenzucker studiert worden. Harnstoff geht mehr als doppelt so leicht über als Zucker, Kochsalz nimmt eine mittlere Stellung ein.

Die Untersuchungen von Lazarus-Barlow führten zu Einwänden gegen die physikalische Theorie, von denen der erste gezogen aus den Resultaten seiner Injektionsversuche mit konzentrierten

Krystalloidlösungen entkräftet worden ist. Der zweite Einwand von Lazarus-Barlow ist der, dass das sogenannte Anfangstriebvermögen in der Lymphe grösser ist, als im Blutserum, d. h. dass der osmotische Druck in der Lymphe höher ist als im Blutserum, wenigstens wenn die Nierentätigkeit ausgeschaltet ist. Eine befriedigende Erklärung findet diese Tatsache darin, dass die Organzellen fortgesetzt ihre Stoffwechselprodukte in die Gewebsflüssigkeit ergiessen (Roth, v. Koranji).

Die Zellen entziehen der Gewebsflüssigkeit Nahrungsmaterial, krystalloide Substanzen und Eiweiss, welches letzteres sie in Körper von kleinerem Molekulargewicht abbauen. Diese kehren in die Lymphe zurück und erhöhen ihre osmotische Spannung.

Starling hatte sich übrigens 1894 bei Kritik der Hamburgerischen Arbeiten in ähnlichem Sinne ausgesprochen: „Since the final result of metabolism in the animal body or in an animal cell is disintegration, a breaking down of large complex unstable molecules of high potential energy into a great number of small simple stable molecules of small potential energy, the total output of an animal cell must have a higher osmotic pressure than the total income so that all the metabolic changes in the tissues would tend to increase the osmotic pressure of the lymph with which they are bathed.“

Asher und seine Mitarbeiter vertreten die cellular-physiologische Theorie der Lymphbildung. Asher kommt in seinen Arbeiten zu dem Schlusse, dass die Lymphe ein Mass der Arbeit der Organzellen ist in weitesten Grenzen unabhängig vom Blutdruck. Bainbridge, unter der Leitung von Starling, stimmt mit Asher darin überein, dass intravenöse Injektion von taurocholsaurem Natrium in mässigen Dosen eine verstärkte Lymphabsonderung aus der Leber zur Folge habe, ohne Erhöhung des Blutdruckes, bedingt durch die vermehrte Tätigkeit der Leberzellen.

In der kritischen Bearbeitung der Frage von Ellinger, erschienen in den Ergebnissen der Physiologie, werden als die bisher bekannten Faktoren für die Lymphbildung angeführt:

1. Die Grösse des Kapillardruckes.
2. Die Grösse des Gewebedruckes.
3. Die Permeabilität der Kapillärwände.
4. Die chemische Beschaffenheit und der osmotische Druck des Blutplasmas.
5. Die chemische Beschaffenheit und der osmotische Druck der Gewebeflüssigkeit.

6. Die chemische Beschaffenheit und der osmotische Druck der Gewebezellen.

7. Die Permeabilität der Zellen in den verschiedenen Geweben.

Der Einfluss der Nerven ist ziemlich allgemein anerkannt, soweit er sich auf die Vasomotoren erstreckt.

Eine sehr gedrängte übersichtliche Zusammenstellung der ausgedehnten neuen Literatur über die Bildung der Lymphe hat kürzlich Asher im Biochemischen Centralblatt, Bd. IV, 1905, veröffentlicht.

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## II. Produktion und Absorption des Kammerwassers.

Über die Frage der Entstehung und Abfuhr des Kammerwassers haben Leber und seine Schüler am intensivsten und konsequentesten gearbeitet. Es ist auch mancher Punkt der Abklärung nahe gekommen.

Als Hauptquellen des Kammerwassers sind heutzutage die Ciliarfortsätze fast allgemein anerkannt. Ob die Ciliarfortsätze die ausschliesslichen Quellen des Kammerwassers seien, wie Leber meint, oder ob ein geringer Bruchteil von der Vorderfläche der Iris geliefert wird, analog dem  $\frac{1}{50}$  der intraokularen Lymphe, welche das Auge durch die Papille verlässt, gegenüber den  $\frac{49}{50}$ , die durch den Kammerwinkel abfliessen; dieser Streit ist zur Stunde nicht ausgetragen. Die Ehrlichsche Ansicht, dass der Humor aqueus wesentlich oder ausschliesslich von der Vorderfläche der Iris geliefert werde, kann als irrig fallen gelassen werden.

Für eine bescheidene Mitbeteiligung der Irisvorderfläche an der Ausscheidung sprechen der Fluoresceinversuch von Hamburger, sowie seine in Heidelberg (1902) demonstrierte Vorderkammer beim Fötus; ferner die Versuche von Nicati und die Beobachtungen von Pflüger über Austritt von grossen grünen Perlen aus zwei Punkten der vorderen Fläche der Iris in der Gegend des Circulus arteriosus major bei Fluoresceinkaninchen. Das Experiment von Leber über die Sekretion des Kammerwassers von der Vorderfläche der Iris bei abgetragener Cornea, setzt derart abnorme Verhältnisse, dass daraus kaum auf das normale Verhalten zurückgeschlossen werden kann.

Die Ansicht von Leber, dass weitaus der grösste Teil des Kammerwassers durch die Fontanaschen Räume austritt und in den Leberschen Venenplexus im Schlemmschen Kanale durchfiltriert, dürfte allgemein angenommen sein.

Die Anschauung über den offenen Zusammenhang dieser Venen mit der vorderen Kammer von Schwalbe, später von Guttmann wieder aufgenommen, hat Leber widerlegt.

„Ich stimme aber mit Guttmann überein,“ schreibt Leber, „dass der Austritt der in Rede stehenden Substanzen durch die Intercellularlücken des Endothels erfolgt. Nur darf dies nicht im früheren Sinn als offener Zusammenhang bezeichnet werden; es handelt sich vielmehr um den Vorgang, den man allgemein Filtration nennt, und auf welchen ich von Anfang an das Zustandekommen der Gefässinjektion bezogen habe.“

Über den Modus der Entstehung des Kammerwassers gehen die Ansichten auseinander, wie wir es ähnlich bei der Entstehung der Lymphe gesehen haben. Leber hat von Anfang an den Anschauungen von Ludwig, der reinen Filtrationstheorie, gehuldigt. „Das Kammerwasser ist ein Produkt der Differenz des Kapillardruckes und des Intraokulardruckes.“ Die Erneuerung des Kammerwassers ist eine träge.

Eine der wichtigsten neueren Arbeiten aus der Leberschen Schule ist diejenige von Niesnamoff: „Über die quantitativen Verhältnisse der Filtration und Sekretion des Kammerwassers“, ausgeführt mit dem verbesserten Manometer von Leber. Seine Resultate waren:

1. Eine vollständig reine physiologische Kochsalzlösung filtriert konstant in unveränderter Stärke durch den Kammerwinkel des toten Auges.

2. Die Filtration wächst proportional mit dem Druck im Apparat, also auch mit dem intraokulären Druck.

3. Die Filtration hängt bei verschiedenen Tieren ab von der Dimension der vorderen Kammer, d. h. von der Grösse der Fläche, durch welche die Flüssigkeit durchfiltriert wird.

4. Bei Anwendung nicht ganz reiner Flüssigkeit — Anwendung von Berlinerblaulösung (Leber, Bentzen) oder unfiltrierter  $\frac{3}{4}\%$  Kochsalzlösung —, in welcher unlösliche Stoffe suspendiert sind, nimmt das Filtrationsvermögen allmählich ab und die Filtration kann vollständig ins Stocken geraten, was auf eine allmähliche Verstopfung der Poren der Gewebe schliessen lässt.

5. Das Pigment des die Ciliarfortsätze und die hintere Irisoberfläche bedeckenden Epithels kann, wenn es in die vordere Kammer hineingelangt, eine allmähliche Abnahme des Filtrationsvermögens und eine Erhöhung des intraokularen Druckes bewirken.

6. Die Filtration in frischen Leichenaugen von Menschen vollzieht sich in ganz der gleichen Weise, wie in den Augen frischer Tierleichen.

7. Die Filtration des Kammerwassers in lebenden Tieraugen ist, bei gleich hohem Druck, der Filtration im toten Auge des betreffenden Tieres ungefähr gleich und entsprechend bei Erhöhung des Druckes im Apparate.

8. Die Absonderung des Kammerwassers ist proportional der Differenz zwischen dem Gefäßdruck und dem intraokularen Druck.

9. Die Absonderung des Kammerwassers vollzieht sich auf dem Wege der Filtration durch die Gefäßwände des Ciliarkörpers, wobei der Druck in den Gefäßen ungefähr doppelt so hoch ist, als der intraokulare Druck.

Zu dem letzten interessanten Satze gelangt Niesnamoff rechnerisch durch die Proportion:

$$\frac{Sp}{Sq} = \frac{v - p}{v - q},$$

wo  $S$  die Sekretionsmenge angibt,  $p$  und  $q$  verschiedene Druckwerte im Manometer und  $v$  den gesuchten unbekanntem Gefäßdruck. Die Werte für die Sekretion unter den verschiedenen Druckhöhen  $p$  und  $q$  fand er, indem er am lebenden Tiere physiologische Kochsalzlösung unter einem Druck  $p$  bzw.  $q$  in das Auge filtrieren liess und die erhaltenen Werte von denjenigen subtrahierte, die er unter dem gleichen Druck an dem toten Auge gewann.

In neuester Zeit haben Leber und Pilzecker wieder über die normale Filtration des Auges experimentiert. Sie fanden, dass die Temperatur der filtrierenden Flüssigkeit von sehr bedeutendem Einfluss auf die Filtration ist; ferner, dass die in das frisch enucleierte Auge einflussende Flüssigkeit nur zum Teil wieder nach aussen filtriert, zum andern Teil in dem an Volumen zunehmenden Auge zurückbleibt. Endlich werde noch beobachtet, dass die Filtration während des Versuches nicht unerheblich zunimmt.

Während Leber und seine Schüler wesentlich die Veränderung der Filtration bei künstlich erhöhtem intraokularem Druck manometrisch studierten, bei unverändertem Blutdruck, kamen von andern Seiten Untersuchungen über die Kammerwasserverhältnisse bei verändertem Blutdruck, sowie über den Einfluss der Nerven auf die Kammerwassersekretion und den intraokularen Druck. — Adamük, v. Hippel, Grünhagen, Jesner, Pflüger, Nicati, Henderson und Starling. —

v. Hippel und Grünhagen erhielten unter anderm bei Ligatur der Aorta unmittelbar unter dem Zwerchfell in einem Fall eine Zunahme des intraokularen Druckes um 31 mm Hg, in einem zweiten Fall eine solche um 50 mm Hg. Bei einer Katze sank der intraokulare Druck nach Carötisunterbindung von 25 mm Hg auf 14, beim Tode auf 8 bis 10 mm Hg. Reizung des Trigeminiursprunges in der Medulla oblongata steigerte den intraokularen Druck viel mehr als alle Veränderungen des Blutdruckes.

Beispiel:

	Intraokulardruck
Katze: Normaler Blutdruck	30 mm Hg
„ bei Kompression der Aorta abdominalis	81 „ „
„ „ Aufhebung der Kompression	60 „ „
„ „ Ligatur der Carotis	34 „ „
„ „ Aufhebung der Ligatur	60 „ „
„ „ Reizung der Medulla oblongata	200 „ „
„ nach Eintritt des Todes	69 „ „

Gegen diesen Versuch, soweit er die Reizung des Trigeminiursprunges in der Medulla oblongata betrifft, werden von verschiedenen Seiten (Leber, Starling) Einwände erhoben, da das nahegelegene Vasomotorenzentrum notwendigerweise mit erregt wurde, so dass der intraokulare Druck in Abhängigkeit vom allgemeinen Blutdruck gesteigert wurde.

Grünhagen und Jesner studierten die Veränderungen des Kammerwassers nach Nervenreizung und Blutdruckschwankungen mit nachfolgenden Resultaten:

Der normale Humor aqueus enthält stets kleine Mengen von Eiweiss und Zucker, nie aber Fibringeneratoren; er koaguliert nicht. Fibrinproduktion und vermehrter Eiweissgehalt in der vorderen Kammer werden erzeugt: a. durch Veränderungen in der Differenz zwischen Blutdruck und intraokularem Druck; b. durch Reize im Gebiet des Trigemini: durch mechanische, chemische und thermische Läsion der Cornea, durch intrakranielle Quetschung des Ramus ophthalmicus, durch Einschneiden der hinteren Ursprungswurzeln des Quintus in der Medulla oblongata.

Der Trigeninus führt dem Auge vasodilatatorische Fasern zu, deren Reizung gesteigerten Blutzufuss zum Auge mit Ausscheidung von Fibringeneratoren und Vermehrung des Eiweisses hervorruft.

Reize, welche den Trigeninus einer Seite treffen, üben einen ähnlichen, nur schwächeren Einfluss auf das zweite Auge aus: Ge-

fässdilatacion und Fibrinausscheidung. Wessely bestreitet zwar diesen Befund, bestätigt aber, dass nach Trigemiusdurchschneidung der einen Seite am andern Auge Fibrin und vermehrter Eiweissgehalt im Kammerwasser auftreten. Grünhagen und Jesner geben ferner an, dass auch die Curarevergiftung bei Einleitung künstlicher Respiration Fibrinausscheidung bewirkt und den Eiweissgehalt vermehrt.

Die Fibrinausscheidung und durch sie bedingte partielle Obliteration der Abfuhrwege im Kammerwinkel sind daher die wahrscheinlichen Ursachen, dass in dem oben citierten Experiment von Hippel und Grünhagen der Augendruck nach aufgehobener Reizung nicht wieder zur Norm zurückkehrte, auch im Tode nicht.

Pflüger berichtete 1880 am 6. internationalen Ophthalmologenkongress in Mailand über Erhöhung des intraokularen Druckes im Kaninchenauge, gemessen mit dem Leberschen Manometer, hervorgerufen durch die Applikation der Kathode auf das Auge, während die Anode auf dem für die künstliche Respiration freigemachten Larynx appliziert war. Bei Stromwendung sank das Quecksilber bis fast zur Norm, um bei erneuter Kathodenwirkung am Auge (Trigeminus) von neuem wieder anzusteigen. So konnte die gleiche Erscheinung bei Stromwendung mehrmals provoziert werden.

Pflüger hatte, bei Anlass einer nicht zu Ende geführten Dissertation eines Frl. Grünberg, schon vor 1880 beobachtet, dass bei Kaninchen, denen grössere Dosen Fluorescein subkutan injiziert worden waren, ohne Punktion der vorderen Kammer nach leichten Sondenberührungen der Cornea oder nach leichter Massage derselben mittels des Oberlides, ein grünes Hypopyon aufgetreten, d. h. dass fibrinhaltiges Kammerwasser ausgeschieden worden ist.

Nicati hat 1891 die Herkunft des Kammerwassers experimentell studiert. Er hält die Manometermethode nicht für genügend rein, weil durch die mit der Einführung der Kanüle in die vordere Kammer verbundene Läsion der Cornea die Art des Kammerwassers geändert werde. Er bediente sich der subcutanen Fluoresceinjektionen in verschiedenen Dosierungen. Seine Schlüsse sind:

Das nicht koagulierbare Kammerwasser des normalen, nicht lädierten Auges kommt zum grössten Teil aus der hinteren Kammer von den Ciliarfortsätzen, zum kleineren Teil von der Vorderfläche der Iris. Kleine Dosen von Fluorescein färben dieses Kammerwasser nicht, und es zeigt das Auge bei der Sektion keinerlei Grünfärbung, auch nicht der Ciliarfortsätze und der Choriocapillaris. Grosse Dosen Fluorescein färben das Kammerwasser des normalen Auges. — Ehr-

lichtsche Linie. — Bei kleinen Fluoresceindosen erscheint 10 bis 15 Minuten nach der Punktion der Hornhaut ein grünes fibrinhaltiges Kammerwasser. Bei der Sektion finden sich die Ciliarfortsätze bis zur Ora serrata, die Choriocapillaris und der Petitsche Kanal grün. Glaskörper, Linse und Retina sind frei von Farbstoff. Nach Exstirpation der Ciliarfortsätze grünt das Auge nicht mehr, trotz der Punktion der vorderen Kammer, ergo sind die Ciliarfortsätze die einzige Quelle des fibrinhaltigen Kammerwassers.

Die fibrinöse Sekretion ist eine Reflexerscheinung, die auftritt, wenn bei gleichem Blutdruck der intraokulare Druck herabgesetzt wird durch Punktion. Herabsetzung des Blutdruckes infolge von Durchschneidung des Rückenmarkes oder von Aderlass verzögert das Auftreten grüner Sekretion. Die Druckschwankung in der vorderen Kammer wirkt aber nicht auf rein mechanische Weise, sondern steht unter einem regulierenden Nerveneinfluss.

Durchschneidung des Trigemini vor und hinter dem Ganglion Gasseri beschleunigt diese grüne Sekretion; erstere mehr als letztere. Ähnlich wirkt die halbseitige Durchschneidung der Medulla oblongata in der Höhe des Calamus — Schöler und Uthoff waren zu analogen Resultaten gekommen; Leber dagegen führt diese Erscheinungen auf neuroparalytische Hyperämie zurück. — Durchschneidung des Rückenmarkes hindert die Beschleunigung der Grünfärbung nicht. Der Trigemini wirkt demnach unabhängig vom Gefäßdruck. Durchschneidung des Trigemini bewirkt die spontane Grünfärbung ohne Punktion der vorderen Kammer auch nach Durchschneidung des Rückenmarkes. Durchschneidung der Ciliarnerven rings um den Optikus bedingt Ausbleiben jeder Grünfärbung des Auges. Das Ganglion ciliare wirkt also als Sekretionszentrum, stets bereit, zu wirken, gehemmt bzw. geregelt durch den Trigemini<sup>1)</sup>.

Nicati ist also ein extremer Gegner der Ludwigschen Schule. Die Ciliarfortsätze sind für ihn eine Drüse mit der Funktion einer Drüse, deren Arbeit durch Nerveneinflüsse geregelt ist.

Während frühere Autoren (Adamük, Leber) die Einwirkung des Trigemini als eine vasodilatatorische, also auf hydrostatischen Verhältnissen beruhende, auffassten, das Mittelglied in lokaler Gefäß-

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<sup>1)</sup> Diesem Gedankengange folgend, hat Rhomer aus Nancy seine neue Glaukoperation aufgebaut: Die Ausreissung des Ganglion ciliare, die er für Glaucoma absolutum dolorosum schon mehrfach am Lebenden ausgeführt hat. (Annales d'Oculistique, Mai 1902.)

erweiterung suchten, so beansprucht Nicati die spezifische Drüsen-tätigkeit des Epithels der Processus ciliares.

Nach derselben Methode mittels Fluoresceinjektionen untersuchte Nicati den Einfluss des Sympathicus und fand nach Durchschneidung oder Exstirpation desselben keine oder nur eine unwesentliche Beschleunigung des Übertrittes; während derselbe auf der andern Seite, infolge der allein zur Wirkung kommenden Blutdruckerniedrigung, verlangsamt war. Schöler und Uthhoff dagegen geben an, dass nach Durchschneidung des Halssympathicus oder Exstirpation des obersten Halsganglions der Übergang des Fluoresceins in die vordere Kammer erheblich beschleunigt sei, und dass grüngefärbte Flüssigkeit in reichlicher Menge durch die Pupille austrete.

Henderson und Starling haben neuerdings (1904) die Bedeutung sowohl des Sympathicus als auch des Trigemini für die Kammerwassersekretion und für den Intraokulardruck geprüft, indem sie dabei jede Reizung des verlängerten Markes (Vasomotorenzentrum) vermieden. Ihre Resultate fassen sie folgendermassen zusammen:

1. Der intraokulare Druck ist eine Funktion des Blutdruckes der Blutgefässe des Auges und ändert sich genau mit dem letzteren.

2. Der intraokulare Druck steigt und fällt mit dem allgemeinen arteriellen Blutdruck und ist nicht nachweisbar beeinflusst durch eine gewisse Erhöhung des intravenösen Druckes.

3. Reizung des Sympathicus (abgeschnittenes Kopfende) verursacht eine vorübergehende Erhöhung des intraokularen Druckes, bedingt durch Kontraktion des ungestreiften Orbitalmuskels, gefolgt von einem langsamen Fall des Druckes, hervorgerufen durch die Kontraktion der intraokularen Blutgefässe (Reizung des Halssympathicus beim toten Tiere — bei aufgehobener Cirkulation — bedingt ebenfalls Erhöhung des intraokularen Druckes, es fehlt aber die nachfolgende Herabsetzung).

4. Stimulation des peripheren Endes der proximal vom Ganglion Gasseri durchschnittenen Trigeminiwurzel verursacht eine Erhöhung des Druckes durch die Kontraktion der ungestreiften Orbitalmuskeln. Dieser Effekt fehlt, wenn die dem Ganglion Gasseri vom Halssympathicus her zukommenden Fasern degeneriert sind infolge vorausgegangener Exstirpation des obersten Cervicalganglions.

5. Es besteht kein Grund zur Annahme vasodilatatorischer Fasern zum Augapfel, weder im Quintus noch im Halssympathicus. Frühere gegenteilige Befunde beruhen auf Versuchsfehlern (Nichtberücksichtigung des allgemeinen Blutdruckes ...).

Indem Henderson und Starling die Wirkung des Trigeminus lediglich auf die in demselben verlaufenden Sympathicusfasern zurückführen, stellen sie sich in Gegensatz sowohl zur Ansicht der meisten Autoren, welche dem Trigeminus vasomotorische und auch vasodilatatorische Fasern zuschreiben, als auch zu derjenigen Nicatis, welcher spezifisch sekretorische Eigenschaften postuliert. Nicht bestritten dagegen sind durch diese Versuche die Veränderungen der Kammerwassersekretion, die durch Reizung der zentripetalen Fasern des Trigeminus beobachtet worden sind.

In der Lehre der Produktion des Kammerwassers gehen also die Ansichten gerade so weit auseinander, wie in der Lehre der Lymphbildung, die rein mechanische Anschauung der Ludwigschen Schule, welche die Differenz des intrakapillaren und extrakapillaren, d. h. intraokularen Druckes als weitaus wichtigsten Faktor ansieht, auf der einen Seite, auf der andern Seite die cellularphysiologische Anschauung (Nicati).

Es wird noch Arbeit kosten, um endgültig festzustellen, wie gross der Anteil der hier in Betracht kommenden verschiedenen Faktoren unter normalen und pathologischen Verhältnissen sein wird.

Als massgebende Faktoren für die Absonderung des Kammerwassers müssen ähnliche wie bei der Absonderung der Lymphe erkannt werden:

1. Die Grösse des Kapillardruckes, abhängig vom arteriellen und venösen Druck.

2. Die Grösse des intraokularen Druckes, der zum guten Teil vom Verhalten der Abflusswege abhängt.

3. Die Permeabilität der Kapillarwände (Erkrankung derselben).

4. Der osmotische Druck des Blutplasmas.

5. Der osmotische Druck des Kammerwassers.

(4 und 5 in normalen Verhältnissen annähernd konstant, ändern in pathologischen Verhältnissen.)

Über den Einfluss des Trigeminus stehen sich drei ganz verschiedene Ansichten gegenüber.

Prof. Starling, welcher grosse Fortschritte in der Lehre der Lymphbildung angebahnt hatte, war so gütig, mich während meines Aufenthaltes in London zu einer Arbeit zu ermuntern, welche die Produktion des Kammerwassers und den Stoffwechsel des Auges unter normalen Bedingungen und unter experimentell veränderten Blutdruckverhältnissen zum Gegenstand hatte. Leider schienen mir die Experimente, die infolge meiner Abreise ziemlich plötzlich abge-

brochen wurden, nicht zureichend, um alle gestellten Fragen mit genügender Sicherheit beantworten zu können. Die nachfolgenden Experimente über die quantitativen Verhältnisse der Kammerwassersekretion, die ich unter der persönlichen Leitung von Herrn Prof. Starling machen durfte, bilden jedoch unter den andern Versuchsreihen ein für sich abgeschlossenes Ganzes und können deshalb für sich behandelt werden.

Als Versuchstiere dienten in den folgenden Experimenten kleine bis höchstens mittelgrosse Hunde (meistens Foxterriers), welche nach vorausgegangener Morphiuminjektion mit der in England gebräuchlichen Mischung von Alkohol-Chloroform-Äther im Verhältnis von 1:2:3, vermittels künstlicher Atmung unter Narkose gehalten und dazu noch curarisiert wurden. Zur Vermeidung einer lokalen Reizung in den Augen wurden vor dem Einstich der Kanüle in die Hornhaut einige Tropfen einer 2% Kokainlösung in den Conjunctivalsack geträufelt. Die Tiere wurden, dem englischen Gesetz über die Vivesektion entsprechend, am Schluss der Beobachtung in der Narkose getötet.

Da wir bei einer andern Reihe von Experimenten durch das Auftreten von Fibrin im Kammerwasser und die Gerinnung desselben sehr erheblich gestört worden waren, so injizierten wir jeweils von vornherein und je nach der Dauer des Versuchs während der Untersuchung noch ein bis mehrere Male 5 cmm einer verdünnten Blutegelextraktlösung in die Venen, welche die Fibrinausscheidung verhindert, wahrscheinlich durch Zerstörung des Fibrinfermentes (Eguet).

Der Blutegelextrakt gehört zu den Lymphagoga I. Ordnung, welche nach Asher keine Vermehrung der Kammerwassersekretion zur Folge haben. Um uns selbst davon zu überzeugen, eröffneten wir einem Hunde, dem vorher die gewöhnliche Dose von Blutegelextrakt injiziert worden war, die vordere Kammer mittels einer Kanüle und liessen das Kammerwasser frei ablaufen. Es sank dabei die Menge des in der Zeiteinheit produzierten Kammerwassers gleichmässig. Nach einer Beobachtungsdauer von 50 Minuten machten wir eine zweite Injektion von Blutegelextrakt und fanden während den nächsten 20 Minuten keine Vermehrung des Kammerwassers, sondern in Übereinstimmung mit dem Verhalten vor der zweiten Injektion eine weitergehende allmähliche Verringerung der produzierten Kammerwassermenge.

Um die Menge des in einem bestimmten Zeitabschnitt secretierten Kammerwassers zu bestimmen, suchten wir die Absonderung desselben vorübergehend gänzlich aufzuheben und den Ausfall der Kammerwassersekretion, bei gleichbleibendem intraokulärem Drucke, durch physiologische Kochsalzlösung zu ersetzen. Dieses Ziel erreichten wir, indem wir den Blutzuffluss zum Auge plötzlich vollständig unterbrachen und den intraokularen Druck manometrisch aufrecht erhielten. Die Menge der Kochsalzlösung, welche nun bei gleichem intraokularem Druck durch den Kammerwinkel nach aussen filtrierte, gab uns dann die Menge des vorher produzierten Kammerwassers an.

Unsere Versuchsanordnung war ähnlich der von Priestley-Smith zuerst angewandten. Eine bewegliche Reservoirflasche von etwa 8 cm Durchmesser war durch ein Rohr verbunden mit einem zweischenkeligen Manometer einerseits und einem graduierten Kappillarrohr andererseits, welches durch einen kurzen dünnen Kautschukschlauch mit der in die vordere Kammer einzuführenden Kanüle in Verbindung stand. Die Kanüle wurde beim Einstich in die vordere Kammer durch eine Klemme von dem übrigen Apparate getrennt. Als Filtrations- und Manometerflüssigkeit benutzten wir eine physiologische Kochsalzlösung (9 ‰), die durch einen geringen Zusatz von Methylblau ganz schwach bläulich gefärbt war, um die als Indikator der Flüssigkeitsbewegungen dienende Luftblase deutlicher erkennen zu lassen. Die Druckhöhen wurden nachher umgerechnet in Quecksilberdruck. Die auffallend niedrigen Werte, die wir in den meisten Fällen für den Intraokulardruck gefunden haben, mögen ihren Grund zum Teil in der Narkose und der Curarisierung, zum Teil auch in den Widerständen in den Röhren des Apparates haben. Nachdem wir den Manometerdruck gefunden hatten, bei dem die Luftblase stationär blieb, also den Gleichgewichtsdruck oder Intraokulardruck, komprimierten wir die beiden Carotiden durch Klammern. In den beiden erstangeführten Experimenten waren die beiden Arteriae subclaviae vor dem Abgang der Arteriae vertebrales mit Hilfe von Ligaturstäben von Anfang an unterbunden; in den andern Experimenten erfolgte diese Unterbindung gleichzeitig mit dem Zuklemmen der Karotiden. Die Vorbereitungen zu diesen Eingriffen waren vorher getroffen worden. Es trat nun, da mit der plötzlichen vollständigen Anämie der intraokularen Gefässe die Kammerwassersekretion vollständig aufhörte, ein Flüssigkeitsstrom aus dem Apparat in das Auge ein, von dem wir auf die Sekretionsmenge vor der Ligatur schliessen dürfen.

In den nachfolgenden Tabellen werden die aus dem Manometer in das Auge einflussenden Flüssigkeitsmengen als positiv, die aus dem Auge in das Manometer austretenden dagegen als negativ bezeichnet. Neben den Gesamtquantitäten für einen bestimmten Zeitabschnitt in Kubikmillimetern (cmm) werden noch die aus denselben berechneten Minutenkubikmillimeter (Mcm) angegeben.

1. Experiment.

Blutgeleextrakt. Alkohol-Chloroform-Äther. Curare. Künstliche Atmung. Subclavien von vornherein und während des ganzen Experimentes unterbunden. Intraokulardruck = 36,8 Hg-mm.

Tabelle 1.

Bemerkungen	Zeit	Manometerdruck im Auge in Hg-mm	Stromquantität	
			total cmm	in Minuten- kubik- millimeter (Mcm)
	11 50	23,5	—	—
	— 55	23,5	— 74,5	— 14,9
	12 —	23,5	— 41,4	— 8,2
	— 01	27,2	+ 5,2	+ 5,2
	— 06	27,2	— 19,3	— 3,8
	— 11	27,2	— 16,5	— 3,3
	— 13	31,0	+ 8,3	+ 4,1
	— 18	31,0	— 16,5	— 3,3
	— 23	31,0	— 13,8	— 2,7
	— 25	34,6	+ 4,0	+ 2,0
	— 35	34,6	— 20,7	— 2,0
	— 37	36,8	+ 9,6	+ 4,8
	— 42	<u>36,8</u>	0	0
Carotiden ligiert	— 43	36,8	0	0
	— 45	36,8	+ 64,8	+ 32,4
	— 50	36,8	+ 34,5	+ 6,9
	— 55	36,8	+ 34,5	+ 6,9
Carotiden wieder frei	— 55	36,8	—	—
	1 —	36,8	— 106,2	— 21,2
	— 05	36,8	+ 6,9	+ 1,4
	— 19	36,8	?	?
	— 50	36,8	+ 4,1	+ 0,1

Da der Apparat für die künstliche Atmung plötzlich nicht mehr funktionierte, musste das Experiment abgebrochen werden.

2. Experiment.

Blutgeleextrakt. Alkohol-Chloroform-Äther. Curare. Künstliche Atmung. Subclavien von vornherein und während der ganzen Dauer des Experimentes unterbunden.

Tabelle 2.

Bemerkungen	Zeit	Manometer- druck im Auge in Hg-mm	Stromquantität	
			total cmm	in Minuten- kubik- millimetern (Mcm)
a. Rechtes Auge. Intraokulardruck = 44,2 Hg-mm:				
Carotiden unterbunden	12 31	44,2	—	—
„ „	— 33	44,2	+ 80,0	+ 40,0
„ „	— 38	44,2	+ 37,2	+ 7,4
„ „	— 45	44,2	+ 37,2	+ 5,3
Carotiden frei	— 46	44,2	—	—
„ „	— 51	44,2	— 77,2	— 15,4
„ „	1 —	44,2	— 5,5	— 0,6
„ „	— 17	44,2	— 27,6	— 1,6
„ „	— 30	44,2	— 2,7	— 0,2
„ „	— 45	44,2	0	0
Carotiden unterbunden	— 49	44,2	0	0
	— 52	44,2	+ 60,7	+ 20,2
	2 02	44,2	+ 52,4	+ 5,2
	— 07	44,2	+ 12,4	+ 2,5
	— 10	44,2	+ 6,9	+ 2,3
b. Linkes Auge. Intraokulardruck mittlerweile auf 23 Hg-mm gesunken:				
	4 10	23	0	0
Carotiden ligiert	— 11	23	0	0
	— 14	23	+ 35,8	+ 11,9
	— 24	23	+ 81,4	+ 8,1
Carotiden frei	— 24	23	—	—
	— 40	23	+ 23,4	+ 1,4
Carotiden ligiert	— 40	23	—	—
	— 43	23	+ 46,9	+ 15,4
	— 53	23	+ 82,8	+ 8,2
Carotiden frei	— 53	23	—	—
	5 —	23	+ 35,8	+ 5,1

### 3. Experiment.

Blutgelextrakt. Alkohol-Chloroform-Äther. Curare. Künstliche Atmung. Die Subclavien befinden sich auf Ligaturstäben und werden unterbunden, während die Carotiden zugeklemmt werden.

Tabelle 3.

Bemerkungen	Zeit	Manometer- druck im Auge in Hg-mm	Stromquantität	
			total cmm	in Minuten- kubik- millimetern (Mcm)
a. Linkes Auge. Intraokulardruck = 23,5 Hg-mm:				
Carotiden u Subclavien ligiert	2 15	23,5	0	0
	— 18	23,5	0	0
	— 20	23,5	+ 69,0	+ 34,5

Bemerkungen	Zeit	Manometer- druck im Auge in Hg-mm	Stromquantität	
			total cmm	in Minuten- kubik- millimetern (Mm <sup>3</sup> )
Carotiden u. Subclavien frei	2 25	23,5	+ 30,3	+ 6,0
	— 30	23,5	+ 38,6	+ 7,7
	— 32	23,5	—	—
	— 37	23,5	0	0
b. Rechtes Auge. Intraokulardruck mittlerweile auf 19,4 Hg-mm gesunken:				
Carotiden u. Subclavien ligiert	4 12	19,4	0	0
	— 14	19,4	+ 69,0	+ 34,5
	— 19	19,4	+ 48,3	+ 9,6
Carotiden u. Subclavien frei	— 24	19,4	+ 42,8	+ 8,5
	— 25	19,4	—	—
	— 27	19,4	— 38,6	— 19,3
	— 37	19,4	+ 11,0	+ 1,1

Die beiden ersten Versuche unterscheiden sich vom dritten dadurch, dass die beiden Subclavien von vornherein unterbunden worden sind und während des ganzen Experimentes nicht wieder geöffnet werden. Durch dieses Verfahren wird eine Hyperämie im Auge verursacht, eine Erhöhung des intrakapillaren und extrakapillaren, des intraokularen Druckes. Im letzten Experiment wird der normale intraokulare Druck, soweit in der Narkose von einem solchen gesprochen werden kann, bestimmt und nachher erst der Blutstrom in den Subclavien und den Carotiden unterbunden.

Im ersten Experiment zeigen die Zahlen zunächst, wie der Gleichgewichtsdruck gefunden wird. Bei dem Anfangsdruck von 23,5 Hg-mm fließen 15 Mm<sup>3</sup> aus dem Auge. Bei dem allmählichen Heben des Reservoirs zu Druckhöhen von 27,2, 31 und 34,6 Hg-mm fließen geringere Mengen aus, bis bei 36,8 Hg-mm die Luftblase stationär bleibt, der Gleichgewichtsdruck gefunden ist. Bei jeder Hebung des Reservoirs stellt sich vorübergehend ein positiver Strom ein infolge der Ausgleichung, d. h. der Ausfüllung des Raumes, welcher durch die unter dem erhöhten Drucke entstehende Skleraldehnung, sowie auch durch mehr oder weniger starke Kompression der Blutgefäße gebildet wird. In den folgenden Experimenten wurde der Kürze halber die Bestimmung des Intraokulardruckes nicht angegeben.

In den ersten Minuten nach jeder Unterbindung der Arterien tritt eine viel bedeutendere Flüssigkeitsmenge aus dem Apparat in das Auge, um den frei gewordenen Raum der Gefäßlumina auszufüllen<sup>1)</sup>. Diese Ausgleichungsquantitäten sind, wenn wir von dem zweiten Auge des zweiten Experimentes absehen, einander ziemlich ähnlich und betragen von 60 bis zu 80 cmm. Auf dem zweiten Auge des zweiten Experimentes finden wir die ausnahmsweise kleinen Werte von 35,8 und 46,9 cmm gegenüber 80

<sup>1)</sup> Auf ähnliche Weise hat Grönholm die Blutmenge des Auges zu bestimmen gesucht.

und 60 cmm auf dem ersten Auge desselben Tieres. Wir müssen für diese Tatsache die Abnahme des intrakapillaren Druckes, welche den intraokularen Druck von 44,2 Hg-mm bei Beginn der Untersuchung auf dem ersten Auge auf 23 Hg-mm, bei der ersten Ligatur auf dem zweiten Auge sinken lässt, verantwortlich machen. Die geringe Blutmenge in den kleinen Gefässen nimmt einen geringeren Raum ein. Wie später gezeigt werden wird, nimmt der intrakapillare Druck während der Beobachtung auf dem rechten Auge zusehends noch mehr ab.

Erst 2 bis 3 Minuten nach jeder Unterbindung stellt sich ein annähernd gleichmässiger Flüssigkeitsstrom ein, nachdem der durch den Wegfall des Blutzufusses freigewordene Raum ausgefüllt ist. Wir müssen daher in unsern Schlüssen von der Filtrationsmenge nach der Ligatur auf die vorher stattgehabte Sekretion, von den in den ersten 2 bis 3 Minuten beobachteten Flüssigkeitsverschiebungen gänzlich absehen.

Die für uns brauchbaren Ablesungen der Stromquantitäten aller drei Experimente stellen wir in Tabelle 4 zusammen.

Tabelle 4.

Experiment	Intraokular- druck Mmmm	Stromquantität	
		getrennte Ab- lesung Mmmm	Mittelwerte Mmmm
1.	36,8	6,9) 6,9)	6,9
2. a. Erstes Auge, 1. Ligatur	44,2	7,4) 5,3)	6,2
2. a. „ „ 2. Ligatur	44,2	5,2) 2,5) 2,3)	4,0
2. b. Zweites Auge, 1. Ligatur	23,0	8,1	8,1
„ „ 2. Ligatur	23,0	8,2	8,2
3. a. Erstes Auge	23,5	6,0) 7,7)	6,9
b. Zweites Auge	19,5	9,6) 8,5)	9,1

In Experiment 1 sind unter demselben intraokularen Druck beide aufeinanderfolgenden Ablesungen gleich. Im zweiten Experiment auf dem ersten Auge nach beiden Ligaturen und auf dem zweiten Auge des dritten Experimentes ergibt die zweite Ablesung einen geringeren Wert als die erste. In 3a. dagegen beobachtet man das umgekehrte Verhalten.

Die Verschiedenheit im Resultat bei den verschiedenen Ablesungen fällt uns nicht leicht in der Erklärung. Es handelt sich hier, die zuführenden Gefässe einmal abgebunden, nur um Filtration, welche uns als Ausdruck der Sekretion gilt. Weshalb aber soll die Fil-

tration unter gleichem Intraokulardruck in so kurzen Zeitintervallen schwanken?

Zunächst kann der oben erwähnte Ausgleich, der sich vielleicht nicht immer in ganz genau derselben Zeit vollzieht, in einigen Fällen noch eine und wahrscheinlich auch die wichtigste Rolle spielen. Dann ist an kleine Ablesungsfehler zu denken, hervorgerufen durch die Parallaxe, die einzig ganz hätten vermieden werden können, wenn wir noch einen zweiten Massstab angebracht hätten. Ferner sind kleine Unregelmässigkeiten im Kaliber des Glasrohrs denkbar. Viertens sind durch die plötzliche Änderung der Krafrichtung infolge Wegbleibens des Blutes, das vorher dem Manometerdruck Gleichgewicht gehalten hat, und durch das Collabieren der Gefässe kleinste anatomische Verschiebungen in den Ausflusswegen nicht ausgeschlossen. Ebenso wenig sicher ausgeschlossen sind minimale Bewegungen der Iris und des Ciliarmuskels.

Angesichts dieser Möglichkeiten ist es wohl richtiger, die Mittelwerte als massgebend zu betrachten. Bei Berechnung dieser Mittelwerte, die in Tabelle 4 in der letzten Kolonne angegeben sind, muss natürlich auf die Zeitdauer zwischen den einzelnen Ablesungen Rücksicht genommen werden.

Bei fast demselben intraokularen Druck wird nach der ersten Ligatur in Experiment 2b. 1,2 Mmm mehr Flüssigkeit filtriert, als in Experiment 3a. Es muss also nach unserer Voraussetzung, dass während der Ligatur die einflussende Flüssigkeit die Menge des vorher secernierten Kammerwassers ersetzte, vor der Ligatur die Sekretion in 2b. ebensoviel mehr betragen haben, als in 3a.

In 2b. ist der intraokulare Druck bei langer Dauer des Experimentes herabgesunken von 44,2 auf 23,0 Hg-mm, sehr wahrscheinlich in Abhängigkeit vom Blutdruck in den intraokularen Gefässen. Es scheint sich also der Blutdruck, der durch die Unterbindung der beiden Subclavien künstlich in die Höhe getrieben worden war, vermittle der physiologischen Regulationsvorrichtungen annähernd wieder auf die normale Höhe eingestellt zu haben. Daneben mag auch die Narkose etwas blutdruckvermindernd eingewirkt haben. In 3a. dagegen beträgt der anfängliche Druck 23,5 Hg-mm. Vor allem ist darauf hinzudeuten, dass es sich in 2b. um ein zweituntersuchtes Auge, in 3a. dagegen um die erste Ligatur bei einem erstuntersuchten Auge handelt.

Es kann in 2b. nur der nach der ersten Ligatur gefundene Wert mit den übrigen verglichen werden. Es sinkt nämlich während

des Experimentes der intrakapillare Druck und damit die Sekretion. Wie die Carotiden das erste Mal losgebunden werden, erreicht der Kapillardruck seinen früheren Wert nicht mehr; wir haben nicht mehr Gleichgewicht, sondern einen positiven Strom von 1,46 Mcmm, nach der zweiten Freimachung der Carotiden sogar einen + Strom von 5,12 Mcmm. Die Filtration nach der zweiten Ligatur stellt uns deshalb nicht den Sekretionswert, sondern eine Summe von Sekretion + der aus dem Manometer zugeströmten Flüssigkeit dar. Dieser Flüssigkeitsstrom ist an Stelle des durch den erniedrigten Blutdruck bedingten Ausfalls der Sekretion getreten. Die ganze Filtration ist ungefähr gleich der Sekretion vor der ersten Ligatur.

Auf der andern Seite finden wir, dass dieselbe Quantität von 6,9 Mcmm Kammerwasser produziert wird unter verschiedenen intraokularen Druckhöhen; bei 36,8 *Hg*-mm (Exp. 1) und bei 23,5 *Hg*-mm (3a.). Ferner wird eine sehr ähnliche Menge Kammerwasser ausgeschieden unter einem dritten differentiellen Druck, unter 44,2 *Hg*-mm in Experiment 2a. Die drei Augen, in denen unter sehr differentem intraokularem Druck gleiche oder ähnliche Kammerwassermengen abgeschieden werden, sind die drei erstuntersuchten Augen der drei Experimente, während die beiden zweituntersuchten Augen einen wesentlich verschiedenen und zwar wesentlich höheren Wert für die Abflussmengen der Flüssigkeit und damit, nach unserer Voraussetzung, für die Kammerwasserabsonderung geben. Trotz der grossen Druckerniedrigung in Experiment 2b. ist auch hier die Abflussmenge grösser als auf dem ersten Auge.

Die gleichen Sekretionsmengen bei verschiedenem intraokularem Drucke würden sich am einfachsten so erklären lassen, dass in diesen Fällen bei steigendem Blutdruck der intrakapillare Druck  $D$  und der intraokulare Druck  $d$  in Abhängigkeit davon in gleichem Verhältnis steigen, so dass ihre Differenz  $D - d$  sich gleich bleibt, und dass dabei die Filtrationswege sich nicht verändern. Es wird sich später noch Gelegenheit zeigen, noch einmal auf diesen Punkt zurückzukommen. Gegen eine Verallgemeinerung dieser These, wonach unter sehr verschiedenem intraokularem Drucke immer gleich viel Kammerwasser produziert werden müsste, sprechen zwar unsere übrigen Beobachtungen, die zeigen, dass bei geringerem intraokularem Drucke mehr Kammerwasser abgeschieden werden kann und beim allergeringsten Druck in unserem Falle gerade am allermeisten, bei dem Intraokulardruck von 19,4 *Hg*-mm 9,1 Mcmm in 3b. Die Tatsache, dass bei demselben intraokularen Drucke bei verschiedenen Hunden

verschiedene Mengen von Kammerwasser produziert werden, sowie auch, dass bei geringerem intraokularem Druck bei dem einen Hund mehr secerniert wird, als bei bedeutend höherem Druck bei einem andern Hunde, können wir nicht zum Einwand erheben gegen das Gesetz, wonach die Sekretion eine Funktion ist der Differenz des intrakapillaren und des intraokularen Druckes ( $D - d$ ), solange wir über die Differenz ( $D - d$ ) selbst nichts anzugeben wissen.

Bei Beginn der Untersuchung auf dem zweiten Auge des zweiten Experimentes ist der intraokulare Druck erheblich kleiner, als er auf dem ersten Auge desselben Tieres war. Es fragt sich nun, ob diese Druckerniedrigung auf eine primäre Herabsetzung des intrakapillaren Druckes zurückzuführen ist. In diesem Falle wäre nämlich der intraokulare Druck in geringerem Masse oder höchstens im gleichen Verhältnis zurückgegangen wie der intrakapillare Druck, da dieser in Abhängigkeit vom arteriellen Blutdruck rascher wechseln und den Intraokulardruck durch veränderte Filtration aus den Kapillaren erst sekundär beeinflussen wird. Der intraokulare Druck wird sich langsamer regulieren, dank der Elastizität der Sklera. Wir finden aber in dem zweiten Auge eine grössere Sekretion als in dem ersten (aus schon angeführten Gründen ist auf dem zweiten Auge nur die erste Ligatur zu berücksichtigen). Wenn die obige Hypothese, dass der Intraokulardruck  $d$  erst sekundär zum Intrakapillardruck  $D$  gesunken ist, richtig wäre, so könnte es sich nicht um eine Vergrösserung der Druckdifferenz  $D - d$  handeln, und das Gesetz, wonach die Sekretion eine Funktion ist der Differenz  $D - d$ , würde dadurch eine nicht unwesentliche Einschränkung erfahren. Es wäre sehr wünschenswert, sich über das Verhalten des intrakapillaren Druckes Aufschluss verschaffen zu können, um diese Frage zu entscheiden.

Leider gestattet uns die Versuchsanordnung nicht, den Blutdruck in einem der Kopfgefässe zu messen, da das Verhalten des zweiten Auges für uns besonders Interesse hat. Wir sehen uns deshalb genötigt, den Blutdruck in der Femoralis beizuziehen, obschon er nur approximativ über die Schwankungen in den Kopfgefässen Auskunft gibt.

#### 4. Experiment.

Hund von mittlerer Grösse, etwas grösser als die früher benutzten Tiere. Alkohol-Chloroform-Äther. Curare. Künstliche Atmung. Blutegelextrakt. Die beiden Subclavien befinden sich auf Ligaturstäben. Der Blutdruck wird in der Arteria femoralis gemessen und registriert sich in einer Kurve auf dem Myographen.

a. Rechtes Auge. Gleichgewichtsdruck = 13,2 Hg-mm. Die beiden Subclavien werden unterbunden, während die Carotiden zu- geklemmt werden. Hierauf werden die Carotiden wieder frei gemacht, während die Subclavien unterbunden bleiben. Der intraokulare Druck steigt, und es muss der Gleichgewichtsdruck von neuem gesucht werden. Nach der zweiten Ligatur werden wiederum alle vier Ar- terien frei gemacht.

Tabelle 5.

Bemerkungen	Zeit	Manometer- druck im Auge in Hg-mm	Stromquantität		Blutdruck in Art. femoralis Hg-mm
			total cmm	in Minuten- kubik- millimetern (Mcm)	
Carotiden u. Sub- clavien ligiert	12 14	13,2	—	—	104
	— 16	13,2	0	0	104
	— 17	13,2	0	0	152
	— 19	13,2	+ 110,4	+ 55,2	139
Carotiden frei. Sub- clavien ligiert	— 24	13,2	+ 38,46	+ 7,7	139
	— 24	13,2	—	—	95
	— 30	13,2	— 93,84	— 15,1	108
	— 30	22,2	—	—	108
Carotiden u. Sub- clavien ligiert	— 34	22,2	0	0	108
	— 34	22,2	—	—	126
	— 36	22,2	+ 55,2	+ 27,6	130
	— 41	22,2	+ 52,44	+ 10,49	130
Carotiden u. Sub- clavien frei	— 41	22,2	—	—	104
	— 51	22,2	+ 57,9	+ 5,79	110

Auf dem ersten Auge finden wir einen Intraokulardruck von nur 13,2 Hg-mm bei einem arteriellen Blutdruck in der Femoralis von 104 Hg-mm. Es ergibt sich dabei nach eingetretenem Ausgleich (110 cmm in 2 Minuten) ein Sekretionswert von 7,73 Mcm. Nach der ersten Unterbindung werden nur die Carotiden frei gemacht, während die Subclavien unterbunden bleiben. Es stellt sich nun ein Flüssigkeitsstrom vom Auge in das Manometer ein, der intrakapillare und der intraokulare Druck ist also durch die Unter- bindung der Subclavien gestiegen und zwar finden wir einen Gleichgewichts- druck von 22,2 Hg-mm, während der Blutdruck in der Femoralis 108 Hg-mm beträgt. Die zweite Unterbindung der Carotiden ergibt dann einen auf 10,49 Mcm gesteigerten Filtrations- bzw. Sekretionswert. Nach Frei- machung aller vier Arterien fließen immer noch 5,79 Mcm in das Auge. Durch das Eröffnen des Gefäßbezirkes beider Subclavien sinkt natürlich der Druck im Auge wieder, deshalb fließt nun bei gleich hohem Mano- meterdruck Flüssigkeit vom Apparat in das Auge.

b. Linkes Auge. Gleichgewichtsdruck = 14,7 Hg-mm. Es werden anfangs nur die Carotiden und erst 7 Minuten später auch

die Subclavien ligiert. Nach 12 Minuten vollständiger Unterbindung des Blutzufusses werden die Carotiden wieder frei gemacht, während die Subclavien ligiert bleiben. Es wird nun der Gleichgewichtsdruck von neuem gesucht und hierauf werden die Carotiden zum zweitenmal unterbunden. Nach einer Beobachtung von 17 Minuten wird die Ligatur aller vier Arterien aufgehoben und, nachdem der Gleichgewichtsdruck wieder kontrolliert ist, eine neue Unterbindung der Carotiden und Subclavien vorgenommen.

Tabelle 6.

Bemerkungen	Zeit	Manometerdruck im Auge in Hg-mm	Stromquantität		Blutdruck in Art. femoralis Hg-mm
			total cmm	in Minuten- kubik- millimetern (Mcm)	
Carotiden ligiert	3 05	14,7	0	0	98
	— 08	14,7	0	0	110
	— 10	14,7	+ 19,3	+ 9,6	110
	— 15	14,7	+ 16,5	+ 3,3	110
Subclavien ligiert (Carotiden ligiert)	— 15	14,7	—	—	110—120
	— 17	14,7	+ 104,8	+ 52,4	120
	— 22	14,7	+ 57,9	+ 11,6	120
	— 27	14,7	+ 57,9	+ 11,6	120
Carotiden frei, Sub- clavien ligiert	— 27	14,7	—	—	100
Gleichgewichtsdruck um 3,39 = 16,5 Hg-mm, Blutdruck = 106:					
Carotiden ligiert	3 39	16,5	0	0	106—124
	— 41	16,5	+ 51,0	+ 25,5	124
	— 46	16,5	+ 55,2	+ 11,0	124
	— 51	16,5	+ 55,2	+ 11,0	124
	— 56	16,5	+ 69,0	+ 13,0	124
Carotiden u. Sub- clavien frei	— 56	16,5	—	—	100
Gleichgewichtsdruck um 4,20 = 16,3 Hg-mm. Blutdruck = 108:					
Carotiden u. Sub- clavien ligiert	4 20	16,3	0	0	108—126
	— 22	16,3	?	?	126
	— 27	16,3	+ 95,22	+ 19,0	126
	— 32	16,3	+ 89,7	+ 17,9	126
	— 37	16,3	+ 92,4	+ 18,5	126

Ungefähr zwei Stunden später war der intraokulare Druck des zweiten Auges bei 14,7 Hg-mm bestimmt, während der Druck in der Femoralis 98 Hg-mm beträgt. Dann werden vorerst nur die beiden Carotiden unterbunden. Es tritt nun ein ganz geringer Flüssigkeitsstrom in das Auge ein, der in den ersten zwei Minuten 19,3 cmm ausmacht und zum Ausgleich des Volumens der weniger gefüllten Blutgefäße dient. In den nächsten fünf Minuten haben wir einen Flüssigkeitsstrom von 3,31 Mcm. Diese Menge gibt natürlich nur den Ausfall der Sekretion an, der durch den ver-

minderten Blutzuffluss zum Auge bedingt ist, denn durch die Unterbindung der beiden Subclavien erhalten wir hierauf während zehn Minuten einen konstanten Filtrationsstrom von 11,6 Memm, welcher Wert uns nun die wirkliche Sekretionsmenge angibt. Wir können also daraus schliessen, dass beim Hunde die vermehrte Blutzufuhr zum Auge durch die Anastomosen von der Arteria vertebralis her zum sehr grossen Teil den durch die Ligatur der Carotis bedingten Ausfall kompensiert. Diese relativ geringe Verminderung der Kammerwassersekretion stellt im Einklang mit der relativ geringen Herabsetzung des intraokularen Druckes bei Kompression der Carotis, wie sie Adamük gefunden hat.

Wie auf dem ersten Auge bleiben nun die beiden Subclavien ligiert, während die Carotiden losgebunden werden. Die Folge davon ist eine Erhöhung des intraokularen Druckes von 14,7 auf 16,5 *Hg*-mm, also nur um 1,8 *Hg*-mm, während der gleiche Eingriff auf dem rechten Auge eine Steigerung des intraokularen Druckes von 13,2 auf 22,3 *Hg*-mm, also von 9 *Hg*-mm bewirkt hat. Der Druck in der Femoralis ist dabei um 8 *Hg*-mm gestiegen. Nach einer zweiten Ligatur erhalten wir während zehn Minuten einen konstanten Flüssigkeitsstrom von 11,0 Memm, der aber in weiteren fünf Minuten auf 13,8 Memm ansteigt. Es werden hierauf wieder alle vier Arterien losgebunden, was jedoch nur eine ganz minimale Abnahme des intraokularen Druckes zur Folge hat. Das Gleichgewicht findet sich jetzt bei 16,3 *Hg*-mm, nachdem es während der Subclavialigatur bei 16,5 sich eingestellt hat. Der Blutdruck ist allerdings gestiegen auf 108 *Hg*-mm bei freier Cirkulation gegenüber 106 *Hg*-mm bei ausgeschalteten Subclavien. Die Herztätigkeit muss sich demnach erheblich gehoben haben. Bei einer dritten Unterbindung konstatieren wir einen Flüssigkeitsstrom von 19,0 bzw. 17,9 und 18,5 Memm durch je 5 Minuten. Während wir bei der ersten und zweiten Unterbindung für zehn Minuten einen völlig konstanten Filtrationsstrom haben, variiert derselbe hier nach der dritten Ligatur etwas. Trotzdem die beobachteten Schwankungen nur geringe sind, so ist es gleichwohl angezeigt, auch hier die Mittelwerte zu benutzen. Die Ausgleichsquantitäten fallen selbstverständlich ausser Betracht.

Dieses Doppelpexperiment ist das massgebendste, weil der Blutdruck gemessen wurde. Auffällig treten uns hier entgegen:

1. Die grösseren Filtrations- bzw. Sekretionswerte gegenüber denjenigen der übrigen Experimente. (Es wurde in diesem letzten Experiment an einem mittelgrossen Hunde, der etwas grösser war, als die früher benutzten Tiere, gearbeitet.)

2. Die Beobachtung, welche wir schon in den vorhergehenden Experimenten gemacht haben, dass auf dem zweiten Auge die Sekretionswerte gegenüber denjenigen des zuerst untersuchten Auges zunehmen.

3. Die hier bei wiederholten Unterbindungen an demselben Auge sich konstant einstellende Zunahme der Sekretionsmenge, während auf dem ersten Auge des zweiten Experimentes nach der ersten

Ligatur die Sekretion von 6,2 auf 4,0 Mcmm zurückgegangen ist. Dieser letztere Fall macht allerdings eine Ausnahme vor allen andern auf Grund des abnorm raschen Fallens des Blutdruckes während des Versuches. Es könnten sich da auch mechanische Hindernisse in den Abflusswegen eingestellt haben. Dagegen ist nicht anzunehmen, dass sich im Verlauf der übrigen und namentlich des vierten Experimentes Filtrationswiderstände eingeschaltet haben, da die Filtration immer besser wird.

Der besseren Übersicht halber werden die Quantitäten der Filtration bzw. Sekretion (Stromquantitäten) in ihren Mittelwerten nochmals neben die Werte des intraokularen Druckes und die korrespondierenden Werte für den Blutdruck in der Femoralis gestellt.

Tabelle 7.

Bemerkungen	Stromquantität Mcmm	Intraokulardruck Hg-mm	Blutdruck Hg-mm
Rechtes Auge:			
1. Cirkulation vorher frei	7,7	13,2	104
2. Nach Ligatur der Subclavien	10,5	22,2	108
Linkes Auge:			
1. Cirkulation vorher frei	11,6	14,7	98
2. Nach Ligatur der Subclavien	12,0	16,5	106
3. Cirkulation vorher frei	13,5	16,3	108

Hervorzuheben ist, dass derjenige Blutdruck massgebend ist, der jedesmal unmittelbar vor der Unterbindung registriert worden ist, da wir ja von der Filtration nach den Ligaturen auf die Sekretion unmittelbar vor denselben schliessen.

Von allen Quantitäten des Kammerwassers dieses Experimentes steht nur die allererste mit 7,7 Mcmm denjenigen der andern Experimente nahe, welche sich zwischen 4 und 9 Mcmm bewegen, während die vier übrigen Werte des letzten Versuches erheblich, beinahe ums doppelte, über diejenigen der übrigen hinausgehen.

Jede der vier folgenden Quantitäten (Mittelwerte) ist regelmässig grösser als die vorhergehenden. Es handelt sich um die zweite Ligatur am ersten Auge und um drei Ligaturen am zweiten Auge desselben Tieres.

Mit der Zunahme der Sekretion hält nicht etwa eine analoge Steigerung des intraokularen Druckes Schritt, ebensowenig eine solche des Blutdruckes. Der minimale Sekretionswert von 7,7 Mcmm entspricht allerdings dem minimalsten intraokularen Druck, nicht aber

dem minimalsten Blutdruck. Dem Maximum des intraokularen Druckes von 22,2 *Hg*-mm korrespondiert der zweitkleinste Sekretionswert von 10,5 Mcmm, dagegen ein grosser Blutdruck von 108 *Hg*-mm, wie er nur noch in der letzten Ablesung in Verband mit der maximalen Sekretion von 18 Mcmm und mit dem drittkleinsten intraokularen Druck beobachtet wird.

Der Anfangsdruck des ersten Auges steigt nach Ligatur der beiden Subclavien von 13,2 auf 22,2 *Hg*-mm, während, wie schon in der Legende betont, am zweiten Auge der erste Gleichgewichtsdruck von 14,7 durch Ligatur der Subclavien bloss auf 16,5 *Hg*-mm gebracht wird, um nach Aufhebung der Ligatur nur um ein Geringes wieder zu sinken.

Wie verhält sich der Blutdruck in der Femoralis während des etwas mehr als 4<sup>1</sup>/<sub>2</sub> Stunden dauernden Experimentes?

Er steht gleich anfangs, einer guten Narkose entsprechend, tief, auf etwa 104 *Hg*-mm, und wird durch die erste Ligatur der Carotiden und Subclavien auf 139 *Hg*-mm gebracht, also etwas höher, als der mittlere Druck beim normalen Tiere beträgt, wenn wir als Normaldruck für die Femoralis beim Hunde etwa 120 bis 130 *Hg*-mm annehmen. Nach der zweiten Ligatur der Carotiden und Subclavien steigt der Blutdruck nur noch auf 130, nach der dritten nur noch auf 120, um nach der vierten wieder auf 124 und nach der fünften auf 126 *Hg*-mm sich zu erheben.

Während der Blutdruck in der ersten Gleichgewichtslage bei ungehinderter Cirkulation 104 *Hg*-mm betragen hat, sinkt er bei der zweiten Gleichgewichtslage mit ungehinderter Cirkulation mehr als 3 Stunden nach Beginn des Versuches auf 98 *Hg*-mm, um bei der dritten fast am Schluss der Beobachtung auf 108 *Hg*-mm zu steigen und so 4 *Hg*-mm über den Anfangsdruck zu kommen. Es handelt sich zweifelsohne zum guten Teil um die von der verschiedenen Tiefe der Narkose abhängige verschieden starke Störung der Herzfähigkeit und Veränderung des Gefässtonus.

Es sei hier noch einmal darauf hingewiesen, dass der Druck in der Femoralis nur ein approximatives Bild gibt von den Druckschwankungen in der Carotis bei Ligatur der Subclavien, und dass diese Schwankungen in der Carotis selbst viel bedeutender sind. In der Carotis selbst kann der Druck nach der Versuchsanordnung nicht gemessen werden.

Welches ist die genauere Beziehung des intraokularen Druckes zum Blutdruck?

Tabelle 7 zeigt ein deutliches, wenn auch keineswegs proportionales Abhängigkeitsverhältnis des intraokularen Druckes vom Blutdruck, so lange man jedes Auge für sich betrachtet. Auf dem ersten Auge werden nach der ersten Ligatur nur die Carotiden wieder freigemacht, während die beiden Subclavien unterbunden bleiben. Die dadurch hervorgerufene Blutdrucksteigerung in der Femoralis um 4 Hg-mm hat eine Erhöhung des Intraokulardruckes um 9 Hg-mm zur Folge gegenüber dem Befunde bei der ersten Gleichgewichtslage. Auf dem zweiten Auge ist die auf die gleiche Weise verursachte Blutdrucksteigerung in der Femoralis um 8 Hg-mm nur von einer Erhöhung des intraokularen Druckes um weniger als 2 Hg-mm begleitet. Eine weitere Steigerung des Blutdruckes, welche nach Eröffnung des Gefäßbezirkes der beiden Subclavien auftritt, um 2 Hg-mm, geht sogar mit einer kleinen Erniedrigung des intraokularen Druckes einher. Dieses geringe Zurückgehen des intraokularen Druckes bei steigendem Blutdruck ist jedoch nur ein scheinbares und stört die Regel nicht. Der Blutdruck von 106 Hg-mm ist gemessen zur Zeit, während beide Subclavien ligiert sind, der letzte Blutdruck von 108 Hg-mm ist dagegen bei ungehinderter Cirkulation notiert. Bei Ligatur beider Subclavien ist aber der Blutdruck im Gebiet der benachbarten Carotiden mehr erhöht als in den entfernteren Femoralgefäßen. Es entspricht also der Blutdruck in der Femoralis weniger demjenigen in der Carotis, als bei völlig ungehinderter Cirkulation. Wir gehen daher sicherer beim Vergleich des ersten und dritten Blutdruckes auf dem linken Auge, hier entspricht eine Steigerung des Blutdruckes um 10 Hg-mm einer solchen des Intraokulardruckes von 1,6 Hg-mm.

Dieses direkte, wenn auch recht unregelmässige Verhältnis des intraokularen Druckes zum Blutdruck hört aber auf, sobald wir aus den fünf Ablesungen beider Augen eine Reihe machen. Dem anfänglichen Blutdruck von 104 Hg-mm entspricht auf dem ersten Auge ein intraokularer Druck von 13,2 Hg-mm, dem Blutdruck von 98 Hg-mm bei Beginn des Versuches auf dem zweiten Auge ein intraokularer Druck von 14,7 Hg-mm.

Es geht zur Evidenz hervor, dass der intraokulare Druck in einem Abhängigkeitsverhältnis zum Blutdruck steht, dass aber dieses Verhältnis kein absolutes und proportionales ist, sondern dass in unsern Experimenten noch ein anderer Faktor eingreift.

Ganz ähnliche Beziehungen wie zwischen Blutdruck und intraokularem Druck finden sich zwischen dem Blutdruck und der Sekretion.

Die geringste Quantität Kammerwasser von 7,7 Mcmm wird unter einem Blutdruck von 104 Hg-mm abgesondert, während unter dem geringsten Blutdruck von 98 Hg-mm 11,6 Mcmm Kammerwasser secerniert werden. Unter dem maximalen Blutdruck von 108 Hg-mm werden auf dem rechten Auge 10,5, auf dem linken Auge dagegen 18 Mcmm Humor aqueus ausgeschieden.

Die Zunahme des Kammerwassers in jedem einzelnen Auge ist verbunden mit je einer Steigerung des Blutdruckes, nicht aber, wenn wir die regelmässige Zunahme des Kammerwassers in der Reihenfolge der fünf Beobachtungen bei diesem letzten Experiment betrachten.

Ausschlaggebend für uns ist das Verhalten der Kammerwassersekretion bei Veränderungen von  $D - d$ , d. h. der Differenz des intrakapillaren Druckes  $D$  und des extrakapillaren oder intraokularen Druckes  $d$ . Wir müssen bei unserer Versuchsanordnung aus dem Blutdruck in der Femoralis auf den Druck der intraokularen Gefässe schliessen, um, wenn auch nur annähernd, einen Begriff zu bekommen von den Veränderungen, welche  $D - d$  erleidet. Die reinsten und eindeutigsten Verhältnisse findet man, wenn man das erste Resultat auf dem rechten Auge mit dem ersten auf dem linken Auge vergleicht, indem es sich eben in beiden Fällen um die erste Unterbindung handelt und indem der Intraokulardruck bei freier Cirkulation bestimmt worden ist. Man findet auf dem zweiten Auge eine Stromquantität (= Sekretionswert)  $S_2 = 11,59$  Mcmm gegenüber  $S_1 = 7,7$  Mcmm auf dem ersten Auge, während auf dem zweiten Auge der Intraokulardruck  $d_2$  um 1,5 Hg-mm höher war als  $d_1$  auf dem ersten Auge, umgekehrt aber der Blutdruck in der Femoralis bei der Beobachtung auf dem zweiten Auge um 6 Hg-mm tiefer war, als bei derjenigen auf dem ersten Auge. Es ist demnach auch der Druck in den intraokularen Gefässen ( $D_2$ ) in dem zweiten Auge kleiner gewesen als derjenige im ersten Auge ( $D_1$ ).

Es ist also  $d_2 > d_1$   
 $D_2 < D_1$ .

Demnach muss  $(D_2 - d_2) < (D_1 - d_1)$ .

Es ist aber  $S_2 > S_1$ .

Man findet also hier eine stärkere Sekretion bei einer geringeren Differenz des intrakapillaren und intraokularen Druckes.

Blutdruck, Intraokulardruck und Kammerwassersekretion stehen in einem nicht zu verkennenden Abhängigkeitsverhältnis, das aber nicht ein mathematisch proportionales und nicht ein absolutes, son-

dem ein relatives ist, und welches durch intervenierende Momente, die noch zu eruieren bleiben, in erheblichen Grenzen verschoben werden kann. Es muss hier ein Faktor intervenieren, bei dem die Dauer der Beobachtung oder die Zahl der Unterbindungen eine Rolle spielt, denn bei jeder neuen Unterbindung wird eine grössere Quantität Flüssigkeit ausgeschieden, bei gleich hohem oder sogar bei kleinerem Blutdruck und Intraokulardruck.

Es liegt natürlich nahe, hier, wie bei jedem physiologischen Experiment, an einen Versuchsfehler zu denken. So könnte es sich um mehr oder weniger vollständige Unterbindung der Blutgefässe handeln, wodurch eine mehr oder weniger vollständige Unterbrechung der Kammerwassersekretion bedingt würde. Diese Annahme wird jedoch von vornherein unwahrscheinlich, wenn man das konstante Ansteigen der Filtrations- bzw. Sekretionswerte bei jeder folgenden Unterbindung betrachtet, mit einer einzigen Ausnahme bei der zweiten Unterbindung auf dem ersten Auge des zweiten Experimentes. Auf der andern Seite scheint mir die Übereinstimmung in den Filtrationswerten bei den ersten Unterbindungen der vier ersten Augen zu beweisen, dass die Bedingungen nicht voneinander abwichen.

Von zahlreichen Autoren ist der Einfluss des Nervensystems auf die Blutgefässe des Auges und den Intraokulardruck hingewiesen worden. In der Deutung dieses nervösen Vorganges stehen sich verschiedene Ansichten gegenüber. Während auf der einen Seite dieser nervöse Einfluss auf vasodilatatorische Fasern im Trigeminus zurückgeführt wird, nimmt Nicati anderseits eine spezifisch sekretorische Wirkung des Trigeminus an. Henderson und Starling hinwiederum negieren beides und führen die Wirkung bei Trigeminusreizung zurück auf die Mitwirkung der im Trigeminus verlaufenden Sympathicusfasern.

Es fragt sich nun, ob nicht vielleicht die Einführung der Kanüle durch die Cornea in die vordere Kammer an und für sich schon ein Reizmoment bildet, welches reflektorisch die Gefässinnervation beeinflusst, oder nach Nicati den sekretorischen Reflex auslöst, und welches dadurch im stande wäre, die nach gewisser Zeit eintretende und sich langsam steigernde Vermehrung der Kammerwasserabsonderung zu erklären. Dass die erste Unterbindung auf dem zweiten Auge einen grösseren Wert ergibt, als die zweite Unterbindung auf dem ersten Auge, wäre dann so aufzufassen, dass während der Beobachtung auf dem ersten Auge auf sympathi-

schem Wege ein Reiz auf das zweite Auge einwirkt, welcher sich mit dem folgenden direkten Reize summiert.

Der Betrag des Flüssigkeitsumtausches in der vorderen Kammer bildet bei jedem Versuchstier eine annähernd gleichmässig ansteigende Kurve, wobei je auf dem zweiten Auge die Kurve des ersten Auges ohne wesentliche Unterbrechung fortgesetzt wird. Eine Ausnahme hiervon macht allerdings das erste Auge des zweiten Experimentes, wo der Betrag bei der ersten Unterbindung um 2,2 Mm höher ist als bei der folgenden. Dieses letztere Verhalten würde nun am ehesten einem nervösen Vorgang entsprechen, bei dem der Reflex im Anfang am stärksten wäre, um nachher bald an Intensität abzunehmen. Ein konstantes Anschwellen des Reflexes bei stundenlanger Einwirkung des auslösenden Reizes widerspricht dagegen in hohem Masse den bei den andern Reflexen beobachteten Ermüdungserscheinungen. Es dürfte also diese Steigerung der Sekretion des Humor aqueus kaum auf einen solchen Reflex zurückzuführen sein. Man hat übrigens einen derartigen kontinuierlichen Reiz, wenn man die vordere Kammer mittels einer Kanüle eröffnet und das Kammerwasser frei ausfliessen lässt. Anfangs erhält man einen ziemlich lebhaften Ausfluss von neugebildetem Humor aqueus, der jedoch allmählich immer geringer wird, um schliesslich auf ein Minimum zu sinken. Dieses Verhalten haben wir auch in einer andern Reihe von Experimenten bei Anwendung von Blutegeleextrakt beobachtet, so dass wir eine mechanische Verlegung durch Fibringerinnsel ausschliessen zu dürfen glaubten. Es nimmt also hier trotz des fortdauernden Reizes die Kammerwassersekretion nicht zu mit der Zeit, sondern wird umgekehrt allmählich und zwar gleichmässig geringer. Ähnlich wie wir am lebenden Auge, hat Leber in seinen neuesten Experimenten am frisch enucleierten Auge während des Versuches eine erhebliche Steigerung der Filtration konstatiert, wo es sich natürlich nicht um nervöse Vorgänge handeln kann.

Bei jeder Unterbindung der vier Arterien machen beide Augen mit ihren Blutgefässen, wie überhaupt der ganze Kopf, eine vollständige Ischämie durch, welche die Permeabilität der Gefässwände und überhaupt der Gewebe erhöht. Es ist deshalb wohl erklärlich, dass nach jeder Unterbindung ein vermehrter Flüssigkeitsaustritt aus den Gefässen der Ciliarfortsätze stattfindet. Trotzdem tritt aber, mit einer einzigen ganz unbedeutenden Ausnahme auf dem zweiten Auge des letzten Experimentes, keine Erhöhung des intraokularen Druckes ein. Es wird die grössere Menge Kammerwasser nach aussen abge-

führt, ohne dass es zur intraokularen Druckerhöhung kommt, was nur auf einer Zunahme der Permeabilität der abführenden Filtrationswege beruhen kann. Es kann diese vermehrte Filtration der Flüssigkeit nach aussen nicht durch die Anämie der Gewebe als solche bedingt sein, da die in Frage kommenden Gebilde, das Lig. pectinatum, ja gar keine Blutgefässe enthalten. Dagegen ist es sehr wahrscheinlich, dass durch den vollständigen Wegfall des venösen Druckes während der Unterbindungen die filtrierende Membran einem ganz einseitigen Druck ausgesetzt ist, wodurch sie kleinste anatomische Veränderungen erleidet. Dasselbe gilt auch für die Filtration am frisch enucleierten Auge. Diese Zunahme der Permeabilität erklärt uns auch, warum nach unsern Unterbindungen, wo es sich eigentlich ja nur um Filtration handelt, bei einem viel geringeren intraokularen Drucke mehr Flüssigkeit nach aussen filtrieren kann als bei einem viel höheren. So haben wir z. B. in unserem zweiten Experiment auf dem ersten Auge, bei einem Intraokulardruck von 44,2 Hg-mm, eine Filtration von 6,2 Mcmm, auf dem zweiten Auge dagegen bei 23 Hg-mm eine solche von 8,1 Mcmm. Ähnlich besteht im dritten Experiment auf dem ersten Auge bei einem Druck von 23,5 Hg-mm eine Filtration von 6,9 Mcmm, auf dem zweiten Auge dagegen bei 19,5 Hg-mm eine solche von 9,1 Mcmm.

In der Zunahme der Permeabilität der Kapillarwände und der abführenden Filtrationswege ist der modifizierende Faktor gefunden, welcher, wie oben einlässlich ausgeführt wurde, im stande ist, das Abhängigkeitsverhältnis der Kammerwassersekretion vom intrakapillaren Druck  $D$  und dem intraokularen Druck  $d$ , sowie besonders von der Differenz  $D - d$  in weitgehendem Masse zu verändern. Unter normalen Verhältnissen dagegen darf jedenfalls die Permeabilität der in Frage kommenden Membranen als konstant vorausgesetzt werden, so dass dieses Abhängigkeitsverhältnis ein absoluteres und proportionaleres sein dürfte.

Wir kommen damit zur Überzeugung, dass wir durch die wiederholten Unterbindungen der Carotiden und Subclavien ganz abnorme Verhältnisse geschaffen haben. Es wird der Flüssigkeitswechsel im Auge gesteigert durch erhöhte Permeabilität der Gefässwände in den absondernden Organen, infolge vorübergehender Anämie. Über das normale Verhalten geben daher nur die bei den ersten Unterbindungen auf den erstuntersuchten Augen gefundenen Werte Aufschluss. Diese vier hier in Betracht kommenden Werte stimmen auch in hohem Masse überein, indem sie 6,9, 6,2, 6,9 und im letzten Experi-

ment, wo ein etwas grösserer Hund benutzt wurde, 7,7 Mm be-  
tragen.

Diese vier Werte werden aber unter sehr verschiedenen Höhen  
des intraokularen Druckes erhalten, welche ihrerseits abhängig sind  
von verschiedenen Druckhöhen der intraokularen Gefässe. In den  
beiden ersten Experimenten sind die beiden Subclavien von vorn-  
herein und bleibend unterbunden. Dementsprechend steigt offenbar  
der Druck in dem benachbarten Carotisgebiet, und wir notieren  
höhere Werte für den intraokularen Druck, nämlich 36,8 und 44,2  
Hg-mm. Bei den übrigen Versuchen wurden bei freier Cirkulation  
intraokulare Druckhöhen von 23,5 und 13,2 Hg-mm gefunden. Trotz-  
dem findet man, wie schon bemerkt, keine höheren Sekretionswerte  
in den beiden ersten Experimenten.

Es wäre nun denkbar, dass der erhöhte Druck in den intraoku-  
laren Gefässen eine vermehrte Kammerwasserproduktion zur Folge  
hat. Da das Auge nicht sofort im stande ist, durch vermehrte Fil-  
tration nach aussen seinen Intraokulardruck auf der früheren Höhe  
zu erhalten, nimmt der auf den Gefässen lastende Gegendruck so  
lange zu, bis die Kammerwasserabsonderung auf ihr früheres Mass  
zurückgegangen ist.

Diese anfängliche und vorübergehende Vermehrung der Kammer-  
wasserproduktion ist jedoch noch durchaus unbewiesen, wie dies auch  
Wessely in seiner neuen Arbeit ausführlich auseinandersetzt.

Dagegen ist nicht zu bezweifeln, dass bei grösserem Blutdruck  
in den intraokularen Gefässen, wenn er, wie in diesen Experimenten,  
lediglich auf grösserer Blutfülle beruht, der Inhalt des Bulbus zu-  
nimmt, was eine Erhöhung des intraokularen Druckes zur Folge hat.  
Es scheint mir deshalb wahrscheinlicher, dass die Erhöhung des  
intraokularen Druckes nicht auf einer vermehrten Kammerwasser-  
produktion, sondern auf der Vermehrung des Bulbusinhaltes durch  
die stärkere Füllung der Gefässe beruht.

Höchst auffallend mag es übrigens erscheinen, dass bei unsern  
Versuchen, wo direkt ja nur die Filtration nach aussen gemessen  
wird, von welcher auf die vorhergehende Sekretion geschlossen wird,  
die Filtration nicht einfach proportional dem Intraokulardruck zu-  
und abnimmt. Ein solches Verhalten wäre doch nach den Filtrations-  
versuchen am enucleierten Auge zu erwarten. Wenn dies aber wirk-  
lich der Fall wäre, so wäre es nicht möglich, durch Veränderungen  
des Blutdruckes eine nur einigermaßen andauernde Erhöhung des  
Intraokulardruckes zu erzielen, denn es müsste durch vermehrte Fil-

tration sowohl ein vermehrter Bulbusinhalt als auch eine erhöhte Sekretion fast momentan kompensiert werden. Ein solcher Ausgleich findet allerdings statt, denn eine dauernde Druckerhöhung im Auge tritt nur bei behindertem Abfluss ein. Dieser Ausgleich benötigt aber ganz ungleich längere Zeit und ist auf jeden Fall nicht allein einer vermehrten Filtration zuzuschreiben, sondern es reguliert sich auch immer der Blutdruck.

Wir haben gesehen, dass mit jeder Unterbindung der Gefässe die Filtration aus dem Auge erhöht wird. Es verändern sich die Filtrationsbedingungen im Sinne einer Verringerung der Widerstände. Auf welche Weise diese Veränderungen erklärt werden können, wurde weiter oben ausgeführt. Es scheinen sich auch im Tode die Filtrationsbedingungen im gleichen Sinne zu verändern, denn es nimmt, wie Leber gezeigt hat, am frisch enucleierten Auge die Filtration während des Versuches nicht unerheblich zu<sup>1)</sup>.

Dass die Verhältnisse nach den wiederholten Unterbindungen denjenigen beim frisch enucleierten Auge ähnlich werden, geht auch aus den quantitativen Ergebnissen hervor. Während Niesmanoff für den Hund einen Flüssigkeitswechsel in der vorderen Kammer von 18,0 Mcmm angibt, bekommen wir nur im letzten Experiment, nachdem vier Unterbindungen der Subclavien und der Carotiden vorausgegangen sind, ein entsprechendes Resultat. Bei den ersten Unterbindungen der erstuntersuchten Augen, wo wir es mit annähernd physiologischen Verhältnissen, soweit von solchen bei einem physiologischen Experiment gesprochen werden kann, schwanken die gefundenen Werte zwischen 6,2 und 7,7 Mcmm.

Die gefundenen Resultate lassen sich zusammenfassen wie folgt:

1. Bei Hunden von kleinerer bis mittlerer Grösse beträgt die Kammerwassersekretion pro Minute rund 6 bis 8 cmm.
2. Der Flüssigkeitswechsel im lebenden Auge nimmt zu, wenn das Auge vorübergehend eine vollständige Anämie erlitten hat.
3. Der Grund dieser Zunahme ist in kleinsten, direkt oder indirekt durch die Anämie bedingten anatomischen Veränderungen, sowohl der Kapillärwände als auch der nach aussen filtrierenden Membranen, zu suchen.

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<sup>1)</sup> Es ist mir leider die grosse Arbeit von Leber und Pilzecker, betitelt: „Neue Untersuchungen über den Flüssigkeitswechsel des Auges“ im v. Graefeschen Archiv. Bd. LIV. 1. S. 1, nicht zugänglich gewesen, da sie während der Drucklegung dieser Arbeit erschienen ist.

4. Angesichts der Inkonstanz der Permeabilität der in Frage kommenden Membranen bei Versuchen am lebenden Auge kann man die am frisch enucleierten Auge gefundenen Resultate den normalerweise im lebenden Auge sich abspielenden Vorgängen nicht völlig gleich zetzen.

5. Beidseitige Unterbindung der Carotis communis bedingt beim Hunde nur eine mässige Abnahme der Kammerwassersekretion.

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Zum Schlusse spreche ich Herrn Professor Starling für die Aufmunterung zu diesen Untersuchungen und für die Anleitung zu den Experimenten, sowie auch meinem hochverehrten Chef, Herrn Professor Siegerist, für die gütige Durchsicht der Arbeit meinen aufrichtigsten Dank aus. Herrn Professor Pflüger sel., meinen unvergesslichen Vater, erreicht leider mein Dank für das Interesse und die Unterstützung, die er mir bei der Ausarbeitung zuteil werden liess, nicht mehr.

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*The Factors which Determine the Production of Intraocular Fluid.*

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In spite of the very numerous researches which have been made during the last half century on the seat and mechanism of production of intraocular fluid, ophthalmologists and physiologists are still far from an agreement on the subject, and a review of the literature reveals many discrepancies in the experimental evidence which it is impossible to clear away without a re-examination of the whole subject. The following paper contains the results of experiments made with the view of determining the weight to be ascribed to different experimental investigations.

As to the seat of production of the intraocular fluid, nearly all authorities are agreed that it is produced by the ciliary processes. From these processes a minute proportion travels backwards into the vitreous cavity, to be absorbed by the lymphatics of the optic disc, while by far the greater part makes its way between the lens and the ciliary processes, through the fibres of the suspensory ligament, into the posterior chamber, whence it passes round the margin of the iris into the anterior chamber. In addition to this mode of production, it has been suggested by Ehrlich that an appreciable amount of intraocular fluid may be secreted directly into the anterior chamber by the anterior surface of the iris. The experiments of Ehrlich (1) were made by the injection of a diffusible substance, fluorescein, and we agree with Leber (2) in regarding them as proving the possibility of diffusion between the vessels in the iris and the anterior chamber, but not the secretion of a normal intraocular fluid by this channel. At any rate, any fluid formed in this way is negligible when compared with that which is produced in the neighbourhood of the ciliary processes.

On the other hand, the place of absorption of the intraocular fluid is universally agreed to be the angle of the anterior chamber. Here the fluid is passed under pressure into the spaces of Fontana, whence it makes its way into the canal of Schlemm, between the endothelial cells lining this canal, and so is carried away into the venous system. This absorption is continuous, and its rapidity is largely determined by the height of the intraocular pressure. Since we have a constant absorption and a constant pouring out of fluid into the eyeball, it is evident that the intraocular pressure must be

a product of the two factors, formation and absorption, and that the maintenance of the pressure at a constant height must be determined by an accurate balance between these two processes. The problem which lies before us is to determine the mechanism of formation of this fluid.

The intraocular fluid is a clear, colourless solution containing a proportion of salts similar to that of the blood plasma, but having an osmotic pressure which is somewhat higher than the blood plasma, and containing the merest trace of proteids.\*

### I. *Methods of Research.*

The animals used were mostly cats. In a few cases dogs were employed, and in one experiment a rabbit. In the case of the cats the anæsthetic used was always ether, with the addition in some cases of a small dose of morphia. In a few experiments, after the induction of full anæsthesia, a small dose of curare was given. The administration of the anæsthetic was continued during the experiment by an air-pump connected with a cannula in the trachea. For the dogs the A.C.E. mixture was employed.

A record of the blood pressure was kept in all experiments. In some it was taken continuously, but in the greater number of experiments a short record was taken every few minutes in order to avoid trouble with clotting in the cannula. In the cats the blood pressure was taken in the lower part of the abdominal aorta, in the dog in the femoral artery.

The apparatus we employed for measuring the intraocular pressure was very similar to that described in a former paper (3). A graduated tube with internal bore of about 0.5 mm., and about 50 cm. long, is provided with a lateral tube near each end. One end of the tube is connected by india-rubber tubing, by means of a T-piece, with a reservoir containing Ringer's solution (or any other fluid the absorption of which is to be determined), and also with a manometer. The other end is connected by a second (glass) tube with a gilt steel hollow needle, which is introduced into the anterior chamber of the eye. The needle may be open at the end, or be closed at the end and provided with a lateral opening. To each of the side tubes a rubber capsule is attached. The capsule nearest the reservoir contains air, while that towards the eye is filled with fluid. By means of screw-clamps, fluid or air may be driven from either of the two capsules into the graduated tube. Before introducing the needle into the anterior chamber, the pressure in the apparatus is adjusted by raising the reservoir to about 25 cm.  $H_2O$ , which represents the average intraocular pressure. While the fluid is dropping from

\* Full details of various analyses of intraocular fluid are given by Leber (2), p. 207, *et seq.*

the end of the needle, this latter is thrust through the lateral part of the cornea, so as to lie in the middle of the anterior chamber. A bubble of air is introduced into the graduated tube by compression of one capsule, and brought to the middle of the tube by relaxing the clamp on the capsule at the end towards the eye. The reservoir is then rapidly adjusted to such a height that the bubble remains stationary.

In some of the later experiments a platino-iridium cannula, with a solid steel point made slightly conical, was found to be an improvement, as, in the event of any leaking occurring, it could be pushed in further.

In introducing the cannula great care must be used, as, should the needle catch in or tear the iris, or wound the lens, the eye would be rendered useless for the purposes of the experiment. The needle, being comparatively large and blunt, requires considerable force for its introduction. We have found it safer to make a small perforation with the point of a cataract knife and, without letting the aqueous humour escape, to introduce the cannula in the hole thus made. Should the exact spot be lost sight of, a little fluorescein will stain it. A fine silk thread passed through the episcleral tissue, as in the operation for advancement of a rectus tendon, gives a better hold than fixation forceps, and is somewhat less in the way.

The fluid employed in the apparatus was usually Ringer's solution, in some cases normal saline. Whichever fluid was employed, it was filtered through a Berkefeld candle before the experiment, in order that no foreign body might be present which could lodge in and block the filtration channels.

The intraocular fluid must play a twofold function in the eye. In the first place, by keeping up the intraocular pressure, it lends rigidity to the supporting structures of the eyeball, and furnishes therefore a fixed point for the intraocular muscles to contract against, besides maintaining the proper distances between the various refractive media. In the second place, it is the only source of nourishment to certain of the structures of the eye, namely, the middle and back part of the cornea, the lens and suspensory ligament, and the vitreous humour. The question that we have to decide is whether this fluid is formed by a process of secretion by the cells covering the ciliary processes, or whether it is a transudation similar to lymph. The question presents many analogies to that with regard to the secretion of urine. In each case we have a possible source of transudation in the capillary blood-vessel network and also an absorbing mechanism. We can only arrive at a conclusion by determining the physiological conditions under which we may alter either the production or the absorption of the intraocular fluid.

II. *The Effect of Changes in the Circulation on the Formation of Intraocular Fluid.*

If the production of intraocular fluid is dependent on a process of filtration through the blood vessels and the epithelium covering the ciliary processes, its rate must vary directly with the difference of pressure on the two sides of the filtering membrane. It must vary, therefore, directly with changes in the capillary blood pressure, and inversely with the changes in the intraocular pressure. In our first series of experiments we sought to eliminate the second factor, namely, that of absorption, by opening the anterior chamber, so that the intraocular pressure could be regarded as zero. A cannula was introduced into the anterior chamber and the fluid allowed to flow off into weighed porcelain capsules. These were changed every 10 or 20 minutes, and the amount of fluid secreted in the time determined by weighing. The fluid drained off during the first minute after insertion of the cannula was regarded as normal intraocular fluid, but the gradual emptying of the eye-ball continues during the first five minutes, so that the figures obtained during this time cannot be regarded as expressing the rate of secretion. In every case the total solids of the intraocular fluid were also determined.

The following experiment, p. 298, shows the results obtained while the blood pressure was approximately constant. It will be seen that there is a constant diminution in the amount of fluid obtained. In these experiments we were at first troubled by the formation in the anterior chamber of clots, which tended to plug the cannula. We found that this difficulty could be obviated by the injection of a dose of leech extract, not large enough to cause a permanent diminution of the blood pressure.\*

The next question to determine was whether it was possible to alter the rate of production or the composition of the intraocular fluid by altering the blood pressure in the vessels of the eye-ball. The experiments on this point were all carried out on dogs. A diminution of the intraocular blood pressure was easily effected by ligature or obstruction of the carotid artery on the same side. In order to produce a maximal rise of pressure in the blood-vessels of one eye, the vertebral and subclavian arteries on both sides were tied. A loose ligature was placed round the thoracic aorta, so as to permit of its being obstructed at any given time. A cannula connected to the mercurial manometer was placed in the carotid artery on the right side. The production of intraocular fluid was determined in the left eye. By obstruction of the

\* This procedure had been previously employed by Mr. E. Pflüger in some experiments carried out in this laboratory in 1902. An account of these experiments will shortly appear as a dissertation in the University of Bern.

aorta a large rise of blood pressure was produced in this eye, since all the blood had to pass through the one carotid artery in order to get back to the heart. On the other hand, an almost complete anæmia could be produced in the eye by obstruction of the one remaining carotid. We give below the results of one such experiment.

Cat, anaesthetised with Ether and the A.C.E. Mixture. A small dose of Curare was injected after anaesthesia was complete. The extract of 2 grammes of dried leech heads was injected.

Time.	B.P. in mm. Hg.	Weight of secretion.	Weight of solids after drying to a constant weight.	Percentage of solids.	Rate of flow per minute.
3.50 cannula inserted.		grammes.	grammes.		grammes.
3.51 .....	130	0·689	0·009	1·3	
3.56 .....	145	0·252	0·007	2·7	0·05
4.16 .....	120	0·756	0·032	4·2	0·037
4.36 .....	100	0·475	0·021	4·4	0·023
4.56 .....	96	0·482	0·024	4·9	0·024

Dog. Weight, 7½ kilos. Anaesthetised with the A.C.E. mixture and morphia. The extract of 2 grammes of dried leech heads was injected. Both subelavians and vertebrals were tied. Temporary ligature round aorta. Cannula in left eye. B.P. observed in right carotid.

Time.	B.P. in mm. Hg.	Amount of secretion in grammes.	Total solids in grammes.	Percentage of solids.	Rate of flow.	Remarks.
3·29	—	—	—	—	—	Cannula inserted. Aorta unobstructed
3·30	110	0·811	0·013	1·5	—	
3·35	110	0·432	0·014	3·2	0·086	
3·45	100	0·550	0·027	4·9	0·055	
3·55	205	1·153	0·068	5·9	0·115	Aorta obstructed. Fluid tinged red.
4·5	100	0·627	0·039	6·2	0·062	Aorta unobstructed.
4·15	198	0·816	0·053	6·6	0·081	Aorta obstructed.

It will be seen that in every case a rise of intraocular pressure caused an increase in the amount of fluid secreted. It is impossible, however, to deduce directly from these experiments that the intraocular fluid is a transudation. The opening of the eye-ball and the consequent diminution of the intraocular pressure to nothing have a serious effect on all the intraocular structures.

Great dilatation of the vessels of the ciliary processes and iris is produced. The fluid, which, in the normal eye, is free from fibrinogen and contains the merest trace of proteid, rapidly acquires the power of coagulation, and its proteid content rises to 3, 4, or 5 per cent. The serious alteration of the vascular structures is shown in many cases by the appearance of red blood corpuscles in the fluid dropping from the cannula, and Greeff has shown that if the lowered pressure be brought about suddenly and maintained for some time, the epithelium covering the ciliary processes may be raised from the surrounding tissue so as to form small blisters, which are filled with coagulable lymph. It has been suggested by Greeff (4) that the change in composition of the intraocular fluid ensuing on opening the eye-ball is determined by the separation of the epithelium, but Bauer (5) has shown that the proteid contents may be raised in the absence of these epithelial changes, and that, on the other hand, the epithelial changes may be well marked on the subsequent day, when the wound in the cornea has closed, and the intraocular fluid has regained its normal composition. He also points out that the amount of change produced depends entirely on the rapidity with which the intraocular pressure is lowered. The change in composition is probably due, as Leber suggests, to the great distension of the capillaries and the consequent separation of their endothelial cells. It represents in fact an alteration in permeability of the filtering membrane.

### III. *Amount of Intraocular Fluid Produced under Normal Circumstances.*

In any investigation of the factors determining the production and absorption of intraocular fluid, it is important to get some idea of the amount of this fluid secreted under normal circumstances, that is at normal intraocular pressure. Since the intraocular pressure is maintained constant so long as the blood pressure is steady, the amount of fluid produced at a given intraocular pressure must be equal to the amount of fluid absorbed at the same pressure. It is therefore a matter of indifference whether we measure the amount formed or the amount absorbed at any given pressure. Le Plat (6) sought to abolish the absorption of the intraocular fluid by filling the anterior chamber with oil or vaseline. A cannula was placed in the vitreous cavity, and the pressure in the cannula maintained at the normal intraocular pressure. It was found that the obstruction of the absorbing angle of the eye-ball carried out in this way caused a rise of intraocular pressure if the eye-ball were closed, or a flow outwards of intraocular fluid by the cannula if the pressure in this was maintained at the normal intraocular pressure. The amount of this outflow was measured, and was regarded by Le Plat as representing the normal rate of formation of intraocular fluid. He arrived at

the conclusion that the amount of fluid normally secreted by the ciliary processes is in the rabbit about 4 c.mm. per minute. We found considerable difficulties in applying this method, chiefly determined by the tendency of the cannula in the vitreous to become blocked. We therefore adopted a method similar to that already employed by Niesnamoff, (7) under Leber's direction. The arrangement of the experiment was as follows:—

The hollow needle, connected by the capillary tube (containing an air bubble as index) to the reservoir and manometer, was introduced into the anterior chamber. The height of the reservoir was then adjusted until the bubble was stationary, showing that the intraocular pressure was exactly balanced by the pressure of the fluid in the tube leading to the reservoir. This intraocular pressure was of course maintained by a constant secretion of intraocular fluid, exactly equal to the amount escaping by filtration through the anterior angle of the eye. The animal was then killed by dividing the heart. This procedure at once stopped the production of intraocular fluid. The intraocular pressure, however, was maintained at its previous height by the connection of the eye with the reservoir of Ringer's fluid; the escape of fluid by the anterior angle was therefore the same as before. The rate of this escape could be determined by noting the rapidity with which the air bubble moved along the capillary tube towards the eye, and this rate must be equal to the rate of *production* of fluid previously obtaining in the eye under normal conditions of circulation. The following table gives the rate of production of intraocular fluid, determined in this way, with varying intraocular pressures:—

Animal.	Intraocular pressure in mm. Hg.	Inflow, after cessation of circulation, in cubic millimetres per minute.
Cat.....	20	12
Cat.....	15	11
Cat.....	26	12
Cat.....	28	10
Cat.....	14	5
Cat.....	20	15
Average.....	20·5	10·8

It will be seen that there is a considerable difference in the case of filtration in various eyes, and therefore a corresponding difference in rate of production of intraocular fluid.

IV. *The Factors Determining Absorption of Intraocular Fluid.*

In the last set of experiments we determined the rate of absorption of intraocular fluid at the normal intraocular pressure, and regarded this as representing the rate of production of this fluid under normal circumstances. In the same experiment it was possible to alter the intraocular pressure by raising or lowering the reservoir, and so to determine the effect of the height of the intraocular pressure on the rate of absorption. The results of two such experiments are given below, and show conclusively that the rate of absorption is determined, in the absence of disturbing factors which we shall have to consider later on, solely by the height of intraocular pressure.

(1) Cat, anaesthetised with Ether. While the anaesthesia was maintained, a small dose of morphia and curare was injected. Atropine was instilled locally into the conjunctival sac.

B.P. in mm. Hg.	I.O.P. in mm. Hg.	Rate of inflow in cubic millimetres per minute.
115	22	0
115	30	4
115	46	7
130	62	8
Heart divided.		
0	22	12
0	36	16
0	46	19
0	62	22

(2) Cat, anaesthetised with Ether. Atropine and cocaine instilled locally into the conjunctival sac.

B.P. in mm. Hg.	I.O.P. in mm. Hg.	Rate of inflow in cubic millimetres per minute.
124	32	0
124	44	5
124	52	11
110	20	0
116	44	10
116	52	20
Heart divided.		
0	52	22
0	44	15
0	20	12

In a previous paper we have shown that the intraocular pressure varies directly as the blood pressure in the vessels of the eyeball. We must therefore conclude that the rate of absorption of intraocular fluid is also determined by the height of the blood pressure, and since the absorption must keep pace exactly with the formation of this fluid, it follows that the formation of the intraocular fluid must also be determined by the height of the intraocular blood pressure. So far then the conditions which we laid down as necessary to be fulfilled in order to justify the filtration theory of the production of intraocular fluid have been fulfilled, and we might conclude with Leber that the formation of this fluid is exactly analogous to that of lymph, and is determined by the difference of pressure between the blood in the vessels and the fluid outside the vessels. There are, however, certain difficulties in this assumption which have so far not been considered by previous workers, but which must be met satisfactorily before we can come to any definite conclusion on the subject.

It has hitherto been assumed by Leber, Niesnamoff, and others, that a fluid having the composition of intraocular fluid might be formed by a process of filtration through the blood vessels of the ciliary processes under any difference of pressure. In this assumption they have neglected the question of the different proteid content of blood plasma and intraocular fluid. It was shown by one of us (E. H. S.) that, in order to separate a proteid-free transudate from a fluid such as blood serum, a certain amount of work had to be done, and that for this separation a minimum difference of pressure on the two sides of the filtering membrane of at least 28 mm. Hg was necessary. The intraocular fluid has such a small content in proteid that it may be regarded as analogous in all respects to the fluid which is supposed to be separated by the glomeruli of the kidney. In order therefore that any fluid shall be poured out in the eyeball, a minimum difference of 30 mm. Hg must be present between intraocular pressure and capillary blood pressure. If this pressure difference is not present, work must be done by the cells forming the filtering membrane, and the formation of intraocular fluid must be regarded in the light of a secretion rather than in that of a transudation. A definite decision on this point could be reached if we had any means of determining the blood pressure in the capillaries of the eyeball. A method for this purpose has been devised by Niesnamoff, (7) and this observer states that the normal intraocular capillary pressure is about 50 mm. of mercury. His arguments, however, involve several fallacies. In his experiments he connected a cannula, attached to a reservoir of salt solution, with the eyeball of a living animal. He found that the fluid neither ran in nor out at 25 mm. Hg, which was therefore the intraocular pressure. He then

determined the rate of inflow when the pressure in his cannula was raised to 50 mm., 75 mm., and 100 mm. Hg. He then killed the animal, and again determined the rate at which the fluid would flow in under these various pressures. He found that above 50 mm. Hg the rate of inflow was the same in the dead as in the living animal. He therefore concluded that 50 mm. Hg represented the intracapillary pressure. In coming to this conclusion he was guided by the assumption that, when the intraocular pressure was raised so as to be equal to the intracapillary pressure, the transudation of intraocular fluid would cease, and above this pressure the rate of inflow for his reservoir would be, therefore, the same in the living and dead eye. It is impossible, however, by this method to determine intracapillary pressure. The globe of the eyeball is practically rigid. As the intraocular pressure is raised, the intraocular fluid will press upon the veins of the ciliary processes, and the blood pressure will therefore rise in the capillaries and in the veins until it is greater than the intraocular pressure. With successive rises in the intraocular pressure the pressure in capillaries and veins must get larger and larger in order that any circulation of blood may be maintained, and the circulation through the capillaries will cease only when the intraocular pressure is very nearly as high as the arterial pressure. If the circulation in Niesnamoff's experiments ceased at 50 mm. Hg, it is evident that the normal intracapillary pressure, when the intraocular pressure is 25 mm. Hg, must be considerably below 50 mm. Hg. How then are we to explain the very definite figures obtained by Niesnamoff? This observer apparently performed very few experiments. In his paper he gives the results of only one such experiment as that here described. On repeating his experiments we found it impossible to obtain anything like such definite figures—and this for various reasons. In the first place, a considerable rise of intraocular pressure, such as to 50 or 70 mm. Hg, exercises an abnormal stretching effect upon the filtering apparatus of the eyeball, so that the channels at the anterior angle of the eye are gradually opened up, and in many experiments we observed a consequent gradual increase in the rate of inflow of the fluid. In most experiments, for example, the rate of inflow was greater with descending pressures than with ascending pressures. This is well shown in experiment No. 2, on p. 301.

The following experiment shows the dilatation consequent on a preliminary raising of the intraocular pressure :—

Cat, anæsthetised with Ether. Eserine applied locally to conjunctival sac.  
Pupil moderately contracted.

B.P. in mm. Hg.	I.O.P. in mm. Hg.	Rate of absorption in cubic millimetres per minute.
110	16	0
110	32	5
110	48	8
108	64	9
112	16	0
112	32	8
112	48	13
112	64	18

Another disturbing factor is the size of the pupil. We shall have to consider this factor more in detail later on, but unless atropin be given at the beginning of the experiment, the observations on the living eye are made with a somewhat contracted pupil, whereas those on the dead eye are made on a widely dilated pupil. Other factors being equal, the filtration in the eye with dilated pupil is always slower than in the eye with contracted pupil. In certain of our experiments we observed an equality of inflow between the dead and living eye at some pressure above 40 mm. of mercury, but on further raising the pressure this equality disappeared, showing that we were dealing with yielding tissues and altering membranes. This fact rendered it impossible to obtain by such methods any definite information of the intracapillary pressure in the eye-ball, or of the level of intraocular pressure at which transudation or formation of intraocular fluid would definitely cease. One other factor which would aid in disturbing the results obtained is the effect of a high intraocular pressure on the general circulation through the eye-ball. If we succeed in raising the pressure to such a height that the circulation is entirely abolished, changes must rapidly take place in the apparatus both for formation and absorption of intraocular fluid, and subsequent results cannot be compared with those obtained before such a cessation of circulation. The raising of the intraocular pressure in itself may act as a stimulus and cause reflexly alterations in blood flow, in the general blood pressure, or in the state of contraction of the pupil. The co-operation of these various factors suffices to explain the varying results obtained in the very many experiments we performed upon this subject, including those of which we have already given details. We are of opinion, therefore, that the results obtained by Niesnamoff must be regarded as accidental, and that a greater number of experiments would have convinced this observer of the fallacies of his method.

Although it is impossible at present to determine the intracapillary pressure in the ciliary processes, we may at any rate inquire whether there is, in all experiments on the subject, the possibility of a difference of pressure of 30 mm. Hg between intracapillary blood pressure and intraocular pressure. In the case of a similar question in the kidney, we have been accustomed to compare the aortic blood pressure with the ureter pressure, and have regarded a difference of 40 mm. between these two pressures as satisfying the necessary conditions for filtration through the glomeruli. A similar comparison of arterial blood pressure and intraocular pressure leads to the same result. Below we give the intraocular pressure and arterial pressure as determined in a series of 20 experiments. It will be seen that in every case there is a difference between the two pressures of at least 48 mm. Hg, the average difference of pressure in all the experiments being 84.8 mm. Hg.

Animal.	B.P. in mm. Hg.	I.O.P. in mm. Hg.	B.P. - I.O.P.
Cat.....	130	16	114
Cat.....	140	25	115
Cat.....	138	20	118
Cat.....	94	24	70
Rabbit .....	74	16	58
Dog .....	112	14	98
Cat.....	104	15	89
Cat.....	106	19	87
Cat.....	106	18	88
Cat.....	120	20	100
Cat.....	150	22	128
Dog .....	84	12	72
Dog .....	58	10	48
Dog .....	70	16	54
Cat.....	115	23	92
Cat.....	124	32	92
Cat.....	110	16	94
Cat.....	138	22	116
Cat.....	94	27	67
Cat.....	110	24	96

So far then our observations tend to support in every particular the view laid down by Leber, namely, that intraocular fluid is produced in the ciliary processes by a process of filtration, and that the sole factor determining the amount of transuded fluid is the difference of pressure between the blood in the capillaries and the fluid in the eye-ball.

#### V. *Influence of the Proteid Content of the Intraocular Fluid on the Intraocular Pressure.*

The fact that the intraocular fluid has to be filtered through the intercellular channels of the endothelium bounding the spaces of Fontana and lining the

canal of Schlemm, in order to escape from the eyeball, suggests that the resistance will be greater if the viscosity of the filtering fluid be increased in consequence of raised proteid content. Indeed, one form of raised intraocular pressure, the glaucoma accompanying inflammation of the ciliary region, has been ascribed to the greater proteid content of the intraocular fluid secreted by the inflamed vessels, and the consequent greater resistance to the filtration of this fluid through the anterior angle of the eye. So far as we are aware, there are no direct determinations of the relative rates of filtration of normal salt solutions with and without proteid. We have, therefore, in a series of animals, determined the intraocular pressure under the two conditions:—

- (a) With normal intraocular fluid.
- (b) After replacing this fluid by blood serum.

We have also compared the relative rates of filtration of normal salt solution and of serum in the living and dead eye.

In our experiments one eye of the animal was connected with a reservoir and manometer containing Ringer's saline fluid, while the other was connected with a similar apparatus filled with filtered blood serum.

In order to determine the intraocular pressure in an eye, in which the normal aqueous humour had been replaced by serum, after introduction of the hollow needle, the aqueous was allowed to escape through the side opening in the cannula. Serum was then allowed to flow in for a time, and then the contents of the anterior chamber again allowed to escape. The side tube was then closed, an air bubble introduced into the capillary tube, and the pressure determined at which the bubble moved neither backwards nor forwards.

In nearly every experiment the intraocular pressure, during the first 5 or 10 minutes after the insertion of the cannula, was higher in the eye filled with serum than in the eye filled with normal fluid. The difference, however, rapidly diminished, so that 15 to 20 minutes after the beginning of the observation the pressures were practically identical in the two eyes, and remained so throughout the rest of the experiment. It must be remembered that with the zero method used by us there is no movement of fluid into the eye. Hence the fluid necessary to replace the loss by filtration and to maintain the intraocular pressure is being constantly secreted by the ciliary processes, and is probably of the normal composition, *i.e.*, practically free from proteid. We should therefore expect a gradual decline of the intraocular pressure in the eye with serum, although hardly so rapid an equalisation of the pressures on the two sides as we actually observed in our experiments.

After the determination of the intraocular pressure, the animal was killed by opening its heart, and the inflow of serum and saline fluid respectively observed, first under the normal intraocular pressure, and then under raised pressures.

The results of two such experiments are given below. It will be seen that there is a marked difference in the rate of filtration of the two fluids, that of serum being, as one might predict, very much slower than that of saline.\*

Experiment 1.—Dog, A.C.E. Morphia. Curare. Vagi cut.

Time.	Blood pressure.	Intraocular pressure.	
		Salt eye.	Serum eye.
4.15	70 mm. Hg.	26.2	29.4 cm. water.
4.20	70 "	24.2	27 "
4.45	100 "	29.2	29 "
Animal killed by opening heart.			

Pressure.	Inflow per minute in cubic millimetres (after 10 minutes).	
	Salt.	Serum.
29 cm.	11.5	6
—	11.5	6
—	11.5	6

Experiment 2.—Cat. Ether, morphia, curare.

Time.	Blood pressure.	Intraocular pressure.	
		Salt.	Serum.
3.0 P.M.	120	14.8	15.1
3.10	116	10.8	12.5
3.20	110	9.2	11.5
Animal killed.			

\* Although serum filters more slowly than normal intraocular fluid or saline, the difference is not sufficiently great to cause any marked variation in the intraocular pressures on the two sides. One cannot, therefore, in view of these observations, ascribe any large part in the production of any form of glaucoma to possible differences in the composition of the aqueous humour which might be determined by inflammatory conditions of the blood vessels.

Inflow three minutes later at same intraocular pressures—

Salt.	Serum.
6	3
5	3·5
5	6
5	4
4	4

Fifteen minutes later—

3·5	1·5
3·0	1·5
3·5	1·5
etc.	etc.

This difference in the rate of filtration of the two fluids becomes greater the higher the intraocular pressure is raised.

#### VI. *The Effect of the Size of the Pupil on the Absorption of Intraocular Fluid.*

In the experiments we made to decide this point, one eye of the animal under observation was treated with eserine and the other with atropine. The instillation of these drugs should be begun before the induction of anæsthesia, as the action of eserine is very uncertain if only instilled after anæsthesia.

We have found, as a result of these experiments, that the intraocular pressure in the two eyes remains the same during the time of observation, but that, if the pressure in the apparatus be raised, the rate of filtration in the eye under eserine is much greater than in that under atropine.

It is difficult to give a precise explanation as to the cause of this difference. Stretching of the filtration spaces at the angle of the anterior chamber may possibly account for it all. If this, however, is the case, we should expect to find the intraocular pressure at a lower level in the eye with the contracted pupil, for the intraocular pressure must of course be the product of the rate of secretion and the rate of absorption of the intraocular fluid. The same objection applies to the explanation of this phenomenon by Grönholm (9), who states that in his opinion it is due to diminished intraocular secretion as a result of the contraction of the intraocular vessels. It may also be possible that at these raised pressures other channels of filtration are opened up—such for instance as the surface of the iris. An important, perhaps the most important, factor, however, must be the crushing of the dilated flaccid iris

into the filtration angle of the eye, thus causing a mechanical obstruction, which will be more marked the greater the intraocular pressure. Hence the smaller amount of filtration in the atropinised or dead eye with dilated pupil, as compared with that in the eye which has been put under the influence of eserine.

The figures of a typical experiment are given.

Cat, anæsthetised with Ether. Blood pressure average 138 mm. Hg, with only trifling variations throughout the experiment.

Intraocular pressure in mm. Hg.	Rate of filtration in eserine eye in cubic millimetres per minute.	Rate of filtration in atropine eye in cubic millimetres per minute.	Rate of filtration in atropine eye <i>post-mortem</i> , in cubic millimetres per minute.
20	0	0	15
35	11	8	20
50	16	11	25
65	23	14	31

#### *Summary of Conclusions.*

1. The intraocular pressure represents the pressure at which the rate of formation of intraocular fluid is exactly balanced by its rate of escape through the filtration angle of the eye.

2. The production of intraocular fluid is strictly proportional to the difference of pressure between the blood in the capillaries of the eyeball and the intraocular fluid.

3. No satisfactory method of measuring the intracapillary pressure in the eyeball has been yet devised. The fallacies of Niesnamoff's method are pointed out. Judging, however, from a comparison of the arterial pressures and the intraocular pressures in a large number of animals under different conditions, there is probably always a difference between the intracapillary pressure and intraocular pressure, which is sufficient to account for the production of the intraocular fluid, without assuming any active intervention on the part of the cells of the capillary walls or of the ciliary processes.

4. An increased proteid content of intraocular fluid slows its rate of absorption in consequence of the mechanical hindrance of the proteid to filtration.

5. Filtration, *i.e.*, the absorption of intraocular fluids, at high intraocular pressures is favoured by constriction of the pupil and hindered by dilatation of the pupil. The difference, however, is barely perceptible with normal or low intraocular pressures.

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ON THE RELATION OF NERVE CELLS TO FATIGUE  
OF THEIR NERVE FIBRES. BY F. H. SCOTT, PH.D.,  
M.B. (Eleven figures in text.)

(From the *Physiological Laboratories of the Thierärztliche  
Hochschule, Berlin, and University College, London.*)

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*I. Introduction.*

IN a former paper<sup>1</sup> I pointed out the similarity, both as regards chemical constituents and function, existing between nerve cells, the cells of the pancreas, and the chief cells of the fundus glands of the stomach. On these similarities, and from the known function of the constituents in the latter two cells, I put forward the hypothesis that in the body of the nerve cell a substance is formed from the nucleus and Nissl bodies which gradually passes into the nerve fibres; and also that stimulation of other cells by a nerve fibre is brought about by the passage of some of this substance into the cells on which the fibre acts. In the present paper I give an account of some experiments on the effect of nerve cells on fatigue which seem to support this hypothesis.

A great difference in the behaviour of nerves as regards fatigue has been noticed in their property of conduction and their power to keep the end organ in activity. Nerves are unfatiguable as regards conduction, but their power of keeping the end organ in activity is more or less

<sup>1</sup> *Brain*, xxviii. p. 506. 1906.

limited<sup>1</sup>. Sherrington<sup>2</sup> has also noted that the reflexes obtained from a given place of the skin soon cease if the stimulus be repeated frequently, although similar reflexes may still be obtained from neighbouring areas. Bethe<sup>3</sup> has also noticed that a cut nerve in frogs loses its sensitiveness much more rapidly if it be subjected to stimulation than if it be left at rest. If all these results do not show the discharge of some substance out of the nerve, they at least emphasise the great difference in the behaviour of nerves when they merely conduct and when they conduct and excite other cells.

We know, chiefly from the work of Mosso, that the central is much more readily fatigued than the peripheral nervous system, and it ought, therefore, to be possible in the former within the limits of a fairly short experiment to localise the actual seat of fatigue, and to determine the factors necessary for recovery of such a part. The stimulation of different parts of the motor tracts affords some evidence in this regard. Levy<sup>4</sup> experimented on the amount and nature of the fatigue, as judged by the muscular movements, resulting from stimulation of different parts of the motor tract. He noticed that, on cutting the cord, the recovery from fatigue caused by stimulation of the lateral columns lower down is much slower than when the cortex itself was excited before division of the cord. I believe this is due to the severance of the synapse from its cells of origin, but, of course, the alteration in blood supply must also be considered.

To decide if there is any recovery after complete fatigue of the severed fibres connecting the brain and cord I made some experiments on the frog. The frog was chosen, since in this animal it is possible to eliminate differences in conducting power or excitability due to changes in temperature. The cord was cut and electrodes applied to its upper end. My experiments showed that it is possible by repeated stimulation to bring these fibres into such a condition that they will not give any more response on stimulation, and such condition remained permanent as long as the frogs lived or were kept (6 to 36 hours), but during all this time stimulation of the central end of the sciatic caused reflexes, thus showing that the fault does not lie in the discharging (motor) cells.

As it was impossible to limit the stimulations to one side of the cord,

<sup>1</sup> Cp. Waller. *Brit. Med. Journ.* ii. p. 135. 1885; Brodie and Halliburton, *This Journal*, xxviii. p. 181. 1902, and papers there quoted.

<sup>2</sup> *Phil. Trans. Roy. Soc. London*, cxc. B. p. 165. 1898; and Schäfer's *Text Book*, ii. p. 831. 1900.

<sup>3</sup> *Allg. Anat. v. Physiol. des Nervensystems*. Leipzig. 1903. p. 164.

<sup>4</sup> *This Journal*, xxvi. p. 210. 1901; and xxviii. p. 1. 1902.

leaving the other side as a control, and since the subject can be better investigated with the dorsal roots, I shall not give any fuller details of these experiments.

## 2. *Fatigue in cut dorsal roots.*

The first series of experiments was carried out to see if a dorsal root cut off from its cells of origin could be permanently fatigued. Two roots were cut close to the ganglion, and one left as a control, while the central end of the other was stimulated. The frog was given ether, its forebrain removed by the cautery, and in some cases the cord was divided below the medulla, also by means of the cautery. The laminae were removed from the lower 3 or 4 vertebræ<sup>1</sup> with scissors and the roots tied proximal to the ganglia with threads and then divided. In most cases the dorsal root of the 10th nerve leaving between the urostyle and last vertebra was secured on both sides but in some cases two roots on the same sides were used. A pair of platinum electrodes were fixed on a stand above the back of the frog so that the nerve could readily be removed for moistening. In this set of experiments the reflex movements of the limbs were observed, but tracings were not taken. Tracings of the movements of the gastrocnemius due to excitation of cut dorsal roots were, however, made in a later series of experiments. Some of these may be seen in the upper tracings of Figs. 1-11.

The reflex excitability was heightened by cooling (Biedermann<sup>2</sup>). By adopting this precaution it is possible to evoke reflex contractions with a much smaller strength of current than would be required by normal frogs. One can in this way avoid all danger of spread of current to adjacent excitable tissues. In most cases the frogs gave reflex contractions with the secondary coil 25-50 cm. from the primary. In some cases this is less than that required to stimulate the motor nerves directly. The weakest possible current was always used. To obtain maximal contractions it is not necessary to increase the current beyond a certain point, as the contractions do not last longer nor are they stronger than with the weaker stimuli. (Sherrington<sup>3</sup>.)

There are variations in the type of muscular response on stimulation of a dorsal root and I have seen the different types described by Wundt, Nothnagel, Sherrington, Biedermann, and others in their various

<sup>1</sup> By snipping off the upper end of the urostyle and then inserting the point of one blade of the scissors, the laminae may be removed without even opening the coverings of the cord.

<sup>2</sup> *Pflüger's Archiv*, LXXX. p. 408. 1900.

<sup>3</sup> *Phil. Trans. Roy. Soc. London*, cxc. B. p. 140. 1898.

publications. Samples of the various types may be seen in the figures. There is usually a tetanic contraction (Figs. 1, 2, 3, 4), but occasionally the contraction is little more than a simple twitch (Fig. 7). Sometimes the prolonged tetanic contraction tends to become alternations of contraction and relaxation, but not always, and often the responses are alternating from the beginning (Fig. 2).

The movement obtained at first usually lasts about a minute, and then the muscles gradually return to rest although the stimulation is continued. After a short pause in the stimulation a second but shorter movement may be obtained. These alternate periods of stimulation and rest may be repeated a certain number of times, and a long time elapses before the last trace of movement disappears. Finally, however, even after long periods of rest no response can be obtained. In most cases the roots were stimulated for given periods, usually 30 seconds and then 30 seconds rest allowed. When the root failed to give a response after the 30 seconds rest, a longer pause ( $\frac{1}{2}$  to 2 hours) was allowed. After 5 or 6 of these periods of stimulation the responses (after a long rest) in a period of stimulation became less and less, and finally no more response could be obtained from it as long as the frog lived (compare upper tracings of Figs. 2, 3, 4, 5, and 6, 7, 8). This condition of ineffectiveness of the root was true, not only for the weak current which had been used for the excitation, but also for the strongest currents which could be applied. There is, of course, great difference in the total duration of movement obtainable from such a severed dorsal root. Ordinarily one can obtain 15-40 minutes' movement (in all), after which no further response can be evoked, however long the frog be kept (2-3 days). A few frogs gave a greater total response than 40 minutes, and quite a number a smaller one than 15 minutes. The smallest total for a cut root equalling  $4\frac{1}{2}$  minutes, and the largest 69 minutes. In three cases the root was not exhausted as rapidly as possible but only a few periods of stimulations given per diem. The totals in these experiments extending over 4 days were 18, 24 and 40 minutes. These numbers were often exceeded in less time, as the table (I) shows, and indeed as much as  $37\frac{3}{4}$  minutes was observed during the first 3 hours of one experiment (No. 4, Table II).

During the time of these experiments the root left as a control loses none or very little of its power to keep the muscles contracted. Table I shows the results of some of these experiments. Many more were made, at first to ascertain the truth of foregoing statements, but as in these earlier experiments the total duration of movement obtained was not

TABLE I.

Number	Mode of preparation	Sensitiveness of roots at commencement of experiment in distance (cm.) of secondary coil from the primary	Number of hours before root became inactive	Total duration (mins.) of movement obtained from stimulated root	Sensitiveness of control root when stimulus strongest current	Number of hours after the stimulated root became ineffective during which the frog was observed
1	R. and L. 10th roots tied and cut at ganglion	Each direct reflex at 9	34	Not observed	8½	24. Killed.
2	"	Each at 12	22	"	11½	48. Died during following night.
3	"	R. = 18 L. = 16	20	15	L. = 15	8. Died during following night.
4	"	Each at 40	23	26	38	48. Killed.
5	"	Each at 42	96	40	35	20. Died during following night.
6	"	Each at 38	98	18	35	36. Killed.
7	9th and 10th roots	Each at 34	94	24	31	5. Died during following night.
8	"	Each at 45	32	49	43	64. Sprang from table to floor and died shortly afterwards.
9	R. and L. 10th roots	Each at 25	33	41	23	48. Killed.
10	"	Each at 30	10	17	30	72. Died during following night.
11	"	Each at 40	44	12	38	24. Killed.

*Note.*—The first three experiments were made with frogs directly from the tank. The remainder with cooled frogs. The numbers of the roots are after the Ecker-Gaupp nomenclature. During the time the frog was observed (after the root became ineffective) the circulation in the web was satisfactory. In some of those cases in which the frog died it was commencing to be feeble. In several cases the circulation was good when last observed but during the night the frogs turned on their backs. They seem to die rapidly in this position.

measured, only two are tabulated. The number of hours before the root became inactive in these earlier experiments varied from 8 to 40. Many other experiments were also nearly completed, viz. the root was giving very short replies after long rests, when some accident to the root put an end to the experiment. These also are not tabulated. The above table shows that for a cut root to become inactive in the short time of these experiments there must be activity. It tends also to show that only a certain amount of movement can be obtained from a cut root, no matter over how many hours the stimulation is extended.

The above experiments show that in a comparatively short time it is possible through activity to bring the reflex arc into such a condition that it no longer gives a reaction on stimulation of the dorsal root. The following possibilities seem to present themselves.

- (1) The muscles are fatigued.
- (2) The cells in the cord are fatigued or dead.
- (3) The root itself is dead and non-conducting.
- (4) Or, as I believe, that all the substance which passes between the root and the cells in the cord, and excites the latter, is used up. The root is therefore ineffective.

In order to guard against either of the first two possibilities the operation was performed with the least possible loss of blood, and the skin kept well moistened the whole time. This latter is most essential, especially if the breathing be feeble.

The condition of ineffectiveness of the dorsal roots, due to their prolonged stimulation, was not due to fatigue in the muscles, since direct stimulation of the sciatic still caused contractions.

On the probable assumption that the motor cells supplying the limb may all be set in action by one dorsal root, it is clear also that the seat of fatigue is not in these motor cells, since movements of the limb were still obtained reflexly by stimulation of other nerve roots (cp. Figs. 8 and 9), and indeed occurred usually on release of the animal from its extended position, and also at times without any obvious stimulation.

The total amount of stimulation had also not injured the conducting property of the fibres. This, I think, is shown by the fact that after prolonged continuous stimulation the roots are still effective if a rest be allowed. Thus in three cases the roots were stimulated continuously for 2, 3½, and 5 hours. After a rest (½ to 1 hour) the root in each case

gave nearly as prolonged contraction as it did before the long stimulation.

Lastly, the absence of effect was not due to loss of irritability in consequence of degeneration. In the frog the dorsal roots, as also other nerves, very slowly lose their irritability. In one case I cut the roots and left them for 10 days. At the end of this time they still caused the normal amount of movement.

It is thus seen that the seat of fatigue is in the root itself, and also for the production of this fatigue there must be excitation of the cells in the cord.

### 3. *Stimulation of roots, one severed and the other unsevered from the spinal ganglion.*

In order to see if the dorsal root would activate the cells in the cord longer if it were still connected with the nerve cells in the spinal ganglion, the following three series of experiments were made.

1. In the first set a pair of electrodes was passed under the uncut root, and the cut root also laid on the same electrodes so that the two sides were stimulated at the same time. At first fatigue is obtained equally rapidly on both sides, and at first recovery is as rapid on both sides. After a time the cut root begins to lag behind, both in its power of recovery and in its effectiveness in keeping the movement prolonged, and finally the cut root is ineffective while the unsevered root is quite good, and in some cases nearly as effective as at first. As the metabolism in frogs is slow, and this slowness is no doubt increased by the low temperature at which these frogs are kept, it is some hours after the experiment has commenced before any difference is noted in the two roots.

Although I tried always to regulate the current so that it gave only the direct reflex, yet one could not be certain that a movement might not be partly due to the impulse from the other root, and as the uncut root is very liable to be detached from the cord by a sudden movement of the frog, this plan was abandoned, but not before four experiments had been completed and several others nearly so.

2. In the second set of experiments the plan was adopted of stimulating the cut root and the corresponding cut nerve in the pelvic plexus. By means of a commutator the two sides were stimulated alternately and approximately equally for equal periods. In order to equalize the sides the two roots (anterior and posterior) of the one side

were cut and laid on the electrodes, but even with this arrangement the root usually responded with a slightly weaker current than the nerve, but if the frog has been well cooled the difference is not marked. Tracings were made by extending the hind legs with elastic bands and then attaching threads to each cut tendo achilles. The threads were passed over pulleys and attached to levers. Signal magnets under the levers marked the point of stimulation. A second battery was used to operate the magnets. In most cases the two sides responded equally well and gave the same number of contractions before failing to respond after 30 seconds rest (Fig. 1). In a few cases the ganglionated or the severed failed a little before the other. If there was very marked inequality the experiment was abandoned, as probably some injury had been done in the preparation. The one side was usually stimulated for 30 seconds and then the other. When one side failed a longer rest was allowed. In all cases, finally, the ganglionated side was the better. The tracings show some of the records obtained in the manner above described. The levers were made as equally sensitive as possible, and were tested by interchanging the levers of the two muscles.

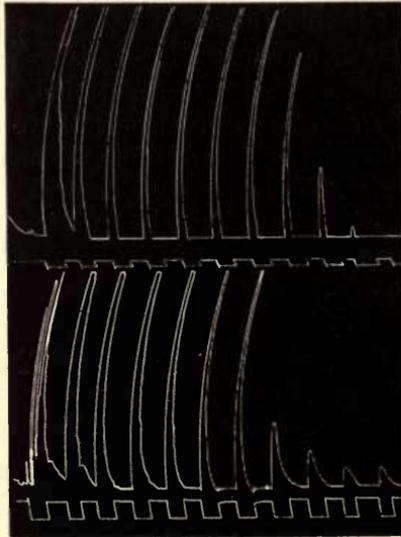


Fig. 1. Upper line from stimulation of cut root, lower line from corresponding nerve in plexus. The fall of the signal indicates stimulation. The time of stimulation equals 30 seconds, when the commutators were reversed into the other electrodes and magnet.

Fig. 1 is a tracing in which the responses are much more regular than usual. It represents the second set of stimulations, one hour after

the commencement of the experiment. Both sides are seen to be practically identical, and the gradual loss of power is well seen. This gradual loss of power is a typical feature, although often not as regularly shown. Figs. 2, 3, 4, and 5 are parts of the record of the

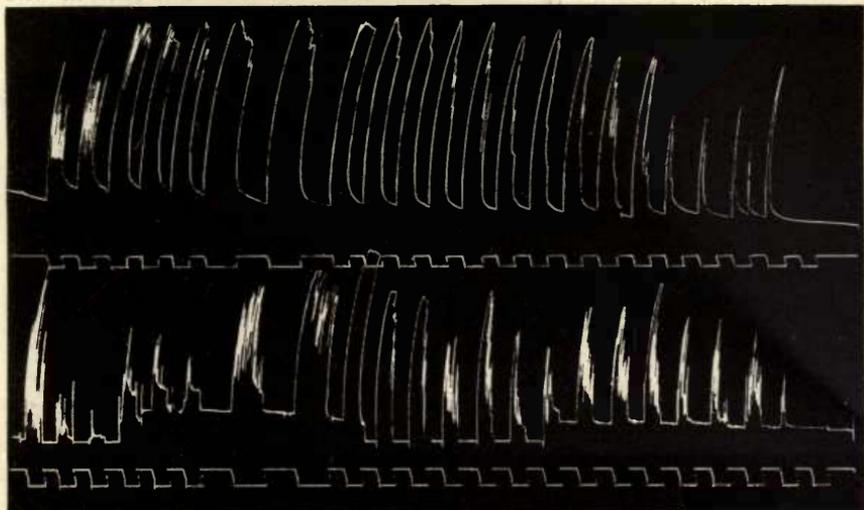


Fig. 2. Upper line from cut root, lower line from corresponding cut nerve in plexus.  
Time of stimulation 30 seconds or twice (in middle) 1 minute.

same frog. This frog had the greatest resistance to fatigue and the greatest power of recovery of any frog met with. Fig. 2 is the first tracing, finished, as the number above indicates, at 2.41 in the afternoon. It was stimulated twice more during the afternoon, and once the next morning before Fig. 3 was obtained. It will be seen that the lower one (ganglionated) gives more prolonged contractions, although its power of recovery at the end to rapidly repeated stimuli (every 10 sec.) is slightly less than the one without the ganglion. One more series of stimulations, in which the ganglionated root was much the better, was made before Fig. 4 was obtained at 12.02 that is 22 hours after the commencement of the experiment. It is now seen that the lower one is undoubtedly the better, and it remained so till the end. The cut root gave a little more response during the afternoon and a few twitches the next morning, when it finally failed. Fig. 5 is a piece of tracing taken 48 hours after the commencement of the experiment. It is seen that the severed root is inactive, while the unsevered root gives fair responses.

During the first three hours of this experiment the total duration of movement from the severed root aggregated  $37\frac{1}{2}$  minutes, and from the unsevered 38 minutes. On the next day the numbers were  $31\frac{1}{4}$  minutes for the severed, and  $40\frac{1}{4}$  for the unsevered, while on the following day a few twitches, aggregating about  $\frac{1}{4}$  minute, were obtained from the

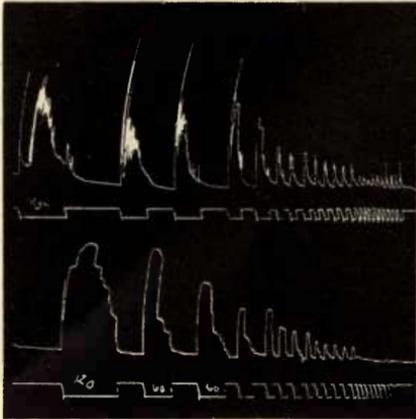


Fig. 3.

Fig. 3. Upper line from cut root, lower from corresponding nerve in plexus. The numbers in the signal line indicate seconds. At end stimulation every 10 seconds.

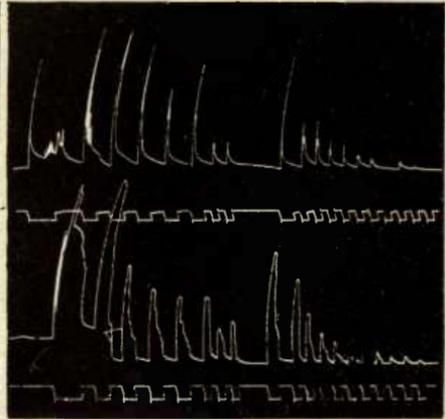


Fig. 4.

Fig. 4. Upper line from cut root, lower from nerve in plexus. Stimulation 1 minute, 30 seconds and 20 seconds. The L indicates a change of level of lever.

severed, and the unsevered gave  $3\frac{1}{2}$  minutes at that time, and was still effective afterwards, as the figure shows.

One does not usually get numbers so large as those in the above experiment, the total of 69 minutes being the greatest observed for a cut root. As before stated it is usual to obtain about 15-40 minutes from the cut root. Figures 6, 7, and 8 are from a frog which did not yield even that amount because most of the time the responses were little better than simple contractions. Fig. 6 is the first series obtained and illustrates the irregularity often met with at first. The contractions with a *V* over them are spontaneous, caused by some other irritation than the stimulation. The commencement of the lower line illustrates a phenomenon sometimes seen, namely, an inhibition of tone on stimulation of the opposite root. Both lines contain a number of crossed reflexes. These are marked in the tracings by a cross. Owing to a slight misplacement the lower two levers were not exactly under the upper two but the stimulation was alternating as in the other experi-

ments. Fig. 7 illustrates the responses after 5 hours (3 stimulations in between), and Fig. 8 after 25 hours (3 more stimulations).

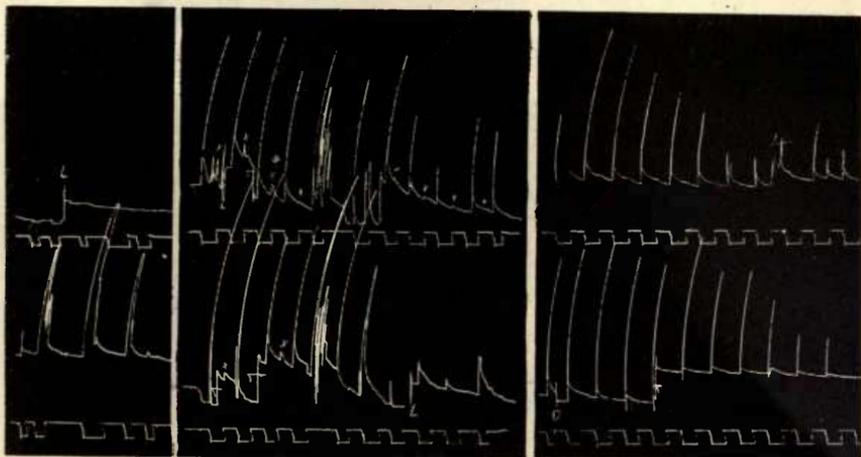


Fig. 5.

Fig. 6.

Fig. 7.

Fig. 5. Upper line from cut root, lower from nerve in plexus. The upper lever was raised at *L*.

Fig. 6. Upper line from cut root, lower from corresponding cut nerve in pelvic plexus. The contractions with *V* over are due to other causes than the stimulation. Crossed reflexes are seen in both lines (marked *X*) and also an inhibition of tone (fall of lever in lower line) on stimulation of opposite root. *L* change of level of lever.

Fig. 7. Upper line from root, lower from nerve in plexus. *V* 'spontaneous' contractions. *D*=stoppage of drum, and *L* change in level of the lever. The momentary response sometimes met with on stimulation of the afferent nerves is well shown, as it is also in Fig. 6.

It will be noticed the upper (cut) root gives a single small response only. All the other contractions in the upper line marked *X* are crossed reflexes. To make this clear the longer interval was allowed. The uncut (ganglionated) root is still effective and remained so for another 24 hours, when the frog died, while the cut root gave only one more twitch. During the first 6 hours of this experiment the two sides gave practically the same total duration of movement, the numbers being  $8\frac{1}{4}$  minutes for the cut root and  $8\frac{1}{2}$  for the ganglionated. On the next day, however, the total amount for the cut root was only  $1\frac{1}{2}$  minutes during 3 hours when it ceased to give more contractions, while the nerve had then given  $4\frac{1}{2}$  minutes and as the tracing shows was still effective.

3. The third set of experiments were made in view of the possibility that the difference in the action of the two roots might be due to a better blood supply and not to the nerve cells themselves. The two corresponding nerves were cut in the plexus and the ganglion on each side carefully dissected free so that both nerves with their ganglia were

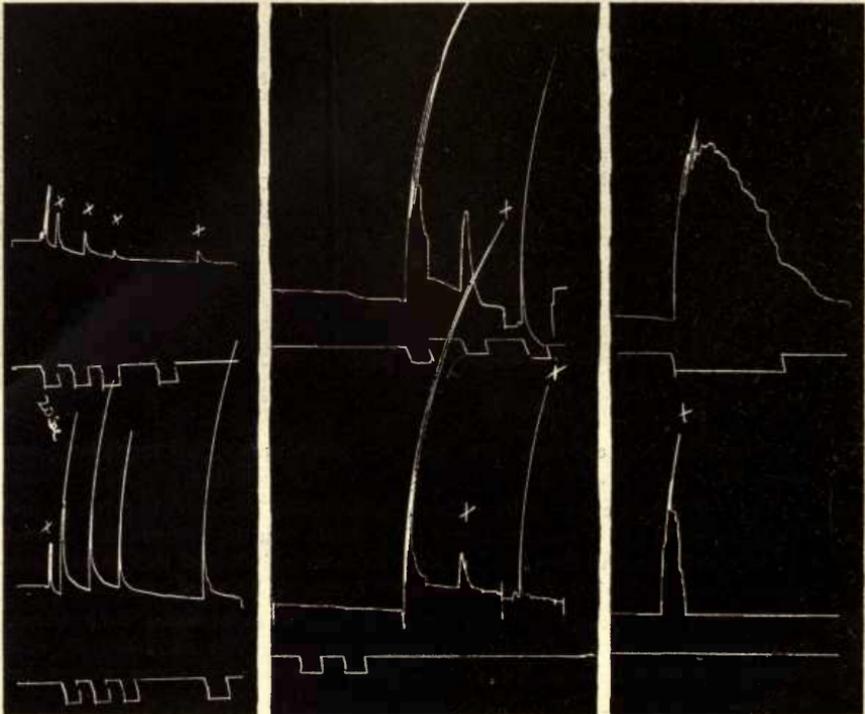


Fig. 8.

Fig. 9.

Fig. 10.

Fig. 8. Upper line from root, lower from nerve in plexus. Only the first contraction in upper line is from the cut root, all the others (marked X) are from the opposite side (crossed reflexes) as can be clearly seen when the longer interval was allowed. The cut root is also seen to have caused a crossed reflex in lower line (first contraction).

Fig. 9. Upper line tracing from unligatured, lower from ligatured root. It is seen there are no contractions from the ligatured root. The contractions (marked X) in the lower line are crossed reflexes from the unligatured root.

Fig. 10. Reflexes from dorsal root belonging to same side as upper tracing. Time of stimulation 2 minutes. Lower tracing is the crossed reflex.

connected to the cord by their roots only and were thus equal as regards blood supply. A thread was bound tightly around one root and the whole dissection kept moistened with oxygenated frog's blood diluted

with Ringer's solution. As it was desired to stimulate the *roots* of each side and as it is not convenient to use 2 pairs of electrodes in the limited space, the plan was followed of stimulating one root and when it did not give a response after 30 seconds rest it was immediately removed and the second root laid on the same electrode. In all cases tried the ganglionated root remained the better. Fig. 9 is a tracing taken 28 hours after the commencement of the experiment. The frog responded throughout by a few (3 or 4) well sustained contractions and then failed. On the first day (6 hours) the responses aggregated the same,  $11\frac{1}{4}$  minutes from each. On the second day the responses up to 12 o'clock, when the ligatured root failed, aggregated  $2\frac{1}{4}$  minutes for the ligatured and  $7\frac{1}{2}$  minutes for the unligatured. After the tracing was taken, which as the times above indicate was nearly two hours after the previous stimulation, the unligatured root gave about two minutes more contraction and a few slight ones the next day before the frog died.

The following table gives the results in those cases in which tracings were taken. Many more experiments were made without tracings but the time was only taken approximately. If the height and power of the contractions were also considered the difference would be greater.

It has been noticed<sup>1</sup> that the reflex applied to one place on the skin soon becomes ineffective. In order to control my numbers a few experiments were made by stimulating with induction shocks a given spot of the skin in frogs whose forebrain had been removed. I usually stimulated for 15 seconds and then waited till the frog quieted. The responses are exactly like those from the dorsal root, in that one can obtain a couple of minutes movement at a time and then no more until a longer rest is allowed. The total response from such a spot during 6 hours was  $6\frac{1}{2}$ , 7, 9, and 11 minutes in the four cases tried. These numbers correspond closely with those obtained during 6 hours from a cut or uncut root.

#### 4. *Effect of stimulating cut and uncut motor nerves.*

As it was suggested that the motor nerves should act in a similar manner to the dorsal roots, some experiments were made with them in the frog. The 3 strands of the pelvic plexus were cut on one side, or the sciatic cut high in the thigh on one side and the electrodes placed under the two sciatics low down in the thigh. The nerves were stimulated

<sup>1</sup> See Sherrington, *Text Book of Physiol.*, edited by Schäfer, II. p. 831.

TABLE II.

Number	Mode of preparation	Time of commencement of experiment	Total duration of movement (in mins.) obtained during first day	Number of hours from commencement before cut root is ineffective	Total duration of movement (mins.) obtained before severed root falls. First day's movement is included in these numbers	Number of hours during which the frog was observed after severed root was ineffective
1	Cut root on one side and corresponding cut nerve in pelvic plexus on opposite side	2.30	Severed root 7½ Unsevered root 7½	46	Severed root 25½ Unsevered root 31½	5. Died during following night.
2	" "	2.50	Severed root 2½ Unsevered root 2½	44	Severed root 16½ Unsevered root 22½	6. Died during following night.
3	" "	10.05	Severed root 11½ Unsevered root 12	24	Severed root 13 Unsevered root 15	31. Died during following night.
4	" "	2.10	Severed root 37½ Unsevered root 38	43	Severed root 69 Unsevered root 82½	8. Died during following night.
5	" "	11.37	Severed root 8½ Unsevered root 8	22	Severed root 9½ Unsevered root 13	26. Died.
6	Both nerves cut in plexus and dissected with ganglion to cord. One root ligatured. Stimulation applied to the roots in each case	2.00	Ligatured root 4 Unligatured root 4	22	Ligatured root 4½ Unligatured root 6	29. Killed.
7	" "	10.30	Ligatured root 11½ Unligatured root 11½	26	Ligatured root 13½ Unligatured root 18½	24. Died.
8	" "	11.35	Ligatured root 4 Unligatured root 5	48	Ligatured root 9 Unligatured root 13	5½. Died during following night.
9	" "	12.05	Ligatured root 23½ Unligatured root 25	53	Ligatured root 56½ Unligatured root 79	22. Died.

*Note.*—Owing to the extra wounds made for attaching the threads and the impossibility of keeping these wounds clean the frogs did not live as long as those recorded in the first table.

alternately, usually in minute periods by means of a commutator. The movement at first is prolonged and practically equal in both sides. After about an hour's stimulation the responses, after the minute's rest, in each side is usually very small. The nerves received this half-hour's stimulation 2 or 3 times a day. Owing to the great amount of muscular fatigue involved in these experiments the blood supply must be a potent factor in the recovery of the muscles. Since the constrictor influence was removed from the cut side the circulation in the web was usually better in that side. Consequently during the second and few succeeding periods of stimulation the muscular responses were usually stronger although not usually longer on the cut side than on the side with uncut nerve. After a time the responses on the uncut side began to last much longer than those on the cut side, and after 3 or 4 days this difference was still more marked. The frogs usually died on the 5th or 6th day so that no experiment was completed and absolute inactivity of the cut nerve was not obtained. On the other hand if a nerve be simply cut and then left for 5 or 6 days it yields equally powerful contractions as the uncut nerve. Bethe<sup>1</sup> has also shown that stimulation causes loss of sensitiveness in such cut nerves when compared with the opposite cut but unstimulated nerve.

5. *Effect on the cells of the spinal ganglion of stimulating its peripheral fibres with cut and uncut dorsal roots.*

On the hypothesis given at the beginning of this paper it might be expected that a change would take place in the spinal ganglion cells on peripheral stimulation of its afferent fibres only if the root fibres were exciting the cells of the spinal cord. This fact has already been observed. The cells of the spinal ganglion were one of the first objects in which Hodge<sup>2</sup> found changes as a result of activity. Changes in these cells as a result of activity have subsequently been found by Levi<sup>3</sup>, Pugnât<sup>4</sup>, and Holmgren<sup>5</sup>. Steinach<sup>6</sup>, however, could find no changes in these cells on stimulation of the nerve peripheral to the ganglion. Pick, who had already<sup>7</sup> made a series of experiments and found changes due to

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Journal of Morphology*, VII. p. 95. 1892.

<sup>3</sup> *Riv. di. pat. nerv. e ment.* I. p. 169. 1896.

<sup>4</sup> *Bibliogr. Anat.* VI. p. 27. 1898.

<sup>5</sup> *Anat. Hefte.* XV. p. 7. 1900.

<sup>6</sup> *Pflüger's Archiv*, LXXVIII. p. 291. 1899.

<sup>7</sup> *Deutsch. med. Wochensch.* XXIV. p. 341. 1898.

activity in the cells of the spinal cord, also examined these cells from Steinach's experiments and he also could observe no changes. The failure of Steinach and Pick to observe changes in these cells is due, I believe, to the fact that Steinach had cut the dorsal roots before stimulating the nerve, and there were, therefore, no changes produced.

I have made a number (12) of experiments on this point and I believe both of these observations are correct. In most cases I cut and excited the central end of one of the nerves in the pelvic plexus, having first in some cases cut the dorsal root close to the cord. The stimulation was applied usually for 30 seconds and then 30 seconds rest. This was repeated as long as reflexes were obtained if the root were exciting or about 10 times if the root were cut. Three or four of these groups of stimulations were given during 4 or 5 hours. The two corresponding ganglia were then put through the same fixing fluids, imbedded together and cut and stained at the same time. In other cases I cut the root on one side and the corresponding nerve in the plexus on that side. On the opposite side two nerves were cut. One corresponding to the cut nerve on the opposite side (this to prevent the muscular movements exciting the cells) and the other nerve for stimulation. The two nerves one on each side were stimulated equally by means of a commutator and then the corresponding ganglia fixed and compared as in the first case. Changes similar to those described by Hodge and Holmgren could be found when the dorsal root had been exciting the cells in the cord, but no changes were observed when the root had been cut before the stimulation. Similar observations were also made in the rabbit, but as all my material has not yet been examined a more complete discussion of this point is reserved for a future occasion.

#### 6. *Some points regarding the double (crossed and simple) reflexes.*

In most cases the direct reflex was obtained with considerably weaker current than that required for the crossed reflex. In some cases this difference was very slight and in two or three cases the crossed reflex seemed easier to obtain. If in a fresh frog a root is stimulated with a current strong enough to obtain reflexes in both legs, the crossed side is observed to come to rest before the direct side (Fig. 10).

The reverse of the above may occur in a frog in which one has fatigued the direct side without having caused the crossed reflex. If the current be then increased, the crossed reflex is obtained while the direct reflex

is absent or very slight. This has been seen 24 hours after the last direct reflex was obtained. These experiments show that a nerve or root, being stimulated with sufficient current and still conducting, can fail to activate cells capable of being stimulated.

Another interesting feature is the character of the muscular response in the two legs. Although there is usually a more or less degree of similarity in the character of the muscular response in the two legs, the tracing (Fig. 11) shows this is not always the case, as we can have one

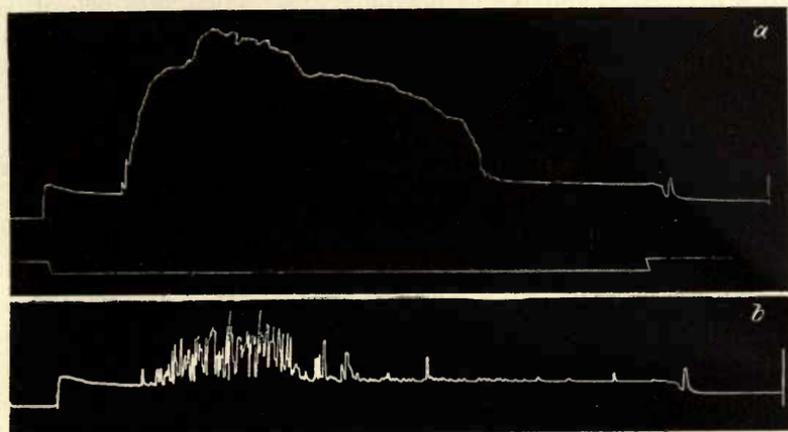


Fig. 11. Double reflex from nerve belonging to lower tracing. This side had been subjected to more or less fatigue before. The drum was moving more rapidly than in other tracings. Time of stimulation  $1\frac{1}{2}$  minutes.

side giving a tetanic contraction while the other side gives an alternating one.

## 7. SUMMARY AND CONCLUSIONS.

It has been shown above that on stimulation of the cut dorsal roots of the frog repeatedly with intervals of rest, reflex contractions are obtained for a period varying from 8 to 98 hours. This variation is due chiefly to the intervals of rest allowed. The total duration of movement in those cases where long rests were allowed was often exceeded in other cases in far shorter periods (cp. Tables I and II). After this time no more movement could be obtained from such a root as long as the animal lived. The duration of this period of ineffectiveness varied from 8 to 72 hours. There is no reason to suppose that these roots have passed into a degenerative state without recovery of function as roots may be cut and left unstimulated for far longer periods

and still be effective. The ineffectiveness of the cut dorsal roots is due to changes in their nerve fibres or nerve endings, but there is reason to believe the roots are still capable of conducting impulses (cp. pages 150 and 161).

It has been shown also that if the spinal ganglion be left in connection with the root recovery of function always occurs after rest. This is true even if its own blood supply be cut off from the ganglion and nourishment supplied only by moistening with diluted blood.

If a nerve is stimulated peripherally to its spinal ganglion, changes occur in the cells of the spinal ganglion (as described by Hodge and others) if the posterior root is allowed to stimulate the cells in the spinal cord, but no changes occur in such spinal ganglion cells if the dorsal roots are cut before stimulation (also observed by Steinach).

Some experiments similar to those on the dorsal roots were made with the sciatic nerve, the nerve being cut on one side and left in connection with its nerve cells on the other. Each nerve was stimulated approximately equally for  $1\frac{1}{2}$ —2 hours a day. After three or four days the cut sciatic gave much weaker contractions than the intact nerve but complete ineffectiveness was not obtained before the death of the animal.

Some observations on the form and duration of the crossed reflex, which bear slightly on the questions discussed in this paper, are also given (cp. sect. 6).

The facts given above seem to me difficult to explain on the common view that the ineffectiveness of a nerve on protracted stimulation (so far as it is not due to fatigue in the cells of the activated organ) is due to abolition of the conductivity of the nerve ending. And it seems to me simpler to suppose that the nerve cells secrete a substance the passage of which from the nerve endings is necessary to stimulation. The recovery of effect after transient fatigue I attribute to the passage of a portion of this substance down the nerve fibre to the nerve ending. The absence of recovery after prolonged stimulation I attribute to the whole of the substance in the nerve fibres being used up, and to their being incapable of making more when severed from their nerve cells.

Before concluding I take this opportunity of thanking Prof. Munk and especially Prof. Starling for their kindly advice as well as the use of their laboratories and instruments, and also to Prof. Langley for suggestions and help in the publication of this paper.





## **A CONTRIBUTION TO THE STUDY OF SECONDARY DESCENDING DEGENERATION IN THE POSTERIOR COLUMNS OF THE SPINAL CORD.**

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SINCE Türck in 1851 first traced degenerated nerve fibres from the brain into the spinal cord, an enormous amount of work has been done on that organ which has enlarged our knowledge of its structure and functions. In this country, Bastian, Ferrier, Gowers, Horsley, Hughlings Jackson, Mott, Sherrington, Schäfer and others have devoted a large amount of valuable time and thought to this subject; whilst abroad, Erb, Edinger, van Gehuchten, Cajal, Obersteiner, Charcot, Déjerine, Pierre Marie, Retzius and a number of others have also given with success a great deal of valuable time to the same purpose. With the advent of fresh discoveries, it has been shown that the nervous system is inconceivably complex, and that systems, tracts, or nerves which formerly seemed simple units, have been analysed into more elaborate details, whilst new structures with necessarily diverse functions have been found as constituent parts of, or in association with, what was formerly thought to be some simple organ. Amongst many instances that could be cited this applies with especial force to the view formerly held of the structure of the posterior columns of the spinal cord which, at

one stage of our knowledge, were supposed to possess merely nerve fibres which, when they underwent secondary degeneration, always did so in an ascending direction (*i.e.* on the cerebral side of the lesion producing the degeneration). It was also supposed that the posterior columns only conveyed afferent impulses (*i.e.* nervous impulses, passing upwards towards the brain).

The first to show that this supposed simple arrangement was not the true one was Dr Charlton Bastian (1), who as early as 1867 figured a small descending tract in the posterior columns of the spinal cord which had degenerated in man after an injury to the cervical region.<sup>1</sup>

About 1876, Flechsig (2) showed by the embryological method, of which he has been so great an exponent, that a small area named by him the oval field could be mapped out on either side of the middle line in the lumbar region of the spinal cord, and that these fibres degenerated in the opposite direction to those around them. In 1880, Kahler and Pick (3), Strümpell (4), and Westphal (5), all published cases which, as a result of compression more or less complete at various levels of the spinal cord, produced descending secondary degeneration in the posterior columns; and three years later, Schültze (6) published similar cases showing the same appearances, and particularly pointed out the presence of a comma-shaped tract, which degenerated downwards as a result of compression of the spinal cord in the cervical region. This tract, larger anteriorly, more pointed posteriorly, he described as extending in the posterior external columns for a distance downwards of two and a half centimetres. But as will be seen below, the tract is much more extensive than Schültze at that time supposed.

In 1889, Howard Tooth (7) found the so-called comma tract (in a case of spinal compression) degenerated from the eighth cervical segment down to the eighth dorsal; and in another case from the seventh cervical to the seventh dorsal segment.

Schmaus (8) in the same year published a somewhat similar case, and Pfeiffer (9) in 1891 described descending degeneration in the postero-median as well as in the postero-external columns,

<sup>1</sup> It is well known that in many animals, *i.e.* the rat, mouse, guinea-pig, squirrel, kangaroo, etc., the pyramidal tracts descend in the posterior columns, but animals with such an arrangement are not, of course, considered in this communication.

whilst Barbacci (10) about the same time showed in a case of transverse lesion at the seventh dorsal spinal segment degenerated fibres from that level downwards on either side of the postero-median septum as far as the lower sacral region. Also Bruns (11) in 1893 figured descending degeneration in the postero-external columns throughout the first five upper dorsal segments.

Moreover, about 1894, Gombault and Philippe (12) discovered a small triangular superficial tract which degenerates downwards in the posterior columns of the sacral region; and two years later Hoche showed, as a result of compression at the level of the seventh dorsal segment, not merely degeneration in the comma tract as low as the fifth lumbar segment, but also degenerated fibres at the peripheral part of the posterior columns (superficial bundle of Hoche), which could be traced continuously through the oval field of Flechsig in the lumbar region, and the triangle of Gombault and Philippe in the sacral region as low down as the conus terminalis. In another case he found the comma tract degenerated from the seventh cervical to the twelfth dorsal segment, and traces of the superficial bundle from the seventh cervical even as low down as the filum terminale.

In the same year (1896), Drs Alexander Bruce and Muir (13) of Edinburgh published a case in which, as a result of a lesion in man in the lower dorsal region, there was definite degeneration along this particular set of fibres, which extended from the twelfth dorsal segment in close proximity to the postero-median septum through the lumbar to the lowest part of the sacral region. To this tract they very happily gave the name of the fasciculus septo-marginalis. A little later Dr Bruce (14) showed that this tract, together with the cornu-commissural tract (of Pierre Marie) is not degenerated in certain cases of tabes. He gave excellent photographs of the course and position of these tracts as stained in such cases by the Weigert-Pal method, reproductions of which are here given for the sake of comparison, and a reference to which will save further description.

Obersteiner (15) has also drawn attention to a tract of fibres occupying an almost identical situation, which he names the dorso-median sacral bundle.

Bischoff (16) in 1896, Flatau (17) in 1897, Zappert (18), and Quesnel (19) in 1898, also threw further light on this

subject. A little later (in 1899), Déjerine (20), who had published several accounts of similar descending fibres, published in conjunction with Théohari (21) a masterly summary of our knowledge of these fibres up to that time. They described and figured as a result of compression at various levels in the spinal cord, not only descending degeneration in the comma tract area and immediately adjoining portions of the postero-external columns, but also similarly affected fibres occupying the superficial area described by Hoche in the dorsal region, the oval field of Flechsig in the lumbar region, and the triangular area of Gombault and Philippe in the sacral region. A reference to Fig. ii., Pl. 3, which is a reproduction of some of their diagrams, be of interest.

According to investigations carried out more recently (1902) by Marburg (22), traces of the posterior cervical roots may be followed as low down as the dorso-median sacral bundle; from the cervical region descending fibres can be traced into the upper sacral region along the lateral part of the posterior columns. These fibres undergo a "ventral thickening" at the neck of the posterior horns, and a "dorsal thickening" in the postero-external portions of the postero-external columns. According to Marburg some fibres also pass in continuity from the dorsal thickening to the posterior para-median septum, and thence along the postero-median septum down into the lowest sacral region. In agreement with many others, he describes some of these fibres as arising in the cells of the neck and apex of the posterior horns (therefore endogenous) and ending at various levels lower down in cells in the same regions (also Hoche).

However, van Gehuchten (23), after section of the first and second posterior cervical roots in rabbits, could not trace by the Marchi method degenerated fibres lower down than three spinal segments, whereas after similar section of the eighth cervical and first dorsal posterior roots he could by the same method trace descending degeneration for eight spinal segments. It is interesting to remember that Flatau (17) in the dog, on section of the same roots, obtained the same extent of degeneration, and that Margulies (24) in the monkey obtained on cutting a posterior root, descending degeneration over nine spinal segments, in each case in the comma tract area. Farquhar Buzzard (25) has also published a case of division of the third lumbar pos-











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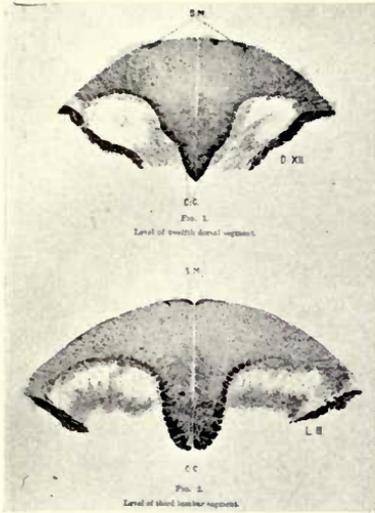


Fig. 1.

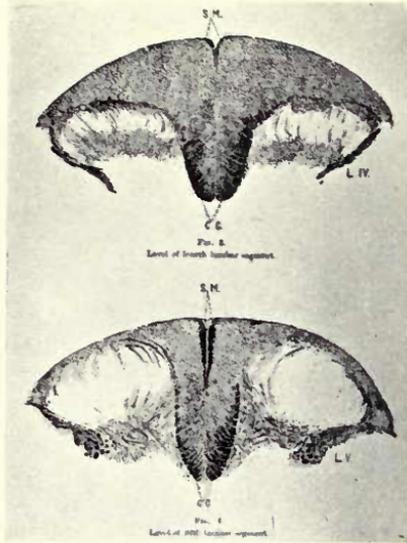


Fig. 2.

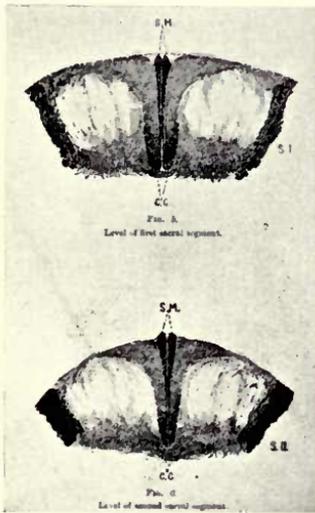


Fig. 3.

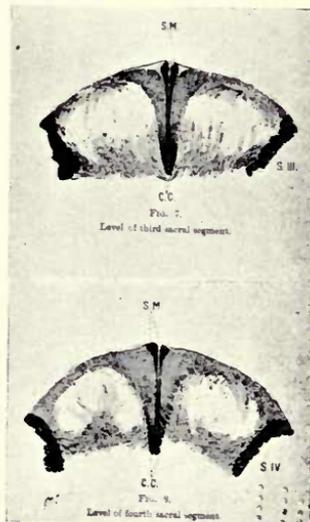


Fig. 4.

After Alexander Bruce.



terior root in man which showed degenerated fibres extending below into the lowest sacral region.

It will be seen from the above that two chief sets of fibres show under certain circumstances descending degeneration in the posterior columns of the spinal cord; one set lying in close apposition to the inner margin of the posterior horns and in the comma tract area; the other set lying more mesially in the posterior median columns.

Whether these fibres are to be considered as being entirely of endogenous origin, as is supposed by Daxenberger, Dufour, Margulies, Pierre Marie, Mayer, Philippe, Schäffer, Tooth, Worotynski, Gombault, etc., or whether with Bruns, Flatau, Redlich, Reimers, Schültze, Singer, Zappert, etc., they are to be considered as exclusively arising from the descending branches of the posterior roots, therefore of exogenous origin; or whether, as the writer considers with Dejerine, Müller, Russell, Sottas, Wallenberg, etc., they are both endogenous and exogenous, need not be gone into here.

The discrepancies in the descriptions by the various authors as to the exact level at which the oval field of Flechsig or other tracts appear or attain their maximum development are, as Bruce has pointed out, no doubt due partly to the differences in the nature and situation of the lesion, and partly to the different methods of investigation employed. To this should, I think, be added the fact that all these tracts contain short, intermediate and long fibres; that, in a word, they are really a series of tracts superimposed; and that therefore it is impossible in one section either to divide all the fibres of a given tract or to produce degeneration in the whole length of any fibre.

#### STIMULATION OF THE POSTERIOR COLUMNS AND POSTERIOR ROOTS.

In a research carried out on monkeys and dogs about ten or twelve years ago, the results of which were published in the *Philosophical Transactions of the Royal Society* for 1895, the writer (26) showed that whereas stimulation, say of the third lumbar anterior root, produced rapid extension of the whole hind limb of the same side in the above animals, stimulation of the corresponding posterior root, or still more of the corresponding part of the posterior columns, gave a strong, though

slow reflex contraction of the whole hind limb. The anterior and posterior columns were inexcitable, and it was proved by a process of division and elimination of the various possible reflex paths for the above reflex movements, that the nerve impulse originated by the stimulus applied to the posterior root or the posterior columns passed outwards to the motor roots partly through the same segment, but largely down the posterior columns and out along several segments below the level stimulated; and that the maximum effect even passed out along the second and third segments below the level stimulated. This result, which has been confirmed in the cervical region by Sherrington, and also by others, seems to the writer to throw a strong side light on the presence and function of some of the fibres in the posterior columns which degenerate downwards.

DESCENDING DEGENERATED FIBRES IN THE CERVICAL AND DORSAL REGIONS OF THE SPINAL CORD EXTENDING DOWNWARDS FROM A LESION IN THE THALAMIC REGION.

About two years ago, at Sir Victor Horsley's suggestion, I did some work for a particular purpose on the thalamic region in several monkeys, etc.; and twelve months later was not a little surprised to find, on examining a cord stained by the Marchi method, some fibres showing degeneration in the cervical and dorsal regions of the spinal cord of a monkey. On examining other specimens of the above animals, I found one in which there was a definite set of coarse fibres, which had evidently degenerated from a bilateral lesion in the upper part of the mid-brain adjoining the thalamus, and were situated on either side of the middle line, forming a wedge-shaped mass of fibres, with the apex anteriorly, and spreading out loosely towards the periphery behind, the bulk of the fibres being situated slightly posterior to the anterior apex of the columns of Goll. A reference to the adjoining untouched photographs of the actual specimens will show, better than any description can give, their exact situation.

It will be seen that in the upper cervical region the degenerated fibres occupy a symmetrical position on either side of the postero-median septum. That in section they consist chiefly of coarse fibres, which occupy a wedge-shaped area, with the apex of the wedge slightly behind the anterior angle of the columns of

Goll, whilst posteriorly the base of the wedge shows scattered fibres, reaching here and there to the periphery of the cord. As the fibres are traced down the cord into the dorsal region they become more scattered, and, as a result of the giving off of collaterals, thinner; whilst they pass for the most part towards the posterior periphery of the cord. At the same time, the angle of the tract opens out so that the fibres, now in section, assume that of a wide angle, or even that of a boomerang.

These fibres had evidently degenerated from at least the upper part of the mesencephalon down to the lower dorsal region. Their path above the decussation of the pyramids will be given in a later communication, in conjunction with some other facts elicited in that research. But the fibres under discussion could be traced in the posterior columns degenerating downwards, and passing into the dorsal region, and in one or two cases traces of similar fibres could be seen actually extending into the lumbar and sacral regions. The specimens, photographs of which are here given, were obtained from the monkey, and a brief note of the fact was sent to the Physiological Society in January last. Since then I have seen, in five or six other cases, fibres similarly degenerated and occupying the same position, though as yet I have not seen them so well marked as represented in the present case, the reason for that being, in all probability, the particular method of operation adopted in the research mentioned above, in which the endeavour was to produce merely a small and limited lesion in the thalamic region or regions. Hence such fibres, if damaged at all, would usually be damaged only in small numbers.

Further work has been carried out with the view of elucidating other points in connection with this particular tract, but it is not yet complete.

The fibres described above must, of course, be long endogenous fibres, which have their origin high up in the mid-brain, or even slightly anterior to this, in the thalamic region, and descend as far as the lower dorsal region, or even lower. The fact that the tract diminishes as it descends in the spinal cord, the fibres becoming smaller and more scattered, occupying a fan-shaped area below the upper dorsal region, would show that the fibres are composed of, as is usually the case, short, intermediate and long fibres; and, as in a somewhat analogous case, that of the posterior longitudinal bundle, it would be

impossible, as Edinger has pointed out, with one section to cause degeneration in all the fibres constituting that tract, or in the complete length of the fibres constituting the tract.

With regard to their function, I feel it necessary to raise a note of warning with regard to the loose employment of the term "descending tracts," or "descending fibres," which is so frequent in text-books and in conversation. It, of course, by no means follows that because a tract undergoes secondary degeneration downwards that therefore it conveys nervous impulse downwards. A good illustration of this is the fact that if a posterior root be cut on the peripheral side of its spinal ganglion, the fibres degenerate towards the periphery (the ordinary Wallerian law), whereas, of course, it is well known that these same fibres must convey nervous impulses in exactly the opposite direction. Therefore, although the fibres described above by various observers, and the set of fibres to which I have called attention in the present paper, degenerate downwards, it by no means follows that they convey impulses downwards. There is a strong presumption that they do convey impulses downwards, but there is no actual proof at present. It may be that they serve to couple up movements of the eyes, or movements which have their centres in the mid-brain, with movements of the hand and lower portions of the body. It may be, also, that they are merely long and short associational fibres subserving in some instances co-ordination, or, of course, their function may be a mixture of the two. Also, van Gehuchten has suggested that they may serve to innervate the lower organic functions. Further work on this point is much needed.

#### CONCLUSIONS.

From the above considerations it follows that apart from the bundle of fibres which under certain circumstances undergo secondary descending degeneration in the posterior columns of the spinal cord as described by many observers, viz. : (1) a set of fibres in the postero-external columns occupying the comma tract area and the immediate vicinity of the inner margin of the posterior horns ; and (2) fibres lying more mesially in proximity to the postero-median septum—there is also, as shown in the accompanying photographs of the monkey's spinal cord, a well-

marked tract, wedge-shaped, in section lying near the middle line in the columns of Goll throughout the cervical region. Above, these fibres originate probably from the thalamus, but in any case as high as the upper (cerebral) end of the mid-brain, whence they can be traced through the brain-stem down into the posterior columns of the cervical region. Below this the fibres become fewer, smaller, and more scattered, and some of them can be traced into the dorsal region, becoming continuous with the superficial bundle described by Hoche, and even occasionally into the oval field described by Flechsig in the lumbar region, and into the triangular bundle described more particularly by Gombault and Philippe in the sacral region.

This tract consists of short, intermediate, and long fibres; but in each area described above there are other scattered fibres which degenerate upwards.

Though the above mentioned fibres undergo secondary degeneration downwards, there is merely at present a strong presumption that they convey nerve impulses downwards, but as yet no proof of this.

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DIE  
CHEMISCHE KOORDINATION  
DER  
KÖRPERTÄTIGKEITEN.

VON

ERNEST H. STARLING F. R. S.  
PROFESSOR AM UNIVERSITY COLLEGE LONDON

DIE

CHRISTLICHE KÖNIGREICHUNG

KÖNIGREICHUNG

ERHART H. STAMM R. L. S.

VERLAGER IN KARLSRUHE

Meine Herren!

Die biologische Rechtfertigung der Wissenschaft liegt in ihrer Nützlichkeit. Wie transzendental und von praktischer Anwendbarkeit entfernt ihr unmittelbares Ziel auch erscheinen mag, unausgesetzt und unermüdlich beschäftigt sich die Wissenschaft mit der Erforschung des folgerichtigen Zusammenhanges der Naturphänomene und deren Einordnung unter die immer weiter ausgreifenden Formeln, die uns unter dem Namen der Naturgesetze bekannt sind.

Die Kenntnis dieses Zusammenhanges macht es der Menschheit möglich, in den Gang ihrer eigenen Entwicklung tätig einzugreifen. Der Mensch hört auf, als ein bloßes Produkt seiner Umgebung zu erscheinen, da es ihm durch die Wissenschaft möglich geworden ist, Naturkräfte zu beherrschen und sein Milieu mit Hinblick auf seine eigenen Zwecke umzugestalten.

Die Herrschaft über die Kräfte der unbelebten Natur ist jedoch keineswegs genügend. Das Fortbestehen des Organismus ist von seiner Fähigkeit abhängig, seine Lebensorgane der Umgebung anzupassen. Es ist unsere Aufgabe, als Physiologen und Mediziner die Bedingungen zu studieren, durch welche die Beziehungen zwischen den verschiedenen Teilen des Organismus bestimmt werden, die sich an dem komplizierten Anpassungsprozess, den wir „Leben“ nennen, beteiligen. Dieses Studium wird es uns vielleicht ermöglichen, in derselben Weise in die Körperfunktionen des Menschen bestimmend einzugreifen, wie wir gelernt haben und noch lernen, die rohen Naturgewalten zu meistern.

Ich habe es mir heute zur Aufgabe gestellt, Ihnen einen kurzen Überblick über eine Reihe von Erscheinungen zu liefern, deren genaueres

Studium verspricht, die Fähigkeit der Einflußnahme auf einige der wichtigsten Vorgänge im Körper in unsere Hand zu legen.

Wir haben uns daran gewöhnt, jeden Lebensvorgang im tierischen Körper als ein Glied in der endlosen Kette seiner Anpassungen an die Umgebung zu betrachten, von denen jeder Anpassungsvorgang sich wieder aus einer ganzen Anzahl einzelner, wechselseitiger Adaptations-tätigkeiten zwischen oft sehr verschiedenen Teilen des Körpers zusammensetzt.

Diese gemeinsame Tätigkeit verschiedener Organe setzt die Existenz eines vermittelnden oder kontrollierenden Mechanismus voraus, welcher letzterer in vielen Fällen durch das Nervensystem repräsentiert wird. In jedem Falle, in dem die Tätigkeit eines Organs sich schnell anderen Körperorganen anzupassen hat, ist die Vermittelung des Nervensystems unumgänglich.

Der *Consensus partium* ist jedoch keine den höheren Tierarten ausschließlich zukommende Eigenschaft; er ist charakteristisch für alle und jede organische Existenz und findet sich ausnahmslos in der ganzen Pflanzen- und Tierwelt vor, in vielen Fällen bei völligem Fehlen eines Nervensystems. In diesen letzteren Fällen müssen die gegenseitigen Beziehungen zwischen verschiedenen Teilen des Organismus durch chemische Mittel herbeigeführt werden. Die auffälligsten Reaktionen bei den niedrigsten Organismen, wie z. B. bei Bakterien, sind jene, welche durch chemische Substanzen bedingt und allgemein als chemotaktische bezeichnet werden.

Chemotaktische Empfindlichkeit ist der bestimmende Faktor bei der Anhäufung von Bakterien und anderen einzelligen Organismen um Nahrungsstoffe, bei der Ansammlung von Phagocyten um fremde Körper und bei der Vereinigung der Geschlechtszellen bei Pflanzen und Tieren. Wenn der Endsproß einer Tanne entfernt wird, so tritt einer der Seitensprossen des nächsttieferen Astkranzes an seine Stelle, der frühen Zerstörung eines Blütensprösslings folgt die Entwicklung neuer Ersatzsprösslinge; Tatsachen, die auf die Wirkung chemischer Substanzen zurückgeführt werden können, welche irgendwo in der Pflanze erzeugt werden und deren Wachstum nach einer bestimmten Richtung anzuregen befähigt sind. Bei Pflanzen und niedrigen Tierarten muß die Übertragung einer Beeinflussung, die durch ein chemisches Mittel dargestellt wird, von einem Teil des Organismus zu einem anderen ein verhältnismäßig langsamer Prozeß sein.

Mit dem Auftreten eines Gefäßsystems und einer kreisenden, alle Körperzellen in gleicher Weise durchtränkenden Flüssigkeit ändert sich dies: es kann keine chemische Substanz gebildet und von irgend einer Zelle ausgeschieden werden, ohne in kurzer Zeit zu allen übrigen Körperzellen zu gelangen. Dadurch wird verschiedenen Teilen des Körpers ein gemeinsames Wirken ermöglicht, indem gewisse chemische

Substanzen im Stoffwechsel eines der zu gemeinsamer Arbeit verbundenen Teile gebildet und von da aus vermittelt der zirkulierenden Flüssigkeit über den ganzen Körper verbreitet werden. Die Vorstellung, daß unter den Bestandteilen der inneren Ernährungsflüssigkeit der Organismen sich gewisse Substanzen befinden, deren Aufgabe es ist, nicht als Nahrungsstoffe im gewöhnlichen Sinne des Wortes, sondern als sogenannte Reizstoffe zu dienen, ist den Botanikern längst geläufig gewesen; trotzdem ist es uns bisher nicht möglich gewesen, eine genaue Grenze zu ziehen zwischen Substanzen, die, wenn auch in kleinsten Mengen, zum Aufbau des Zellsystems selbst notwendig sind, und solchen, deren Aufgabe es ist, die Funktionen des bereits gebildeten Protoplasmas zu modifizieren.

Der Wert der Nahrungsstoffe steht im Verhältnis zu ihrer Fähigkeit, dem Organismus Energie oder aber Material zu seinem Aufbau und Wachstum zuzuführen. Die erwähnten Reizstoffe aber sind, soweit uns bekannt ist, nicht assimilierbar und liefern auch keine nachweisbaren Energiemengen. Ihre Bedeutung liegt in ihrem dynamischen Einfluss auf die lebende Zelle. Sie bilden in dieser Hinsicht eine Analogie mit den Substanzen, aus welchen die gewöhnlichen Heilmittel unserer Pharmakopöen bestehen. Da es ihre Aufgabe ist, bei normaler Körperfunktion sehr häufig in den Blutstrom hinein ausgeschieden zu werden, durch welchen sie jenen Organen zugeführt werden, auf welche sie ihre spezifische Wirkung entfalten, so können sie nicht zu jener Klasse von komplexen Körpern tierischer oder pflanzlicher Herkunft gehören, welchen wir die Toxine zuzählen. Diese Toxine, welche nach EHRLICH'S Anschauung die Rolle der Nahrungsstoffe nachäffen und dergestalt beim Aufbau der lebenden Zelle selbst Verwendung finden, verursachen, wahrscheinlich infolge dieser selben Eigenschaft, nach Injektion in den Blutstrom die Bildung der Antikörper. Die Bildung von Antikörpern würde in Fällen, wo ein Zusammenwirken durch ein chemisches Medium bedingt ist, dessen physiologische Wirkung vernichten. Wir müssen daher diese letzteren Substanzen, die während des normalen Stoffwechsels gewisser Zellen entstehen, als Körper von bestimmter chemischer Konstitution auffassen und sie in Bezug auf chemische Natur und Wirkungsweise mit Heilmitteln, die eine bestimmte Wirkung ausüben, beispielsweise mit den Alkaloiden, vergleichen. Diese Schlußfolgerung erhält ihre Bestätigung durch einige Untersuchungen über die Natur der chemischen Boten, welche gewisse wohl charakterisierte Beziehungen zwischen Funktionen im Organismus höherer Tiere vermitteln. In Anbetracht der ausgesprochenen charakteristischen Eigenschaften dieser Körpergruppe und der wichtigen Aufgaben, die derselben im Organismus der höheren Tiere zufallen, schlage ich vor, diesen Substanzen einen eigenen Namen zu geben, und ich werde sie deshalb fernerhin in diesem Vortrage als

Hormone (von ὀρμάω = ich reize oder rege an) bezeichnen. Die mir zur Verfügung stehende Zeit gestattet mir nicht, Ihnen eine vollständige Aufzählung aller Beziehungen verschiedener Funktionen zu liefern, welche innerhalb des Körpers durch chemische Mittel bewerkstelligt werden. Einige Beispiele aus dem Gebiet dieser hormonischen Reaktionen werden genügen, Ihnen die Wichtigkeit dieser Klasse von Reaktionen vorzuführen.

Das einfachste Beispiel auf dem Gebiete der chemischen Korrelation wird durch den Mechanismus geliefert, vermittelt dessen ein sich kontrahierender Skelettmuskel mit der notwendigen Sauerstoffmenge versorgt wird. Vor vielen Jahren lehrte MIESCHER, daß die Tätigkeit des Atemzentrums durch die Kohlensäurespannung im Blutplasma und letztere wieder durch die Spannung der Kohlensäure in den Lungenalveolen bestimmt wird. Diese Theorie ist kürzlich durch HALDANE und PRIESTLEY und, wie ich glaube, durch die von ZUNTZ und seiner Schule erhaltenen Resultate bestätigt worden. Innerhalb physiologischer Grenzen erhöhte Muskeltätigkeit vermehrt die Ausscheidung von Kohlensäure durch die Muskeln und erhöht so die Spannung dieses Gases im Blute. Als unmittelbare Folge stellt sich erhöhte Tätigkeit des Atemzentrums ein. Die Atemzüge werden tiefer und schneller, bis die erhöhte Ventilation gerade genügt, um die Kohlensäurespannung des Blutes auf ihren Normalwert zurückzuführen. Wird die Muskeltätigkeit exzessiv gesteigert, so daß die Sauerstoffzufuhr den Sauerstoffbedarf der Muskeln nicht mehr zu decken vermag, so findet ein Übertritt von sauren Substanzen, wie Milchsäure, ins Blut statt. Diese sauren Substanzen werden eine weitere Erhöhung der Kohlensäurespannung im Blute und in noch gesteigertem Ausmaße im Atemzentrum verursachen, der Einfluß auf die Atembewegungen wird somit noch ausgesprochener als zuvor. In diesem Falle wird das Hormon von einem der gewöhnlichsten Produkte des Stoffwechsels dargestellt. Diese chemische Korrelation, die Anpassung der Tätigkeit des Atemzentrums an die Bedürfnisse des Muskelsystems, wird durch die Entwicklung einer speziellen Empfindlichkeit des Atemzentrums gegen Kohlensäure ermöglicht. Es ist wahrscheinlich, daß auch die anderen Hormone, deren Tätigkeit ich heute besprechen möchte, ursprünglich gewöhnliche Stoffwechselprodukte einiger Gewebe darstellten, und daß die Entwicklung der chemischen Korrelation nicht durch die Hervorbringung einer besonderen Substanz, die als chemisches Medium zu dienen hat, zustande kam, sondern durch die Erwerbung einer spezifischen Empfindlichkeit seitens eines anderen funktionell verwandten Gewebes.

Im Verdauungstrakt finden wir die anschaulichsten und am meisten typischen Beispiele chemischer Anpassung. Vergegenwärtigen wir uns z. B. den Verdauungsprozeß im Duodenum. Die Forschungen von HIRSCH, v. MERING und anderen haben uns gelehrt, daß eine halbe Stunde bis

drei Stunden nach einer Mahlzeit der Sphincter pylori in regelmäßigen Intervallen sich öffnet, um den stark sauren Chymus, welcher die ersten Produkte der Magenverdauung enthält, in das Duodenum übertreten zu lassen. Sobald diese saure Flüssigkeit den Darm betritt, ergießen sich in ihn drei Säfte, welche an der Darmverdauung teilnehmen: der Pankreassaft, die Galle und der Succus entericus. Der letztgenannte Saft ist ein Produkt der Drüsen, welche sich an der Innenseite der Darmwandung selbst befinden, seine Ausscheidung könnte somit ganz wohl durch direkte Einwirkung des sauren Chymus auf die Darmschleimhaut angeregt werden. Eine reflektorische Kontraktion der Gallenblase ist zweifellos wichtig für den Zufluß der Galle. Wenn wir jedoch eine Gallen fistel herstellen, so finden wir, daß dem Eintritt des Chymus in das Duodenum nach ein oder zwei Minuten eine wirkliche Steigerung der Menge der von der Leber selbst sezernierten Galle folgt. Wir haben hier somit zwei Drüsen, deren sekretorische Anteile sich in beträchtlicher Entfernung von dem primären Orte des Reizes, i. e. von der Duodenalschleimhaut, befinden. Welcher Natur ist der Konnex zwischen der Schleimhaut und den beiden Drüsen?

CLAUDE BERNARD beobachtete reflektorische Absonderung von Pankreassaft nach Einführung von Äther in den Dünndarm, und diese Erscheinung wurde sowohl von ihm, als auch von späteren Forschern der Mitwirkung des Nervensystems zugeschrieben. Wenn man von einigen positiven Resultaten, die HEIDENHAIN durch Reizung der Medulla oblongata erzielte, absieht, waren alle Versuche, die Bahnen dieses Reflexes zu bestimmen, erfolglos, bis PAWLOW die Physiologie durch eine neue Versuchstechnik bereicherte, mit deren Hilfe er bewies, daß eine Sekretion von Pankreassaft bei Hunden (und zwar ohne die Tiere zu narkotisieren, und ohne daß damit Schmerz verbunden wäre) durch Reizung der peripheren Enden der durchschnittenen Nervi vagi erhalten werden kann. Nach PAWLOW würde dieser Konnex durch die Nervi vagi und die Medulla oblongata gebildet. Der Ausgangspunkt des Reflexes wäre die Reizung der Duodenalschleimhaut durch Säuren und Fette. Sieht man von der Nahrungsaufnahme ab, so läßt sich nach PAWLOW Abscheidung von Pankreassaft am leichtesten durch Einführung von verdünnter Salzsäure, sei es direkt ins Duodenum oder indirekt vom Magen aus, erzielen.

Im Jahre 1900 zeigten unabhängig von einander WERTHEIMER und POPIELSKI, daß Einführung von Säuren in das Duodenum oder den Anfangsteil des Dünndarms, selbst nach Durchtrennung beider Nervi vagi und splanchnici und nach Zerstörung des Rückenmarks, eine Sekretion von Pankreassaft hervorruft. Die genannten Forscher schließen daraus, daß wir es mit einem auf dem Wege des peripheren Nervensystems allein zustande kommenden reflektorischen Vorgang zu tun haben. Um die Bedingungen dieses peripheren Reflexes fest-

zustellen, begann ich in Gemeinschaft mit BAYLISS das Studium der Pankreassekretion. Es wurde uns bald klar, daß das Nervensystem an diesem sogenannten Reflex wohl kaum beteiligt sein kann. Es gelang uns z. B., an einem Stück des Dünndarms im oberen Teile des Jejunums jegliche nervöse Verbindung zu zerstören und es gleichzeitig durch die unverletzten Gefäße im Zusammenhange mit dem Körperkreislauf zu belassen. Der Einführung von 0,4 Proz. Salzsäure in eine derartig isolierte Darmschlinge folgt die Ausscheidung einer gleichen Menge von Pankreassaft, wie wir am Anfang des Experiments erhielten, als die Säure in den intakten, vom Nervensystem noch nicht abgelösten Darm eingeführt worden war. Wir wußten bereits aus WERTHEIMERS Experimenten, daß direkte Einführung von Säuren in den Blutkreislauf ohne Einfluß auf das Pankreas bleibt. Der einzig mögliche Schluß, den unser Experiment zuläßt, ist: daß die Säure auf die Darmepithelzellen wirkt und die Anregung zur Bildung einer Substanz innerhalb dieser Zellen gibt. Diese Substanz wird vom Blute absorbiert und der Drüse zugeführt, auf deren Sekretionszellen sie als spezifischer Reiz wirkt.

Der Beweis dieser Annahme war unschwer zu erbringen. Ein kleines Stück Darmschleimhaut wurde abgeschabt, mit Säure verrieben und der rasch filtrierte Extrakt in die Vena jugularis injiziert; innerhalb zweier Minuten beobachteten wir eine mächtigere Sekretion von Pankreassaft, als wir als das Resultat der Einführung der Säure in das Darmlumen erhalten hatten.

Es war somit klar erwiesen, daß der Nexus zwischen Duodenalschleimhaut und Pankreas nicht nervöser, sondern chemischer Natur sein muß. Unter dem Einfluß der Säure wird eine neue Substanz in den Epithelzellen gebildet, die wir „pankreatisches Sekretin“ nennen wollen, und deren Aufgabe es ist, als spezieller chemischer Bote zur Anregung der Pankreastätigkeit zu dienen. Obgleich unsere Beobachtungen durch spätere Forscher auf diesem Gebiete völlig bestätigt wurden, ist es den Physiologen doch noch nicht gelungen, das Sekretin zu isolieren. Die Tatsachen, daß es durch Kochen, selbst in stark saurem Medium, nicht zerstört, daß es durch Magensaft nicht angegriffen wird, daß es leicht diffundiert und durch die gewöhnlichen Reagentien für Proteine und Peptone, wie Gerbsäure und Phosphorwolframsäure, nicht gefällt wird, weisen auf einen verhältnismäßig stabilen Körper von bestimmter Konstitution und wahrscheinlich von niedrigem Molekulargewicht hin. Er gehört mit einem Worte zu den physiologisch wirksamen Agentien, die wir als Hormone bezeichnet haben. Obenerwähnte charakteristische Eigenschaften des Sekretins, zusammengehalten mit seiner Unbeständigkeit bei Anwesenheit von Sauerstoff oder oxydierenden Agentien, genügen, um die Unhaltbarkeit von POPIELSKIS Ansicht, nach der Sekretin nicht mehr und nicht weniger als ein Pepton ist, darzutun. Der geringe und unbeständige Effekt,

den die Injektion einer großen Dosis von käuflichem Pepton auf das Pankreas hervorbringt, ist nicht zu vergleichen mit der starken Absonderung von Pankreassaft, die nach Injektion minimaler Dosen von Sekretin stattfindet. Es ist auch möglich, daß mitunter eine Spur von Sekretin selbst sich im käuflichen Pepton vorfindet, im Falle letzteres durch Einwirkung von künstlichem Magensaft auf Gewebe, die etwas Darmschleimhaut enthielten, hergestellt wurde. Sekretin ist eine Substanz, deren Vorkommen streng begrenzt ist. Es wird durch die Einwirkung von Säuren (vermutlich aus einem Vorläufer, dem Prosekretin) auf die Schleimhaut des Duodenums und des oberen Abschnittes des Dünndarms gebildet. Saure Extrakte aus dem unteren Abschnitte des Ileums, des Dickdarms oder aus irgend einem anderen Gewebe des Körpers bleiben ohne Wirkung auf das Pankreas.

Da das Zusammenwirken der drei Säfte: Pankreassaft, Galle und Succus entericus, zum normalen Ablauf des Verdauungsprozesses im Duodenum notwendig ist, wäre es offenbar ein ökonomischer Mechanismus, wenn die Tätigkeit aller drei beteiligter Drüsenarten durch ein und dasselbe Mittel angeregt würde, d. h. wenn das Sekretin, welches durch Einwirkung von Säure auf die Duodenalschleimhaut gebildet wird, sekretomotorisch nicht nur auf das Pankreas, sondern auch auf Leber und LIEBERKÜHNsche Krypten wirken würde. Daß dies bei der Leber der Fall ist, wurde von BAYLISS und mir bewiesen. Es ist nötig, bei der Prüfung der Wirkung von Darmextrakten auf dieses Organ etwa darin enthaltene Gallensalze, die an sich bereits cholagog wirken würden, auszuschließen. Aus diesem Grunde behandelten wir in unseren Versuchen über den Einfluß des Sekretins auf die Leber vor allem die Schleimhaut mehrmals mit kochendem absoluten Alkohol, in welchem Prosekretin unlöslich ist. Dadurch wurden alle Gallensalze entfernt. Darauf wurde die alkoholkoagulierte Schleimhaut mit verdünnter Säure extrahiert und so eine Lösung erhalten, die bei intravenöser Injektion nicht nur Absonderung von Pankreassaft hervorrief, sondern auch die Gallensekretion auf das Doppelte steigerte.

Mit Bezug auf die Sekretion des Succus entericus ist der Nachweis nicht ganz so unzweideutig. Nach DELEZENNE verursacht intravenöse Injektion von Sekretin eine Absonderung von Darmsaft, jedenfalls im Duodenum und oberen Teile des Darms. Andererseits betrachtet PAWLOW die mechanische Dehnung und die Anwesenheit von Pankreassaft als die wirksamsten Reize für die Absonderung des Succus entericus, während FROUIN behauptet, daß die Sekretion dieses Saftes durch Injektion desselben selbst oder auch durch Injektion eines alkalischen oder neutralen Extraktes von Darmschleimhaut in den Blutstrom angeregt werden kann.

Zweifellos ist die Tätigkeit der oberen Darmpartien von der der unteren Darmabschnitte wesentlich verschieden. In den ersteren ist

die Sekretion, in den letzteren die Resorption vorherrschend, und es wäre ganz gut möglich, daß die abweichenden Resultate, zu denen die verschiedenen Beobachter gelangt sind, sich auf verschiedene Abschnitte des Dünndarms beziehen.

Ich möchte hier noch einen anderen im Verdauungskanal vorkommenden chemischen Anregungsvorgang erwähnen. PAWLOW hat uns gelehrt, zwei Phasen bei der Magensaftsekretion, welche auf eine Mahlzeit folgt, zu unterscheiden. Die erste wird gänzlich vom Zentralnervensystem aus beherrscht und wird hauptsächlich durch das Hungergefühl und durch Geschmacksvorstellungen auf dem Wege Gehirn—Nervi vagi erregt. Die zweite Phase kann durch Einführung von Fleischextrakt oder der Anfangsprodukte der Magenverdauung, selbst nach Durchtrennung sämtlicher Magennerven, hervorgerufen werden.

Diese zweite Phase, die wir nach PAWLOW als lokalen Reflexvorgang aufzufassen haben, ist, wie EDKINS bewiesen hat, der im pylorischen Abschnitt des Magens erfolgenden Resorption einer besonderen Substanz zuzuschreiben, einem gastrischen Sekretin, welches durch die Wirkung der safttreibenden Bestandteile der Nahrung auf die Pylorus-schleimhaut produziert wird. Von den Zellen dieser letzteren gelangt das gastrische Sekretin in das Blut, wird so allen Organen zugeführt und erregt bei seiner neuerlichen Passage durch die Magenwandung die Tätigkeit aller dieses Organ auskleidenden Drüsen.

In all diesen Beispielen von chemischer Korrelation äußert sich die Wirkung der Hormone vorerst darin, daß sie das reagierende Organ zu erhöhter Tätigkeit anregen. Eine solche Steigerung der funktionellen Aktivität kann nicht ohne Einfluß auf die Ernährung der in Betracht kommenden Gewebe bleiben. Wir wissen, daß das wirksamste Mittel zur Erzeugung von Hypertrophie in irgend einem Organ darin besteht, daß die Ansprüche an seine Aktivität gesteigert werden, das heißt, daß die ihm zufallende Arbeit erhöht wird. Wir müssen somit erwarten, daß der indirekte Einfluß dieser Hormone oder Reizstoffe sich in einer verbesserten Ernährung, vielleicht auch in erhöhtem Wachstum der betreffenden Organe äußern könnte. Ich muß nunmehr Ihre Aufmerksamkeit einer Gruppe von Korrelationen zuwenden, bei der erhöhte Aktivität nur als indirekter Effekt sich geltend macht, während das primäre Resultat der Tätigkeit des Hormons Verminderung der Aktivität bei gleichzeitig gesteigerter Assimilation und Gewebshypertrophie zu sein scheint. Die zwischen den Sexualorganen und den übrigen Teilen des Körpers bestehenden Korrelationen bieten die auffälligsten Beispiele von Vorgängen, bei denen als primäre Wirkung eines chemischen, von einem räumlich entfernten Organ ausgehenden Reizes Wachstum auftritt. Obwohl man sich schon seit vielen Jahren spekulativ mit dem Studium der Art und Weise, in der diese Korre-

lationen hervorgebracht werden, beschäftigt hat, wurde doch erst vor ganz kurzer Zeit der Versuch gemacht, diese Beziehungen mittels experimenteller Methoden zu ergründen. Ich möchte Sie besonders auf die Tätigkeit der Brustdrüsen aufmerksam machen. Diese Organe finden sich bei beiden Geschlechtern zur Zeit der Geburt in unentwickelter Form vor. In den ersten Lebenstagen kommt es bei beiden Geschlechtern häufig zu einer Vergrößerung der Drüsen, die sogar mit echter (als Hexenmilch bekannter) Sekretion einhergehen kann. Diese Drüsentätigkeit hört nach ein bis zwei Wochen auf. Erst nach erreichtem Pubertätsalter zeigt sich ein Unterschied zwischen den Brustdrüsen beider Geschlechter, indem sich beim weiblichen Geschlecht — gleichzeitig mit dem Beginn der Ovarialtätigkeit — ein schnelles Wachstum der Drüsen einstellt. Während der ganzen Dauer der Geschlechtsreife verharren die weiblichen Brustdrüsen unverändert auf der gleichen Entwicklungsstufe, solange keine Gravidität eintritt. Der Beginn der Gravidität gibt den Anstoß zu weiterer beträchtlicher Vergrößerung der Drüsensubstanz, ein Wachstum, welches mit stets zunehmender Intensität während der ganzen Schwangerschaftsperiode andauert. Dieses Drüsenwachstum hört nach erfolgter Entbindung mit einem Schlage auf, und zwei bis drei Tage später finden wir, daß die Tätigkeit, die sich vorher im Drüsenwachstum äußerte, nunmehr als Milchsekretion sich kundgibt und bei regelmäßiger periodischer Entleerung der Drüsen viele Monate hindurch andauern kann.

Da es möglich ist, diesen ganzen Zyklus von Veränderungen durch Exstirpation der Ovarien hintanzuhalten, so müssen wir zunächst diese Organe für das Wachstum der Brustdrüsen verantwortlich machen; ob sie aber die unmittelbare Quelle der Impulse sind, durch welche ihr spezielles Wachstum während der Gravidität bedingt wird, oder ob diese Impulse vom Uterus, von der Placenta oder vom Foetus ausgehen, muß durch Experimente festgestellt werden. Daß diese Impulse unmöglich nervöser Natur sein können, erscheint durch die Versuche von ECKHARD und RIBBERT und besonders durch jene von GOLTZ und EWALD an des Rückenmarks beraubten Tieren klar bewiesen. Da nach diesen Versuchen selbst bei gänzlichem Fehlen jeglicher nervöser Verbindung zwischen Beckenorganen und Milchdrüsen Schwangerschaft eine Hypertrophie der Mamma verursacht und die Entbindung von Milchsekretion gefolgt ist, so ist es klar, daß das korrelative Wachstum der Brustdrüsen durch chemische Substanzen verursacht wird, welche in den Beckenorganen entstehen und vom Blutstrom den Drüsen zugetragen werden. KNAUER hat nachgewiesen, daß, obwohl doppelseitige Ovarialexstirpation die periodischen Veränderungen im Uterus, welche die Erscheinungen der Brunst bedingen, zum Verschwinden bringt, es möglich ist, beide Ovarien nach Durchtrennung all ihrer nervösen Verbindungen zu transplantieren, ohne die oben erwähnten Erscheinungen

zu vernichten. Daher muß in diesem Falle das verbindende Glied wohl chemischer Natur sein.

Es ist vorerst unsere Aufgabe, darüber klar zu werden, weshalb die Milchsekretion in den Brustdrüsen erst am Ende der Schwangerschaft beginnt, und dann den Ursprung des Reizes festzustellen, welcher während der Gravidität für das Wachstum dieser verantwortlich ist.

Was die erste Frage betrifft, so ist HILDEBRAND der Meinung, daß während der Schwangerschaft eine Substanz im Blute kreist, welche die Veränderungen dissimilatorischer Natur in den Drüsenzellen hemmt. Diese dissimilatorischen Vorgänge selbst sieht HILDEBRAND als eine Art Autolyse an. Wenngleich es im höchsten Grade unwahrscheinlich ist, daß die chemischen Veränderungen, welche Organtätigkeit im allgemeinen charakterisieren, mit den autolytischen Veränderungen, welche in Drüsenzellen unmittelbar nach dem Tode einsetzen, identisch sind, so ist doch die Idee, daß eine Substanz dadurch Wachstum verursacht, daß sie in einer Beziehung hemmend wirkt oder nach HERINGS Nomenklatur assimilatorische Wirkung ausübt, sehr wertvoll. Dieser Ansicht gemäß muß, solange diese hemmende Substanz im Blute zirkuliert, das Wachstum des Brustdrüsengewebes fortschreiten. Mit der bei der Entbindung stattfindenden Entfernung der Quelle, aus der das hemmende Hormon hervorgegangen ist, wird das Drüsengewebe, dem nunmehr ein hoher Grad von Leistungsfähigkeit innewohnt, in einen Zustand von autonomer Dissimilation übergehen, das heißt, es wird eine Periode langdauernder Tätigkeit einsetzen. Fräulein LANE-CLAYPON und ich haben gefunden, daß künstlich herbeigeführte Unterbrechung der Schwangerschaft beim Kaninchen innerhalb der ersten vierzehn Tage, das heißt bevor Bildung von Sekretionsalveolen stattgefunden hat, bloß regressive Veränderungen in der Drüse verursacht. Wird die Gravidität in irgend einem späteren Zeitpunkte unterbrochen, so werden die sekretorischen Alveolen in Tätigkeit versetzt, und es resultiert die Absonderung von Milch. Daß diese Sekretion in der Entfernung eines Reizes und nicht in der Erzeugung einer neuen stimulierenden Substanz ihren Grund hat, wird durch die den Klinikern wohl bekannte Tatsache bewiesen, daß auch Totalexstirpation des schwangeren Uterus und seiner Nebenorgane von Laktation gefolgt sein kann.

Was die Frage über die Herkunft des hemmenden Hormons anlangt, so schließt die Tatsache, daß doppelseitige Ovariectomie während der Schwangerschaft das Wachstum der Brustdrüsen nicht unterbricht, die Ovarien als direkte Quelle des Reizes aus. Sorgfältiges Studium klinischer Beobachtungen hat HALBAN zu der Ansicht geführt, daß die Quelle des Hormons in den Chorionzotten und in der Placenta zu suchen ist. Seine Beweisführung ist jedoch nicht absolut zwingend, und wir suchten deshalb zur Lösung dieser Frage zu gelangen, indem wir vaginalen Kaninchen Extrakte von Embryonen, von Ovarien, Placenten und von

Uterusschleimhaut injiziert, in der Hoffnung, dadurch eine ähnliche Mammahypertrophie, wie sie während der Gravidität zustande kommt, herbeizuführen. Es war uns von Anfang an klar, daß es sehr schwierig, wenn nicht gar unmöglich sein würde, einen dem normalen Stimulus entsprechenden Reiz für die Brustdrüsen zu gewinnen. Wir haben ja anzunehmen, daß, wo immer auch das Hormon erzeugt wird, seine Erzeugung kontinuierlich vor sich gehen muß; daher müssen wir auch ein fortwährendes Durchsickern der wirksamen Substanz in das Blut annehmen, und es ist sehr wahrscheinlich, daß die Menge der produzierten Substanz mit der Dauer der Schwangerschaft zunimmt. Die Brustdrüse wird somit in jedem Zeitpunkt der Einwirkung dieses spezifischen Reizes unterworfen sein. Andererseits war zu erwägen, daß, wie immer wir auch unsere Gewebsextrakte darstellen mochten, wir nicht erwarten konnten, mehr als die eben in den Geweben befindliche und auf der Wanderung durch die Placenta in die mütterlichen Blutgefäße begriffene Menge der Substanz so zu sagen abzufangen. Diese Menge konnten wir zwar dem Kaninchen injizieren, aber es war wohl anzunehmen, daß sie schon längst in den Kreislauf übergegangen und resorbiert worden war, bevor wir zur nächsten Injektion bereit waren. Somit konnten wir, während unter normalen Bedingungen die Brustdrüsen während der Schwangerschaft fortwährend zur Hyperplasie angeregt werden, in unseren Versuchen der Drüse nicht mehr als eine Reihe von kurzen Anstößen in der gleichen Richtung erteilen.

Ungeachtet der diesen Versuchen anhaftenden Schwierigkeiten gelang es uns doch in sechs Fällen, ein Wachstum der Brustdrüsen bei virginalen Kaninchen zu erzielen, welches dem während der ersten Phasen der Trächtigkeit stattfindenden gleicht. Es bestand in Proliferation der die Drüsengänge auskleidenden Epithelien und Neubildung von Drüsengängen durch Verzweigung der alten Gänge. In einem dieser Versuche, in welchem die Injektionen fünf Wochen lang fortgesetzt wurden und dem Kaninchen im ganzen Extrakt von 160 Embryonen injiziert worden war, kam es sogar zur Bildung wirklich sezernierender Acini im peripheren Anteil der Drüse. In allen diesen Fällen stammte das Extrakt von Embryonen. In einer Anzahl von Versuchen, in denen wir Extrakte aus Uterus, Placenta oder Ovarien einspritzten, kam es zu keinerlei Wachstum. Wir dürfen demnach die Schlußfolgerung ziehen, daß unter normalen Verhältnissen das Wachstum der Milchdrüse während der Schwangerschaft durch eine chemische Substanz, ein Hormon, bedingt ist, welches hauptsächlich im heranwachsenden Embryo erzeugt und durch die Placenta hindurch auf dem Wege des Blutstroms der Drüse zugeführt wird. Das im Verhältnis zu der großen Menge des zu den Versuchen verbrauchten Materials klein erscheinende Resultat beweist, daß die zu einer gegebenen Zeit in den Geweben vorhandene Hormonenmenge minimal sein muß, und daß wir, wenn wir

Extrakte aus Embryonen injizieren, höchst wahrscheinlich nur die geringfügige Menge der Substanz einverleiben, welche in die Säfte diffundiert ist und sich auf dem Wege zu den Blutgefäßen und zum mütterlichen Kreislauf befindet. Unsere Experimente liefern keine Aufklärung über die Bildungsstätte des Mammahormons im Embryo, und es ist gleichfalls noch unbekannt, ob es etwa mit Hilfe irgend einer einfachen Methode aus einer im embryonalen Gewebe vorkommenden Vorstufe abgespalten und so in größerer Menge erhalten werden könnte, wie dies beim pankreatischen Sekretin der Fall ist. Wir können es als bis zu einem gewissen Grade wahrscheinlich ansehen, daß das Brustdrüsenhormon in einer Hinsicht dem Sekretin oder dem Adrenalin verwandt ist, insofern es Erhitzen verträgt, ohne seine Eigenschaften einzubüßen. Es muß künftiger Forschung überlassen bleiben, die übrigen Fragen, und zwar sowohl in Bezug auf Bildungsstätte und Natur der spezifischen Substanz, als auch in Bezug auf die Fähigkeit verschiedener Reagentien, sie aus einer eventuellen Vorstufe abzuspalten, zu beantworten.

Diese drei Beispiele mögen genügen, um Sie zu überzeugen, daß es möglich ist, auf chemischem Wege die Funktions- oder die Ernährungsbedingungen eines Gewebes im Sinne erhöhter oder verminderter Tätigkeit zu beeinflussen, und daß sich der tierische Organismus dieses Mittels normalerweise bedient, um Funktionen und Wachstum räumlich weit distanter Organe zu koordinieren. Ich habe die oben erwähnten drei Beispiele gewählt, teils weil ich mich mit zwei derselben während der letzten Jahre eingehend beschäftigt habe, hauptsächlich aber, weil sie die besten Beispiele einer Koordination liefern, die, obwohl auf chemischem Wege herbeigeführt, dennoch den zahlreichen Koordinationsvorgängen ungemein gleicht, die vom Zentralnervensystem ausgeführt und allgemein als Reflexvorgänge bezeichnet werden.

Andere Beispiele für von einem Organ auf andere Körperteile ausgeübte chemische Beeinflussung dürften Ihnen wohl geläufig sein. In diesen gleich zu besprechenden Fällen ist jedoch der Endeffekt in seiner Wirkung nicht bloß auf ein Organ beschränkt, sondern macht sich allenthalben im Körper geltend, obwohl, wenigstens in manchen Fällen, diese Ausbreitung der Reaktion über ein so weites Gebiet dem Umstande zuzuschreiben ist, daß das spezifisch reagierende Gewebe oder die spezielle Funktion allenthalben im Körper anzutreffen ist.

Ich brauche in dieser Hinsicht nur auf die Rolle hinzuweisen, welche die Nebennieren, die Schilddrüse, das Pankreas und die Hypophyse bei den allgemeinen Stoffwechselforgängen im Körper spielen. Was den erstgenannten Fall anlangt, so wissen wir, daß die Marksubstanz der Nebennieren einen arzneimittelartigen Körper, das Adrenalin, in den Blutstrom hinein sezerniert. Dieser Teil der Nebennierensubstanz entwickelt sich aus dem sympathischen Nervensystem und

ist nur ein Teil einer ganzen Gruppe ähnlicher Organe; LANGLEY und ELLIOTT haben gezeigt, daß Adrenalin auf jedes Gewebe im Körper, das vom sympathischen Nervensysteme versorgt wird, einwirkt, und daß ausnahmslos der durch Injektion dieses Mittels hervorgebrachte Effekt derselbe ist, als würde der das betreffende Organ versorgende Nerv elektrisch gereizt. Demgemäß verursacht es Erweiterung der Pupille, Absonderung von zähem Speichel, Kontraktion der Blutgefäße, Beschleunigung der Herzaktion, Erschlaffung der Muskulatur von Dünn- und Dickdarm, Kontraktion der Valvula ileo-coecalis, des Uterus und entweder Kontraktion oder Erschlaffung der Harnblase, je nach dem bei verschiedenen Tierarten verschiedenen Einfluß, den der betreffende sympathische Nerv auf dieses Organ hat.

Bei der Schilddrüse ist es schwer, sich darüber auszusprechen, ob das wirksame Prinzip, welches anscheinend in dem jodhaltigen, von BAUMANN zuerst dargestellten und Jodothyrim genannten Körper enthalten ist, mehr dissimilatorische oder assimilatorische Wirkung hat. Es steht fest, daß beim wachsenden Tier seine Anwesenheit in den zirkulierenden Säften zur normalen Ausbildung aller Gewebe des Körpers, ganz besonders der Knochen, notwendig ist. Seine Einverleibung in den erwachsenen Organismus jedoch steigert die dissimilatorischen Vorgänge. Die Harnstoffausscheidung wird vermehrt, und es kann zu rapidem Fettschwund kommen.

Der seitens des Pankreas auf den Kohlehydratstoffwechsel ausgeübte Einfluß wurde vor fast zwanzig Jahren durch MINKOWSKI und v. MERING aufgedeckt, welche bewiesen, daß totale Pankreasexstirpation von tödlich verlaufendem Diabetes gefolgt ist. Sowohl die Experimente dieser Gelehrten, als auch jene späterer Forscher haben es fast zweifellos gemacht, daß vom Pankreas aus auf dem Wege innerer Sekretion irgend eine Substanz den zirkulierenden Körpersäften beigemischt wird, deren Anwesenheit zur Assimilation von Zucker, sei es durch die Leber oder durch die Muskeln, unumgänglich notwendig ist. Alle Versuche, die Wirkung des lebenden Pankreas durch aus diesem Organ gewonnene Extrakte nachzuahmen, sind bisher erfolglos geblieben. Sollte jedoch auch diese innere Sekretion derselben Art sein wie die anderen Körper, welche ich unter der Bezeichnung Hormone zusammengefaßt habe, so sollte es wohl möglich sein, das wirksame Prinzip der Drüse zu isolieren und durch Einführung der Substanz in den Blutkreislauf Fälle von menschlichem Diabetes, welche durch Pankreaserkrankung bedingt sind, günstig zu beeinflussen.

Es ist den Physiologen längst klar geworden, welche wichtige Rolle diese inneren Sekretionen bei der Regulierung der Tätigkeiten des ganzen Körpers spielen. Ich hatte es mir zur Aufgabe gestellt, in diesem Vortrage ganz besonders den einen Punkt zu betonen, daß diese inneren Sekretionen, Hormone, wie ich sie genannt habe, Sub-

stanzen von verhältnismäßig einfacher chemischer Zusammensetzung sind, daß sie ganz wohl isoliert und selbst — wie das Adrenalin — synthetisch dargestellt werden können, und daß ihre Wirkung nicht der eines Nahrungsmittels, sondern der eines Arzneimittels vergleichbar ist, da sie, wie dies tatsächlich der Fall ist, von der physiko-chemischen Konfiguration des Moleküls abhängt und nicht von der Anwesenheit haptophorer Gruppen, welche die Assimilation dieser Substanzen in das lebende Protoplasmamolekül bedingen würden. Ich habe Ihnen Gründe für die Annahme angeführt, daß die Hormone in Bezug auf Vorkommen und Wirkung weit verbreitet sind, und daß zu hoffen steht, daß weitere in dieser Richtung fortgesetzte Untersuchungen uns ein Rüstzeug wirksamer Faktoren in die Hände liefern werden, durch die es uns möglich werden könnte, die meisten Funktionen des Körpers zu beeinflussen.

Doch selbst, wenn wir alle im Körper wirksamen Hormone entdeckt haben werden, und wenn uns die Aufdeckung ihrer chemischen Konstitution und ihre Synthese gelungen sein sollte, würde unsere Aufgabe noch nicht erschöpft sein. Wir hätten dann noch immer die Art und Weise zu ergründen, in welcher diese chemischen Substanzen ihre spezifische Wirkung auf das komplizierte Molekularaggregat, welches wir Protoplasma nennen, auszuüben vermögen. Nach den Worten Ludwigs „hat die wissenschaftliche Physiologie nicht nur die Aufgabe, die Leistungen des Tierleibes festzustellen, sondern sie auch aus den elementaren Bedingungen desselben mit Notwendigkeit herzuleiten“.

Wir sind gezwungen, das Protoplasma als ein Riesenmolekül aufzufassen, dessen vielfältige Reaktionen durch die Kompliziertheit seines Aufbaues bestimmt werden, und welches, dank seiner Größe, sich der Abhängigkeit von den Gesetzmäßigkeiten entzieht, welche wir mit Rücksicht auf Moleküle von unendlich kleinen Dimensionen angenommen haben. Jedes physiologische Problem ist somit in letzter Linie auf ein chemisches zurückzuführen. Es befaßt sich ja mit der Wirkung von Körpern bekannter Konstitution auf ein komplexes Molekül, das bereits „molare Größe“ erreicht hat, und dadurch chemische Phänomene durch solche, die auf Oberflächenwirkung und Organisation zurückzuführen sind, noch weiter kompliziert. Hier reichen sich Physiologie und Pharmakologie die Hände, und die älteste unter den Forschungen, die in Verbindung mit der Heilkunde erscheinen, nämlich jene, welche sich mit der Wirkung der Arzneikörper befaßt, wird uns vielleicht die Handhabe zur Aufklärung der fundamentalen Lebensprobleme liefern.

Das Streben der modernen Wissenschaft löst sich mehr und mehr in ein Ringen um immer weiter reichende Einflußnahme auf. Chemiker und Physiker sind bestrebt, immer mehr Macht sich anzueignen, die in der Materie schlummernden mächtigen Kräfte frei zu machen, und die das Weltall durchströmenden Energiemengen in den Dienst der Mensch.

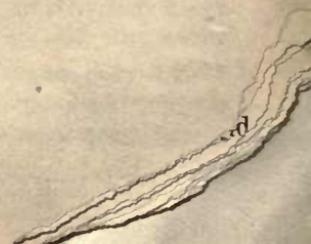
hervor zu stellen. Uns ist die noch schwierigere Aufgabe zuteil geworden, die Bedingungen der in uns selbst waltenden Tätigkeiten zu erforschen und Einfluß zu gewinnen auf Funktion und Wachstum jedwedes Organs in unserem eigenen Körper. Obwohl wir noch weit von der Erreichung eines solchen Zieles entfernt sind, werden Sie mir doch darin zustimmen, daß die während der letzten Jahre auf biologischem Gebiete gemachten Fortschritte, von denen der Gegenstand meines heutigen Vortrages ein Bruchstück bildet, uns die Erreichung eines Zeitpunktes verheißen, in dem wir Ärzte — im Besitze vollständiger Kontrolle über die Funktionen unseres Organismus — die Herrschaft über den menschlichen Körper wirklich antreten werden.

Dies ist der zuversichtliche Glaube, der unsere Arbeit leitet.

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