T H E S I S

THE ZYMOLYSIS OF TISSUES, PHYSIOLOGICAL AND PATHOLOGICAL, WITH A HISTORICAL RESUMÉ OF THE NATURE AND ACTION OF ENZYMES

by

J. SOUTTAR McKENDRICK

October, 1899

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

C O N T E N T S

PART II.

CHAPTER VI. Description of method adopted in carrying out research with the process of extraction and tests submitted to each extract.

CHAPTER VII. A description of the fallacies that may occur in the experiments, and how these may be overcome.

CHAPTER VIII. Physiological Tissues - Enumeration of tables of extracts, with results obtained and a comparison of these results.

CHAPTER IX. Pathological Tissues - Enumeration of tables of extracts, with the results obtained, and a comparison of these results.

CHAPTER X. A comparison of the results obtained from the analysis of physiological and pathological extracts, with remarks on striking reactions.

CHAPTER XI. A discussion on certain problems that arise in this research.

CHAPTER XII. Is our knowledge of disease helped in any way by the analysis of extracts from particular tissues?

CHAPTER XIII. Summary of this contribution with enumeration of the possibilities for further research in this direction.

I N T R O D U C T I O N

The present research has been carried on by me during the last twelve months, partly in the Physiological Laboratory of Glasgow University, and partly in a private laboratory of my own. The paper, however, does not deal solely with the results of my own observations, as I considered it would be of some scientific interest and value to devote the first portion of the paper to a general survey of the question of Zymolysis, introducing shortly the theories and conclusions that have been arrived at by various physiologists, before commencing the second portion, which is more strictly the original part of this paper.

As the title of the paper denotes, the chief

enquiry lay in the endeavour to obtain, or to shew the absence of, unorganised ferments or enzymes in the tissues themselves, quite apart from their presence or absence in the juices, or organs which secrete those juices, and have to do with digestion.

For example, to ascertain whether pepsin, which is known to exist in the gastric juice, existed in other tissues or organs of the body, even though possibly in less amount: or again whether there was present in the tissues of the body an enzyme similar in character to ptyalin which is known to exist in saliva; or amylopsin, which is present in the pancreatic juice, and which would be capable of converting starch into sugar. I have endeavoured to carry out the chemical tests as accurately as possible, so that the reactions, whether they be due to enzymes or not, might at least be looked upon as facts either positive or negative.

Only one method has been adopted in making all my observations so that the absence of a particular reaction must not be looked upon as a positive proof that no enzyme exists. The method, however, consisted in a slightly modified form of that advocated by Von Wittich, and we know that most enzymes, if not all more or less, are soluble in glycerine.

Glycerine extracts have been made from physiological and pathological tissues, the latter being examined in this way with the view of ascertaining whether tumours had the power of secreting enzymes or contained a larger proportion of the parent "Zymogen" than existed in physiological normal tissues. One could in this way possibly account for the rapid growth of carcinomata and sarcomata in particular tissues.

Various difficulties have presented themselves in carrying out this research, and many fresh ideas have occurred to me which I have mentioned towards

the end of the paper.

I have to thank Professor Buchanan, and Doctors Renton, Gray, Jardine and Ferguson for supplying me with the material, without which the research could not have gone on.

In writing the bibliography on enzymes and their actions, and also in discussing various scientific problems as they arose, I have been greatly helped by the admirable writings of Dr. Halliburton, more especially in his "Text Book of Chemical Physiology and Pathology;" of Dr. Gamgee in his "Physiological Chemistry of the Animal Body;" of Dr. Moore in his article on enzymes in the latest edition of "Schäfer's Physiology;" of Dr. Sheridan Lea in his "Chemical Basis of the Animal Body;" and also the writings of Langley and others that have appeared from time to time in the "Journal of Physiology - Cambridge and London."

I have endeavoured to consult German and

French literature as much as possible, and I may mention here more especially the help I have obtained through articles that have appeared by Dr. Moraczewski in the "Archir für gesammte Physiologie" and by Professor Maly in the "Hand Buch der Physiologie" - (Hermann).

Both these articles enter pretty fully into the history of enzymes, and yield many valuable references. I have consulted many of the German journals, but my one regret has been that I have had the greatest difficulty, and sometimes failed completely in obtaining some of the earlier standard works. The literature on the subject is chiefly German, but many valuable contributions have come from the English, French and Americans. I have endeavoured to give most of the references, although in some cases I have been unable personally to refer to them.

P A R T I.

CHAPTER I.

FERMENTS, ENZYMES, ETC. WITH DEFINITION OF TERMS

Although the subject of enzymic action is closely allied to the process of fermentation, I do not intend to enter into this great question. Still, it may be safely stated that although the results of fermentative processes had been observed for many hundreds of years, a true explanation of the cause for these processes could not be given until far

into the present century.

In 1838, Berzelius[1] expounded the catalytic theory of fermentation, which was followed, and slightly modified by Liebig[2] in 1848.

These theories were interesting, but had little or no foundation and it was not until 1857 that the true nature of fermentation was brought to light.

Pasteur[3] found that an organism was the cause of fermentation, and he shewed that various forms of fermentation depended upon the activity of different organisms. When all the world then was quibbling over mere speculative theories, Pasteur was able to shew the true facts. Fermentative processes produced by the action of living organisms

(1) Lehrbuch der Chemie 1840.

(2) "Rechtfertigung der Contact-Theorie" Annalen, Vol. 35 (1840)

(3) Ann. de Chim. et Phys. 3, Ser. I, 58.

were said to depend upon "organised" ferments. This, however, is not the only kind of fermentative change that can occur, for during the process of digestion we now know that certain substances are eliminated that have the power of transforming food stuffs into a form more suitable for assimilation. These substances are called "unorganised" ferments, as they are the products of the action of living cells which exist in tissues and not of living organisms.

This distinction can be carried still further as we can speak of an "organised" form of ferment when the action of fermentation is direct and occurs in the living cell itself, while we must speak of an "unorganised" ferment when the action is indirect and occurs quite apart from the living cell, as a soluble material secreted from a living cell.

These classes, however, in some cases partly overlap, for we know that certain organisms which have a particular action, are capable even after

death, of secreting a soluble ferment which has another action; for example, yeast produces alcoholic fermentation, but it also liberates a soluble ferment which has the power of converting cane sugar into dextrine and levulose. It is this latter circumstance that has made it so difficult to exclude the possibility of soluble ferments being secreted from organisms, and thus yielding a series of reactions which would otherwise be enzymic. Still by the aid of certain antiseptics not only can we kill the organism itself, but we can prevent the activity of the soluble ferment which it produces.

Kühne[1] in 1878 first used the term enzyme to denote the soluble "unorganised" ferment.

The term "ferment" he wished to remain for the "organised" agents.

(1) Verhandl. d. naturh-med. Ver. zu Heidelberg, 1879, N. F. Bd. 1, S. 236.

How is an enzyme or soluble unorganised ferment to be defined? One cannot do better than take the definition of Sheridan Lea which is, that "substances which can be extracted from animal and vegetable cells, and which can produce in a more striking degree certain other substances without themselves undergoing any observable alteration are called enzymes or soluble ferments."

These enzymes, as we shall see, play a very important part in the processes of digestion, and it is an astonishing fact that an almost infinitesimal quantity of an enzyme can convert a very large quantity of material, the rapidity of its action, however, depending on the quantity, the degree of dilution, the reaction, and the temperature. Enzymes, then, are soluble substances engaged in transforming material, "the process of the action" of enzymes, has been called by Roberts(1)

(1) Proc. Royal Society, Vol. XXXI, p. 145

Enzymosis, a term, however, which is a mere lengthening of the word enzyme introduced by Kühne, and which has received little popular favour.

In 1890 Sheridan Lea[1] used the term "Zymolysis" to denote "Changes produced by the enzymes in their action upon other substances," and he applied the old term "fermentation" to the action of the organised ferments.

In this way he says "Zymolysis" corresponds to the German "Ferment-wirkung," and "Fermentation" to "Gahrung."

Following up this term introduced by Sheridan Lea, I have called this paper "Zymolysis of Tissues" meaning thereby the actions of particular enzymes in the tissues, producing changes on other substances. For example, I wished to see whether enzymes were

(1) Journal of Physiology - Cambridge and London, Vol. XI, p. 254.

secreted by the tissues, and, if so, if they were capable of producing changes in certain substances, such as starch and fibrin.

We know through the labours of Heidenhain and others that most enzymes do not exist as such in the cells of their respective tissues. They are stored up in the cells as a parent zymogen, which is the inactive progenitor of the true enzyme. It is often necessary to treat the tissue with some substance which will convert the zymogen into the active enzyme. Heidenhain(1) in 1875 shewed that the proteolytic enzyme of the pancreas did not exist as such in the tissue, but as a zymogen which is stored up in the cells, and which finally liberates the enzyme trypsin.

This substance was called by Kühne "the Zymogen of trypsin," but more recently it has received

(1) Pflüger's Archiv, Vol. X, pp. 557-632

the name of "trypsinogen."

Similarly the works of Ebstein, Grützner[1], Langley and Edkins[2] shew us that pepsin does not exist as such in the gastric glands, but as a zymogen which liberates pepsin, and which has received the name of pepsinogen. Again, the enzyme, which has been called rennin by Sheridan Lea[3], and chymosin by Deschamps[4], does not exist in this form in the glands of the stomach, but as a zymogen which has received the names of rennet zymogen or Renninogen[5]. Liversidge[6] believes that he obtained a zymogen for the pancreatic diastatic enzyme, but

(1) Pfluger's Archiv, Vol. XI, p. 1

(2) Journal of Physiology, Vol. VII, p. 371

(3) "Chemical Basis of the Animal Body," p. 65.

(4) Lehr. d. physiol. chem. Wiesbaden, 1891, T 153

(5) Journal of Physiology, Vol. III, 1881 (Hammersten).

(6) Journal of Anatomy and Physiology, Vol. VIII, p. 23

this result has not been generally accepted.

So that one sees that quite a number of enzymes are merely liberated from a parent zymogen that is stored up in the cells of their respective tissues. This depends upon some chemical change. No one has found the parent zymogen of certain other enzymes, but failure in this does not prove that they do not exist.

Although the parent zymogens are closely related to the enzymes, they have been recently distinguished by Langley[1] who has written a lengthy article on this subject in special relation to pepsin and pepsinogen.

He finds that the destruction of pepsinogen by alkalies is slower than that of pepsin: that pepsinogen on the addition of one per cent. of H.Cl.

(1) Loc. cit. "Langley and Edkins."

is very soon converted into pepsin: that a glycerine extract of the gland containing pepsinogen may remain unchanged for almost any length of time: that carbonic acid destroys pepsinogen more speedily than it does pepsin: and that both are rapidly destroyed by high temperatures.

CHAPTER II.

SHORT HISTORY OF DIGESTIVE FERMENTS WITH THEIR METHODS OF EXTRACTION

No subject probably in the whole range of physiological science has so interested the minds of workers as that on the process of digestion.

It is by this act that all the food we eat is converted into a form which is suitable for assimilation, and so able to maintain life.

From the earliest times theories have been expounded as to the probable explanation of the digestion of food, in what form it was assimilated, and how it was absorbed by the blood, and stored up

in the tissue cells. I think I am right in affirming that up to one hundred years ago little or no information was known concerning the juices that have to do with digestion, and even fifty years ago little was known of the enzymes that exist in those juices, and are the chief factors in the digestive process. We see then with what rapid progress physiological chemistry has advanced within recent years, and even at the present time we are constantly hearing of the new substances that are being discovered in the tissues, and which play such an important part in cell metabolism. Much of the knowledge that we possess has been derived from the careful, accurate, and laborious researches of Brücke, Kühne, V. Wittich, Hoppe Seyler, Heidenhain, Claud Bernard, Hammersten, Chittenden, Sheridan Lea, Langley, Halliburton, and many others. All these investigators have made analyses of the juices concerned in digestion, and have succeeded in placing our knowledge on a strictly scientific basis. These soluble

enzymes play the most important part in digestion. Dr. Moore has given a concise and modern definition of digestion when he says "that digestion might be described as the physical and chemical alteration of the food stuffs into forms better fitted for absorption by the action of certain soluble ferments, the digestive enzymes."

As enzymes are so all important in the chemistry of digestion, I have considered it to be of interest to give a short historical sketch of the more important digestive enzymes, and to see how the methods adopted for their extraction have been gradually perfected.

We shall consider these in the following order:-

A	Ptyalin -	that exists in	Saliva
B	Pepsin	do	Gastric Juice
C	Rennin	do	do
D	Trypsin	do	Pancreatic Juice
E	Amylopsin	do	do
F	Pialyn	do	do
G	Intestinal Enzymes		

A. PTYALIN

Leuchs[1] in 1831 observed that the spittle or saliva had the power of converting starch into sugar. He may be said to have first given the name ptyalin to this enzyme. Schwann[2] shortly afterwards confirmed his result, but neither of these observers were aware of the probable reason for such a reaction. Mialhe[3] however found that by diluting the saliva with several times its weight of absolute alcohol he was able to extract a substance that could convert starch into sugar. Mitscherlich[4] and Berzelius[5] were able to obtain the same substance by a slightly modified method.

(1) Arch. f. d. ges. Naturh. Nürnberg 1831

(2) Annal. d. Phys. & Chem. Leipzig 1836, Bd. XXXVIII, S. 358

(3) Compt. rend. Acad. d. Sc. Paris 1845, Tome XX, pp. 654, 1483.

(4) Ann. Chem. v. Pharm. Bd. 27

(5) Thierchemie, 1881

No one had yet, however, obtained pure ptyalin, and even today we cannot be sure whether we have succeeded in this respect.

Cohnheim[1] perhaps has been able to obtain the purest form in a comparatively easy manner. He added lime water to the saliva which had been previously acidulated with phosphoric acid up to the point of neutralization.

The precipitate of phosphate of lime brings down with it all the ptyalin which is subsequently purified by frequent extractions with water, and finally precipitated with absolute alcohol. The ptyalin so produced was found to have strong diastatic properties.

The method was a serviceable one, both for practice and scientific purposes, although the

(1) Virchow's Archiv, Vol. XXVIII, S 246.

ptyalin was not absolutely pure. The enzymic action of saliva has been carefully studied by Jacoubowicz, Magendi and Claud Bernard.

B. PEPSIN

None of the digestive juices have received so much attention as the gastric juice. The stomach was even in the pre-historic times looked upon as the chief organ concerned in digestion, and after the researches of Beaumont on Alexis St. Martin, the minds of physiologists were more alive to this prevailing idea.

Scientific observation, however, has of recent years tended to the opposite extreme. We have heard quite recently that the stomach may be looked upon merely as a receptacle for food, with little power either for its digestion or absorption. This view has been in part confirmed by the extirpation of the entire stomach for cancerous and

other affections with no appreciably disturbing symptoms to the patient. I think, however, that this subject is too modern a one to draw any final or hasty conclusions, but it may safely be said that the stomach is not such an important organ in the process of digestion as it was at one time supposed.

The first to enter the field in the study of the action of the gastric juice was Réaumur[1] who in 1752 found that on introducing small metallic tubes, which were closed at one end, and covered by muslin at the other, into the stomach of a buzzard, the food which they contained on examination after the regurgitation of the tubes was partially dissolved.

Stevens[2] in 1777 introduced perforated

(1) "Hist. Akad. roy. d. sc. de Paris" 1752, pp. 266, 461

(2) "De alimentorum concoctione" Edin. 1777

silver balls containing food into the stomach of a Hungarian, who had the power of regurgitating the various articles which he swallowed as he desired.

He confirmed the views of Réaumur, but went a step farther, as he was able to obtain some of the gastric juice itself, and which he found had the power of digesting food outside the stomach, as well as inside of it.

Spallanzani(1) in 1783 experimented on birds, and noticed many points of interest in the action and nature of the gastric juice. Then came the remarkable paper by Beaumont(2) who described his experiment on Alexis St. Martin. This case is known by everyone, so I need not enter into it, further than to say that owing to an injury on the right side of the chest received by a bullet, a

(1) A Genève 1783

(2) "Experiments and observations on the gastric juice, and the physiology of digestion."

gastric fistula remained patent which allowed Beaumont to observe with his naked eye the secretion of the gastric juice, and the complicated movements of the stomach wall. So accurately were Beaumont's observations made, that most of the statements that have come from his pen have been proved and generally accepted. He was not aware, however, that an enzyme existed in this juice. In 1836 Schwann(1) gave the name of pepsin to a substance which he was able to separate from the mucous membrane of the stomach. His process consisted in making an aqueous solution of the mucous membrane of the stomach treating the same with ferro-cyanide of potassium, and subsequently filtering and neutralizing with potassium carbonate. Corrosive sublimate was added to the solution and the precipitate was suspended in H.Cl. and decomposed by H_2S. Pepsin was not, however, by this process isolated.

(1) Muller's Archiv, 1836, pp. 90-138

In 1839 Wassmann[2] attempted to do so. He precipitated the watery extract of the pig's stomach with acetate of lead. The precipitate was decomposed with H_2S as in Schwann's method, and from the filtrate pepsin was obtained in an impure form. At this stage Brücke[2], whose researches have always been recognised as so accurate, entered the field of enquirers. He isolated the enzyme pepsin, although he himself admits that it was by no means in a perfectly pure form. His method consisted in washing the mucous membrane of the pig's stomach: finely powdering it, and digesting it in a five per cent. solution of tribasic phosphoric acid at a temperature of 35° C.; the fluid was then neutralized by addition of lime water; the precipitate of calcium phosphate which was formed

(1) Diss. Inaug. Berolini 1839

(2) Sitzungsber, Wien. Akad. XLIII, p. 602

brought down with it pepsin; this was dissolved in H.Cl, the solution being poured slowly into a tube containing cholesterin; a precipitate of cholesterin was formed which brought down with it most of the pepsin; this was collected and shaken up with pure ether when two layers of fluid formed, the lower one containing water which held the pepsin in solution.

V. Wittich[1] found that most enzymes were soluble in glycerine, and he succeeded in obtaining a glycerine solution of pepsin. The process is a simple one, and in most cases is satisfactory, and thoroughly reliable.

I may state here that in describing the above methods for obtaining the enzyme pepsin I have made free use of Dr. Gamgee's[2] treatise on this subject,

(1) Pflüger's Archiv, Vol. II, p. 193

(2) Physiological Chemistry of the Animal Body, Vol. II, pp. 85-88.

in some cases indeed referring to the methods almost verbatim.

Many interesting researches in regard to the character of the gastric juice, and to its enzymes, as well as the action of these enzymes on proteids, have been made by Tiedemann, Gmelin, Eberle, Meissner, Kühne, Schützenberger, Chittenden and Neumeister.

C. RENNIN

The history of this enzyme commences comparatively within recent years.

The name, as we have already seen, was given by Sheridan Lea[1] while Deschamps[2] used the term chymosin. Heintz[3] was the first to show that a

(1) Loc. cit.

(2) Loc. cit.

(3) Journ. f. prakt. Chemie. Neue Folge Vol. VI, p. 374

substance could be extracted from the mucous membrane of the stomach which had the power of curdling milk.

Hammersten[1] however first described to us its true nature. He was able to shew, as he did in the case of trypsin, that rennin did not exist in the gastric glands in that form, but as renninogen which set free the enzyme rennin by the presence of H.Cl.

Various methods have been described for the extraction of this enzyme, but one of the most serviceable is that of Erlen-Meyer[2] who extracted with an aqueous solution of salicylic acid and afterwards precipitated with alcohol.

Most are now agreed that the glands of the cardiac end of the stomach have the power of secret-

(1) Maly's Jahresbericht Vol. II p. 118

(2) Maly's Jahresbericht, Vol. V p. 267

ing or liberating a larger quantity of both pepsin and rennin than those of the pyloric end. Schiff[1] Ebstein[2], V. Wittich[3], and Fick[4] go so far as to say that the cardiac glands secrete twice the quantity of the pyloric[5]. I believe that many of the difficulties which have presented themselves in the relative strengths of these enzymes made by different observers, may have originated in their experimenting on different portions of the mucous lining of the stomach.

D. TRYPSIN

The action of trypsin on proteids corresponds

(1) Digestion, Vol. II p. 287

(2) Jahresber. d. ges. Med. 1870, I. S. 99

(3) Jahresber. d.Thierchemie II. S. 207, 1872

(4) Ebenda 1. S. 192, 1871

(5) The above four references appear in Hermann's Handbuch der physiologie, Vol. V - Article by Maly, p. 89

very closely to the action produced by certain organisms and the ferments which they secrete. This subject will be discussed in Chapter III, when the methods of preventing any possibility of confusion will be described.

Trypsin is one of three or four ferments that exist in the pancreatic juice, and consequently different methods are adopted for bringing the action of different enzymes into play. We must here, however, consider only the proteolytic enzyme of the pancreas, and after describing briefly the history of the other enzymes, to mention in a word how these ferments have been separated in their extraction.

The pancreatic juice may be said to have been first collected by De Graaf.(1) Claud Bernard(2)

(1) Lugd. Batar 1664

(2) Archiv. de Méd. Vol. 19 p. 60 and Leçons, Paris, 1865, p. 334

however made a fistulous opening into the pancreas, and may be said to have been the first to study the characters of this juice.

Bidder and Schmidt[1] also made analysis of this juice, but none of these observers attributed sufficient importance to the proteolytic property that existed in it. Bernard, for example, noticed that this juice converted starch into sugar, emulsified fats, and he considered that these were the two principal functions of the juice. Corvisart[2] however clearly proclaimed the proteolytic activity of the pancreatic juice, and he was so convinced that this was one, if not the greatest property of this juice, that he set on foot a band of physio-

(1) Die Verdauungssäfte und. der. Stoffwechsel, Milan and Leipzig 1852

(2) "Collection de Memoires sur une fonction peu comme du pancréas, la digestion des alimentes azotés - Paris 1857

logical chemists who were entirely opposed to him. In 1859 Meinsner[1] corroborated Corvisart's statement, and Kühne[2] in his elaborate treatise on the proteolytic enzyme of the pancreas which he named trypsin, considered that the activity of this enzyme was even greater than that of the pepsin of the gastric juice. One of Kühne's pupils, Danilewski[3] by name, was the first to isolate clearly and unmistakably the tryptic enzyme. He found in 1862 that he was able to isolate a proteolytic enzyme which acted on fibrin in neutral or faintly alkaline fluids. He precipitated aqueous solutions of the pancreas by collodion and found that this brought down the proteolytic ferment in a gelatinous form. Various other methods of preparing trypsin

(1) Zeitschrift f. rat. Medizin, 3d Ser. Vol. VII, p. 17.

(2) Virchow's Archiv. Vol. 39, p. 130

(3) Virchow's Archiv. Vol. 25, p. 267

were employed, the chief of these being by Heidenhain and Kühne (for description of these methods see Gamgee's work, Vol. II, pages 221-222).

We have already seen that Heidenhain[1] discovered that trypsin did not exist as such in the pancreatic cells, but as trypsinogen, so that it is necessary, as in other cases to treat the tissues with some re-agent which will have the power of converting trypsinogen into its active enzyme (Lea).

The question of the solubility of enzymes in glycerine will be treated later, but it may be here mentioned that the consensus of opinion at the present time is, that trypsin is not soluble in glycerine, although its zymogen is. For this reason Von Wittich's method has been used with advantage in separating the pancreatic enzymes, for V. Wittich himself found that the glycerine extract he obtained

(1) Loc. cit.

had strongly diastatic properties, but no action on fibrin[1]. This result, as we shall see later, has been questioned by Hufner[2] and others.

Without entering into detail on the works on the pancreatic juice I shall merely mention here those physiologists who have written most on this subject. They are Ludwig, Weinmann[3], Bernstein[4], Roberts[5], Cohnheim[6], Langley[7], and Lea[8].

E. AMYLOPSIN

There is considerable doubt as to who was the first to discover that the pancreatic juice possessed

(1) Loc. cit.

(2) Jr. f. prakt. Chemie N.F. Bd. V (1872) S, 372

(3) Ztsch. f. rat. Med. N.F. Bd. III, S.248 (Ludwig v. Weinmann).

(4) Ber. d. Sächs Gesell. d. Wiss. Math. phys. Cl. 1869, S. 97

(5) Proc. Royal Society, London 1881 Vol. XXXII (Ludwig v. Bernstein)

(6) Virchow's Archiv, Vol. XXVIII

(7) Journal of Physiology III

(8) Verhandl. d. Heidelberg, Naturhist. Med. vereins. N.F. I, Heft. V.

diastatic properties. In Halliburton's text book, and also in Maly's article in Hermann's Handbuch, Valentine is mentioned as the discoverer of this important property of this juice.

Gamgee, however, enquired into this subject, and he believes that Bouchardat and Sandras[1] in 1845 were the first to observe the amylolytic properties of the juice. They obtained pancreatic juice from hens and geese and found that this rapidly converted starch into glucose. Brücke[2] fed animals on starch, and killed these in from four to five hours after. He found little sugar or starch in the stomach, but a large quantity in the great intestine. Maly[3] in a summary on the re-

(1) Comptes Rendus, de l'académie des sciences, Vol. XX.

(2) Sitzungsberichte. d. Wiener. Acad. LXV. 1872.

(3) Hermann's Handbuch der Physiologie, p. 238.

sult of Brücke's experiments, believes that this depended upon the highly amylolytic properties of the pancreatic juice. He also believed that the diastatic property of this juice was more active than that which existed in saliva.

Ptyalin converts starch into ptyalose, which has recently been found to be almost identical with maltose, although a small quantity of dextrose is produced after prolonged action. Amylopsin, however, converts starch into maltose and dextrose, the latter being formed in considerable quantities in a comparatively short time. The method of separating the diastatic ferment which was adopted by Bouchardat and Sandras consisted simply of making a watery infusion of the pancreatic tissue, and subsequently extracting with alcohol. It was by no means a satisfactory process as all the other pancreatic ferments existed in the extract. Dali-

necwski[1] endeavoured to separate the amylolytic enzyme by precipitating aqueous solutions of the pancreas with collodion, the filtrate being afterwards treated with absolute alcohol.

Cohnheim[2] adopted a method similar to that which he employed for the isolation of ptyalin from saliva. V. Wittich[3] dehyrated with alcohol, and afterwards with glycerine. Roberts[4] has done much in the perfecting of methods for preservation of pancreatic ferment, many of which have proved useful also as a means for their extraction.

He adopted three systems:-

1 Boracic Solution.

This solution contains three to four per cent. of a mixture of two parts of boracic acid and one of borax.

(1) Virchow's Archiv. Vol. XXV, p. 279

(2) Virchow's Archiv. Vol. XXVIII, p. 251

(3) Pflüger's Archiv, Vol. II.

(4) "Digestion and Diet" p. 17

He found that amylopsin and the other pancreatic enzymes were well preserved in this medium.

2 Dilute Spirit.

This solution consisted in water being mixed with 12 to 15 per cent. of rectified spirit. This has proved to be an exquisite method for the extraction of the pancreatic enzymes.

3 Chloroform Water.

He found that this solution formed a good medium for the preservation of the pancreatic enzymes.

F. PIALYN

Of recent years the fat splitting ferment has received considerable attention.

No doubt this is in part due to our vague knowledge of its action, and in part to the difficulty that is experienced in its separation. The first physiologist to note the fat splitting properties

of the pancreatic juice was Claud Bernard[1], and it is to his researches that we owe most of our knowledge as to its character. It is a curious fact, however, as pointed out by Rachford[2] in his elaborate research on the influence of bile on pialyn, that Claud Bernard, who correctly observed all that occurred when the pancreatic juice acted on a neutral oil, failed to see that the emulsion which formed, and the liberation of the fatty acid that was produced, were one and the same process. Now, it is generally believed that pialyn performs one function, and that is the formation of a fatty acid and glycerine, while the emulsion is due merely to the action of the liberated fatty acid which developed in the process. In short, we believe now, that there

(1) Compte. rend. de l'acad. de Paris, XXVIII. Mémoire sur le Pancreas, Paris, 1856.

(2) Foster's Journal of Physiology, Vol. XII (1891) p. 72

is no emulsive ferment.

The term steapsin is frequently used to denote this enzyme, but more recently the term pialyn, which was first introduced by Sheridan Lea has received more popular favour. It is derived from the Greek πιαρ = fat and λυειν = to split up or decompose. Roberts undertook many researches on this subject, and he did not believe that there was a fat splitting ferment. The extract, however, must be made from a fresh gland, and before that gland has become acid in reaction, so that it is quite possible, and indeed probable, that Roberts' failure in obtaining this ferment was due to its unstable condition, the tissues having become acid probably before its extraction. Bidder and Schmidt(1), Grützner(2) and others, have proved that the least acidity prevents

(1) Die Verdauungssäfte, p. 250.

(2) Pfluger's Archiv. Vol. XII (1876) p. 302.

its action.

Paschutin[1] observed that the bi-carbonate of sodium was an excellent salt for the extraction of pialyn.

Researches on the nature and action of this enzyme have been made by Brücke[2], Gad[3], Bidder and Schmidt[4], Bernstein[5], Grützner[6], Heidenhain[7] and Rachford[8].

There is also a milk curdling ferment in the pancreatic juice, but it has received little attention and is considered of small importance, as all the milk is curdled by the rennin in the gastric juice before passing into the intestine.

(1) Arch. of Anat. v Physiol. Leipzig 1873 S. 382

(2) Sitzungsberichte der Weiner Acad. der Wissensch. Bd. LXI, p. 362

(3) Archiv. für Anat. v. Physiol. 1878, p. 181

(4) Loc. cit. (5) Loc. cit. (6) Loc. cit.

(7) Loc. cit. (8) Loc. cit.

From the above then we see that there exist in the pancreatic juice four ferments, each with its own function, and each being capable of extraction in a more or less pure form.

The presence of two ferments in the same extract prevents the accurate analysis of either, and it is in order if possible to eliminate all ferments except the one under consideration, that has led to so much investigation on the question as to whether enzymic action is increased or destroyed in certain media, or by the presence of certain salts. This question will be shortly discussed in the next chapter.

G. INTESTINAL ENZYMES

Our knowledge of the chemical characters of the intestinal juice dates back to the researches

of Vella[1] and Thiry[2] who made intestinal fistulae on various animals for the purpose of obtaining this juice as it was secreted by the intestinal glands, to note the period of the maximum secretion, to observe how the quantity was affected by stimulation with acids, alkalies etc., and also to see what action this juice exerted on the food stuffs that were mechanically introduced into the intestine.

Two recognised enzymes at least exist in the intestinal juice, the one an invertive ferment, which was described in 1871 by Paschutin[3] and called by him "inversin", the other an enzyme which has the power of converting starch into sugar. The

(1) Moleschott's Untersuchungen Vol. XIII, p. 40

(2) Sitzungsberichte. d. Wiener, Akademie, Vol 50, p. 77.

(3) Archiv. of. Anat. v. Physiol. 1871, pp. 305-384

former enzyme inversin has the extraordinary capacity of converting cane sugar into grape sugar.

It is the most important enzyme of the intestinal juice, and can be obtained from any portion of the intestinal mucous membrane. It has been observed that the extract obtained from the mucous membrane itself has a more active inversive power than that from the juice. The other enzyme, which converts starch into sugar, exists in greater abundance in the upper portion of the intestine.

Thiry, Leube and Schiff could not find this enzyme, but Brown and Heron[1], Rohmann[2] and others, were certain of its existence, and they observed likewise that the maltose which is formed as a result of its action is rapidly converted into dextrose.

(1) Annal. d. Chemie v. Pharmacie Vol. CCIV, pp. 228-251
(2) Pflüger's Archiv. Vol. 41, p. 424

Some observers have considered that a fat splitting ferment is present in the intestinal juice, but the general belief is that no such ferment exists. Again in the upper portion of the small intestine a fluid is secreted from Brünner's glands which contains an enzyme with an action similar to that of pepsin(1).

One of the most interesting questions that has occurred for many years in the history of chemical physiology is whether a proteolytic enzyme exists in the intestinal juice or not.

Thiry(2), Leube(3) and Schiff(4) found a proteolytic enzyme, but their views were strongly opposed

(1) Grützner, Pflüger's Archiv. XII. p. 288

(2) Loc. cit.

(3) Centralblatt. f. d. Med. Wissensch. 1868 p. 289

(4) Centralblatt. f. d. Med. Wissensch. 1868 p. 357

by V. Wittich, Paschutin and Quinke. More recently Masloff[1] and Wentz[2] have proved that if every precaution be taken to prevent the influence of putrefactive organisms, then no such proteolytic action will proceed. This last sentence has accidentally raised in my mind the question whether organisms have powers of digesting fibrin and converting starch into sugar, and as this is such an important point in making observation on tissues I will discuss this question briefly.

Organisms exist in great abundance in the intestines, and have to do with the putrefactive changes that occur in the bowel. Putrefactive germs are capable of acting on fibrin in much the same way as trypsin, but with the formation of a larger quantity of leucine and tyrosin, but not only

(1) Kühne's Untersuchungen Vol. II, p. 920

(2) Zeitsch. f. Biol. Vol. XXII. p. 1

so, for they have been observed as well to have the power of converting starch into sugar(1).

Dr. Halliburton says "proteids are easily decomposed by putrefactive germs. Insoluble proteids like fibrin are first dissolved, forming a solution of globulin: the change is like that produced by digestion with formation of peptone, then amido-acids (leucin, tyrosine etc.) ammonia, $C.O_2$, amines and sometimes indole, and skatol are formed."

From this it will be seen how easy it is to confuse proteolytic reactions, as these may depend upon enzymes and organisms. Moreover, even on the death of the organism a ferment is sometimes liberated from its substance which is readily taken up by an extractive. In the words of Sheridan Lea "Organisms to whose activity the fermentation is due,

(1) Bienstock-Zeitsch f. Klin. Med. Bd. VIII and Jakowski - Archiv. des. sciences Biologiques, St. Petersbourg.

do not discharge their enzyme into the surrounding medium; when killed, however, by alcohol etc. they yield it readily to a suitable extractive."

Various methods of differentiation have been carried out between the enzymes proper and the ferments secreted from organisms and these have been of service in considering the presence or absence of a proteolytic enzyme in the intestinal juice.

A few of these methods may be here enumerated:-

1 Peroxide of Hydrogen kills organised, but not unorganised ferments.

2 Borax has little or no effect on the organised, but destroys the unorganised ferment.

3 Salicylic Acid (.1 per cent.), thymol, (. 5 per cent.) /Sheridan Lea/ kill organised but do not influence the unorganised ferment.

4 The researches of Kühne, Harris and Tooth(1) shew that 1 to 2 per cent.

(1) Journal of Physiology Vol. IX, No. 4

$HgCl_2$; .24, .5 and 1 per cent. CO_2; 1.25 per cent. thymol, 1 per cent. sulphate of quinine and solutions of salicylate of soda, and iodine, kill organised ferments, but allow the action of the unorganised (as in this case trypsin) to proceed. (The above is taken almost verbatim from McKendrick's Physiology, Vol. II, page 128.)

5 Chloroform inhibits the activity of organised ferments, but has no action on the unorganised(1).

6 Kühne showed that acetic acid possessed the same power as salicylic acid in killing organised ferments, while sulphuric acid had not.

By the above methods one is able to distinguish between the organised and the unorganised ferments.

This question will present itself in the second part of this paper when considering more especially the extracts of the pathological tissues.

(1) Muntz. Compt. Rend. T. LXXX, (1875) p. 1255.

CHAPTER III.

CHARACTERS OF ENZYMES AND HOW THEY ARE INFLUENCED. ARE ENZYMES PROTEIDS?

All enzymes are soluble in water. When a particular tissue, such as the mucous membrane of the stomach, or a portion of the pancreas, is finely minced and placed in water, gradually the zymogens are liberated from the tissues and become dissolved in the solution, while there is a still more gradual conversion of the zymogen into the active enzyme. Enzymes are precipitated by ammonium sulphate and by excess of alcohol, but as Roberts points out they differ from proteids in that they are not coagulated by alcohol. Their action is unaltered by alcohol,

for when this fluid is removed the enzymes are not found to have lost their chemical activity.

Pepsin is rather an exception to this rule, and it has been found to become inactive after prolonged immersion in alcohol.

All enzymes are soluble in glycerine except trypsin, so that V. Wittich's was a great discovery as it enabled physiologists in a comparatively short time to study the action of almost any enzyme.

As I have already said his method failed to extract from the pancreas an enzyme that had proteolytic activity, whereas Hüfner[1] discovered, by this method, a distinct proteolytic ferment. Kühne[2] after a series of experiments finally agreed with V. Wittich and considered Hüfner's mistake lay in using dilute glycerine as he believed that it was the water

(1) Loc. cit.

(2) Lehrb. d. physiol. chem. 1868. S 120.

in the glycerine that dissolved the enzyme trypsin.

Sheridan Lea considers that any method for the elimination of pancreatic enzymes consists in an extract being obtained which has chiefly diastatic properties, but which is by no means free from other enzymes.

Tripsinogen again is found to be soluble in glycerine and will remain in that form for an indefinite time without being converted into its enzyme.

Gamgee[1] himself believes that all pancreatic enzymes, including trypsin, can be more or less extracted by glycerine, whereas Langley[1] thinks it hardly proved that any enzymes are soluble in glycerine, as he believes that the element of water

(1) Loc cit.
(2) Dr. Gamgee's Work - Vol. II p. 4 (foot-note)

in the glycerine is in all cases the cause of the enzyme being in solution. However, I think we can accept the general opinion which is that trypsin is the only ferment which remains insoluble in pure glycerine although its zymogen tripsinogen is dissolved in it.

Enzymes act best at a temperature slightly above that of the animal body, or at that point which we call the "pointum optimum," which if exceeded, rapid destruction of the enzyme takes place. Different enzymes, however, act better at different temperatures.

This subject was investigated by Roberts(1) who finds that ptyalin has its greatest activity between 30° and 45° C. while it is destroyed between 65° and 70° C.; pepsin acts best between 35° and 50° C.; rennin between 38° and 40° C.;

(1) Loc. cit.

trypsin between 50° and 60° C. while it is destroyed above 75° C.; amylopsin acts best between 30° and 45° C. while it is destroyed above 65° C.; pialyn acts best between 38° and 40° C.

Von Tamman's[1] experiments prove that warmth always favours enzymic action, in the same way as it does in other chemical processes.

It is a curious fact that certain ferments when dry can be heated to a very high temperature without destruction: for example, pepsin and trypsin when dry can be heated to 170° C. without injury, whereas when wet they are destroyed at 100° C.[2]

High temperatures destroy all ferments, whereas low temperatures slow the action of the ferment, and if low enough stop their action, but the enzyme is

(1) Pfluger's Archiv. (Bd 10)

(2) Chem. Centralblatt 1881 p. 745.

not destroyed, for its activity returns when the temperature is again raised.

Enzymes differ from all other substances in possessing the power of converting an infinite amount of material with a finite amount of substance. Their action, in the words of Moore[1] "is in all respects analogous to that of catalytic agents: there is the passage from a less stable to a more stable condition, which is brought about by an agent which is not itself altered in the process."

In short, as described by Sheridan Lea the enzyme unites with fibrin and forms an unstable substance, which afterwards unites with water, and becomes still more unstable. This substance splits up into new bodies that give various reactions for peptones etc. and also into the original enzyme again.

(1) Schäfer's Text-book of Physiology

For example, if A stands for the original enzyme and B the fibrin, they unite and form C, which with water becomes D. D splits up into E, F, G, etc. and the original A which is again ready to act on more fibrin.

The activity of the enzyme is modified also by the reaction of the fluid in which it acts, and in some cases it is even destroyed in certain media.

PTYALIN acts best in neutral or slightly acid solutions, but sparingly in alkaline.

PEPSIN again is only active in acid solutions, the most efficient acid being H.Cl. at a strength which normally occurs in healthy gastric juice, viz.·2 per cent.(1) It is rapidly destroyed by alkalies. Brücke has demonstrated that anything which prevents the swelling of fibrin causes a re-

(1) Ad. Mayer. Zt. of Biol. Bd. XVII (1881) S 356

tardation in the activity of pepsin, and for this reason, it seems to me that we can account for the inactivity of pepsin in alkaline media. The fibrin in alkaline solutions would tend to erode, and not to swell as is the case with trypsin.

RENNIN also acts only in acid solutions, and is rapidly destroyed in alkaline.

TRYPSIN acts more or less in all solutions, although the presence of .1 per cent. H.Cl. destroys its action, especially in presence of pepsin(1). It has its greatest activity in a 1 per cent. bicarbonate of soda solution.

AMYLOPSIN acts most efficiently in neutral or faintly acid fluids, while

PYALIN is only active in alkaline or neutral

(1) Kühne - Virchow's Archiv. Bd. XXXIX (1867) S. 130

solutions. As we have seen, an acid stops its activity immediately.

All strong acids and alkalies kill enzymes and remove their action.

"Man faud allgemein, dass sowohl starke säuren uri starke alkalein die enzyme zerstören und deren Wirkung aufheben[1]." (Moraczewski)

Certain enzymes act more powerfully in presence of salts while others are rapidly destroyed by them. For this reason salts have been used for purposes of extraction for different kinds of enzymes.

A great amount of investigation has been done in this direction, and I shall briefly mention the conclusions which have been arrived at by physiological chemists. Dr. Maly in his article on en-

(1) Archiv. fur. gesammte physiologie 1898, p. 36

zymes (see Hermann's hand-buch Vol. V. page 72) enters into this subject very fully. He enumerates the experiments that have been made on the actions of alkalies on digestive juices by Nasse[1], Chittenden, Detmar, Heidenhain[2], Schmidt, Hammersten[3], and Jacobson[4].

Mineral salts further enzymic action, and chloride of ammonium invariably strengthens it. Alkaloids such as quinine, morphine etc. have various actions on enzymic activity. In some instances they increase it, while in others they hinder it. Salts of the heavy metals, such as those of silver, mercury and lead, if used in large quantity destroy

(1) Pflüger's Archiv. Bd. II

(2) Pflüger's Archiv. Bd. X

(3) Zeitschr. f. physiol. Chemie. Bd. XXII S. 333

(4) Zeitschr. f. physiol. Chemie. Bd. XVI (16)

enzymic action, while it has been shewn that they have little effect when present in minute quantity.

Vazilieff[1] found that chloride of mercury when used in small quantities had no harmful effect. Lead acetate, copper sulphate, and chloride of mercury if used in concentrated solutions entirely stop peptic digestion.

Chloride of sodium, sulphate of sodium, sulphate of magnesium and iodide of potassium, all hinder peptic digestion while arsenious acid, hydrocyanic acid, carbolic acid and salicylic acid have little or no effect.

It has been shewn recently that formaldehyde rapidly destroys peptic digestion[2].

Tryptic digestion is increased by the presence

(1) Zeitschr. f. Physiol. Chemie Bd. VI.

(2) Journal. f. pr. Chem. N.F. XXXVII p. 101

of .02 per cent. of lactic acid, and 1 per cent. of bicarbonate of soda, especially the latter when in presence of bile.

Chittenden and Cummins[1] have also shewn that trypsin is extremely active in a solution of borax or cyanide of potassium, but not in one of mercury or iron. Paschutin[2] has made use of certain salts for the extraction of the pancreatic enzymes, and he finds that sodium chloride, sodium sulphate and chlorate of potassium extract all of these with almost an equal degree of success, while the carbonate of sodium has the greatest power in the extraction of pialyn, the iodide and sulphide in the extraction of trypsin, and the arseniate of potassium in the extraction of amylopsin.

As we have already seen, thymol, salicylic acid,

(1) Lab. Physiol. Chem. New Haven (1885) Vol. I p. 100

(2) Archiv. f. Anat. v Physiol. Leipzig (1873) S. 382

and other antiseptics have little action on the true enzyme proper, while they have a markedly destructive action on the organised ferment (Sheridan Lea).

Enzymes are mostly all unaffected by exposure to light[1] (Mayer).

The action of enzymes is hindered by the accumulation of substances formed by their activity. If these substances be removed, then the ferment will act as powerfully as ever, and convert an almost infinitesimal amount of material. If the products, however, are allowed to remain, then the activity of all enzymes gradually diminishes until it is finally destroyed. The experiments of Sheridan Lea[2] on the action of ptyalin on starch will be mentioned in the next Chapter, but it may be said here that they strongly emphasise the fact that if

(1) Enzymologie, Heidelberg 1882

(2) Journal of Physiology Vol. XI.

all products which are produced by enzymic activity and are injurious to the action of the enzyme, be removed, the activity of the enzyme will not suffer.

The action of an enzyme has been shewn by Hoppe-Seyler to be akin to the process hydrolysis[1]. As has been mentioned the unstable condition of the enzyme itself is increased by the union with water, which causes the compound thus formed to split up afresh into new substances.

Schutz[2] has shewn that enzymic activity depends upon the concentration of the enzyme in the solution, and on experimenting, more especially on pepsin, has proved that the amount of conversion of proteids into peptones was proportional to square root of the quantities of pepsin present.

(1) Archiv. f. d. ges. physiol. Bonn (1876)
Bd. XII, S. 1

(2) Ztschr. f. physiol. Chemie. Strassburg (1885)
Bd. IX, S. 577

From what has been said above one cannot fail to see with what thoroughness the subject of the nature of enzymes has been investigated, and although these have not as yet been isolated in sufficiently pure form to make accurate analyses of their constituents, yet, in most cases at least their characters are well known. They have all a definite action to perform. They each pick up that portion of the food on which they can create a change, and thus render it suitable for assimilation.

We are now met face to face with the question - What is the true chemical nature of these enzymes?

They contain exactly the same elements as proteids, but are they proteids?

This question is one that will be always difficult to solve, until methods are devised for obtaining enzymes in the perfectly pure state.

At present, as they exist possibly along with

peptones or other albuminous substances it is impossible to make accurate analyses of their constituents.

Within recent years, some of the enzymes have been stated to be proteids, but these results have not yet been accepted by all authorities.

Halliburton[1] declares that the fibrin ferment is a true proteid; Langley and Edkins[2] say the same about pepsin, and Loew[3] the same about malt diastase. Moraczewski discusses this question, and shews that the researches of most physiological chemists prove that there is a too deficient quantity of N and C to call these substances proteids, although they shew that they are closely allied.

(1) Journal of Physiology IX p. 229

(2) Journal of Physiology VII p. 371

(3) J. pr. Chem. XXXVII p. 101

He mentions a paper by Hüfner[1] where an analysis of the various enzymes have been made by different observers.

It is as follows:-

C	H	N	Asche	Enzyme	Author
43.6	6.7	14.0	.88	Trypsin	Hüfner
48.8	7.13	14.16	1.2	Emulsin	Schmidt
43.9	8.4	6.0	.6	Invertin	Barth
46.6	7.3	10.4	1.0	Diastase	Libner
46.6	7.1	14.9	.9	Pancreatin	
43.9	6.9	9.5	.6	Invertin	Donath
43.5	7.0	11.6	1.3	Emulsin	Bull

Maly also in his article on enzymes mentions the results of four observations by Hüfner on the analysis of the pancreatic ferments. These results varied as follows:-

C	H	N
40.3 to 40.5%	6.5 to 6.9%	13.3 to 13.6%

Asche - Considerable quantity.

(1) Jahrb. f. pr. Chem. N.F. Bd. V. S 372.

How do the results of these tables compare with the analysis of true proteids?

Let us take the analysis of proteids according to the experiments of Hoppe-Seyler[1] and Drechsel[2]:-

	C	H	N
Hoppe-Seyler	51.5 to 54.5%	6.9 to 7.3%	15.2 to 17.0%
Drechsel	50.0 to 55.0%	6.8 to 7.3%	15.4 to 18.2%

In both cases considerable amount of asche.

In comparing these tables we find the average percentage for proteids is:-

C	52.75 per cent
H	7.075 "
N	16.45 "

whereas the average percentage for particular enzymes is:-

(1) Handbuch. d. Physiol. path. chem. Anat. (1885) S. 258

(2) Ztschr. f. Biol. München (1886) Bd.XXII, S.452

C	40.3	to	48.8	per cent.
H	6.5	to	8.4	"
N	6.0	to	14.16	"

We see then that there is a distinct deficiency in the amount of carbon and nitrogen in the constitution of enzymes although the quantity of hydrogen in both cases is closely similar.

Even though the percentages were closer than they appear to be, the question would still remain - Are the enzymes pure?

Brücke and Sundberg[1] were against the opinion that enzymes were proteids, but it must be admitted that their methods were not so perfect as those of today, and probably their enzymes not so pure. Although certain enzymes may be proteids, few of these give proteid reactions.

(1) Ztschr. f. physiol. Chem. Strassburg (1885) Bd. IX, S. 319

The question is one of great interest and importance, and one that is by no means solved yet.

CHAPTER IV.

CLASSIFICATION OF ENZYMES WITH THEIR RESPECTIVE FUNCTIONS

A classification is scarcely necessary as most of the enzymes have already been mentioned. However, although we know the names of enzymes and in what juices they occur, it is of advantage to group them together if possible, in order to see whether various enzymes may have a similar action, and consequently are in a position to assist one another in the digestive process. One could not do better in making such a classification than to refer to the table given by Halliburton, which is as follows:-

1 Proteolytic - Pepsin, Trypsin and Papain

2 Amylolytic - Ptyalin, Amylopsin, and Diastase

3 Steatolytic - Pialyn

4 Inversive - Invertin of intestinal juice

5 Emulsin or Synaptase

6 Coagulative - Fibrin, Myosin and Rennin

This is a complete classification of our present knowledge of the digestive enzymes, arranged according to their particular action. It now remains for me to describe briefly what the various actions are. My object, however, is not to enter into the most elaborate and complicated results obtained by the action of these enzymes, but merely to refer generally to their action in special relation to their final products. For a full account of the intermediate bodies formed by the proteolytic enzymes or proteids, one may refer with advantage to such text books as those of Gamgee and Halliburton,

and the exceedingly lucid article by Moore in Schäfer's new text book of physiology. In these books a full description of the cleavage theory, introduced by Kühne[1], is given, with a full account of the characters of the intermediate bodies that occur before peptones are formed.

FIRSTLY: How is the digestion of albumin by pepsin accomplished?

The general opinion is that the action is one of a slow process of hydrolysis. Water is constantly being absorbed and the substance is gradually becoming more unstable.

Pepsin does not act by itself on fibrin, but in the presence of dilute H.Cl. it becomes most active. H.Cl. again digests fibrin extremely slowly

(1) Verhandl. d. Naturh. Med. Ver. zu. Heidelberg, 1877, N.F. Bd. I, S. 236

when alone, but with pepsin the action is most rapid.

An interesting experiment has been performed recently by Matthes[1]. He applied H.Cl. (3%) to a frog's leg: the skin became inflamed, irritated and reddened, but little or no other change occurred until pepsin was added, when the leg commenced to become digested.

The action of pepsin with acid on albumin is as follows:-

Acid albumin is first formed then albumoses, proteoses and finally peptones.

In undergoing this change the proteid gradually swells up, becomes transparent and dissolves. The peptones which are found in solutions, are chemically identical with proteids, and it is only by their behaviour with particular salts etc. that they can be

(1) Centralblatt. für. physiologie 1894

distinguished from them. They can be recognised also by certain colour tests as, for example, the Biuret reaction - where a rose pink hue develops on adding a drop of copper sulphate and excess of caustic potass to the fluid - and also the xantho-proteic reaction.

SECONDLY: How is the digestion of proteid by trypsin accomplished?

The process is practically the converse of that which occurs from pepsin. The fibrin does not swell up, but is attacked from the outside, and gradually becomes eroded. Peptones are formed which have the same characteristics as those mentioned above, but besides these, other substances are formed, the chief of which are leucin and tyrosin.

Kühne[1] has shewn that only one half of the proteid is converted into peptone, while the other

(1) Loc. cit.

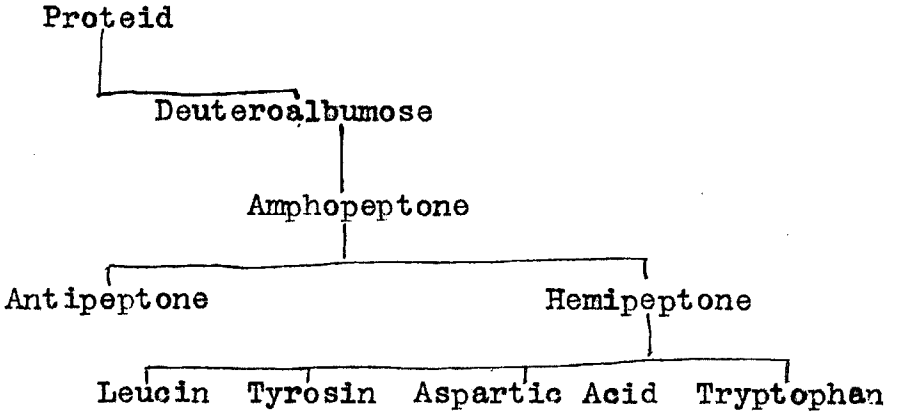
portion is changed into simpler substances.

To show the close relation that exists between the peptones resulting from peptic and tryptic digestion he has used certain terms that are common to both. He finds, for example, that trypsin first forms peptones and then goes a stage further with the production of other substances. He calls the peptones formed from peptic digestion ampho-peptones. Pepsin can convert them no further, while trypsin converts a portion of these peptones into new substances. The portion that is not changed by tryptic digestion he calls antipeptones, while the portion that is converted he calls hemipeptones - the conversion being into a series of new substances, the chief of which are leucin and tyrosin.

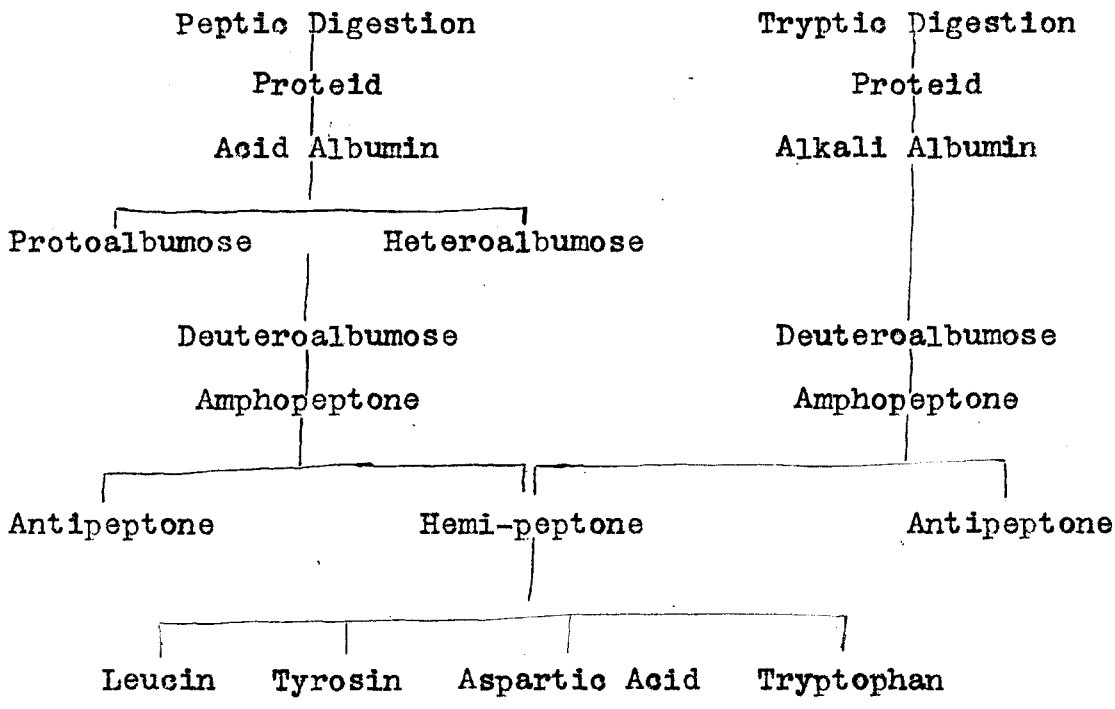
If one compares the schema of Neumeister[1] on the action of trypsin on proteids we see that ampho-

(1) "Lehrbuch. der. Physiol. Chemie." Jena. 1893 Th. 1, S. 200

peptones are first formed as in the case of peptic digestion, that portion which is not altered being called antipeptones, the portion that is, being called hemipeptones.



If we make a conjoint schema of peptic and tryptic digestion, the changes resulting from pepsin being marked by blue, and those from trypsin by red, we can see clearly where the change takes place.



Most of our knowledge on the nature and action of trypsin and on the substances which it produces, are due to the elaborate and multiple investigations of Kühne. He was the first to see the formation of leucin and tyrosin by the action of trypsin on proteids. His experiments shew that the average amount of these substances produced by digestion is

9.1 per cent leucin, and 3.86 tyrosin. The chief substances formed by the action of trypsin on albuminous substances are:-

I	Peptones
II	Leucin, which is an amido caproic acid
III	Tyrosin, which is a paroxyphenylamido-proprionic acid
IV	Tryptophan
V	Lysine
VI	Lysatine
VII	Ammonia

Apart from the treatises of Kühne, the other important investigations have been conducted by Chittenden(1), Schützenberger(2), Neumeister(3),

(1) Ztschr. f. Biol. München 1884, Bd. XX, S. 11

(2) Bull. Soc. Chim. Paris 1875 - Tome XXIII.

(3) Loc. cit.

Brücke[1], Henningen[2], and Hofmeister[3].

The nature of the final products has been most exhaustively treated by Gamgee in his text book.

THIRDLY: What is the action of ptyalin on starch?

The process is one of hydrolysis and can be expressed as follows:-

$$10\ (C_{12}H_{20}O_{10}) \quad 8\ (H_2O) \quad = \quad 8\ (C_{12}H_{22}O_{11}) \quad 2\ (C_{12}H_{20}O_{10})$$

Starch Water Maltose Dextrin

The starch is first rendered soluble, and then is decomposed into erythrodextrin and maltose, the erythrodextrin again is decomposed into achroodextrin and maltose: so that maltose is the final product

(1) Sitzungsber. k. Akad. d. Wissensch. Wien. 1859

(2) Comptes rendus (1878), Vol. LXXXVI, p. 1413.
Bd. XXXVII

(3) Ztschr. f. physiol. Chem. Bd. II S. 206

along with a certain amount of dextrin.

The question then arose as to how much dextrin and maltose respectively resulted from the experiment, and up to within recent times the percentages were widely various as given by different observers.

We have already stated that the action of an enzyme is hindered by the products which it forms unless they be quickly removed.

Sheridan Lea[1] considered that if an instrument could be devised which would remove speedily all the substances which would be injurious to enzymic activity, the enzyme would act unceasingly, and that as a consequence all dextrins would be converted into maltose.

He thus attacked the Views of Musculus[2] who

(1) Journal of Physiology - Vol. XI.

(2) Chem. Centralblatt - 1860

considered that the final products were 33 per cent. of maltose and 67 per cent. dextrin, and those of Payem[1] who reckoned the percentage as 52.7 per cent. of sugar and 47.3 per cent. of dextrin.

Sheridan Lea invented the dialyser, which allowed of a continuous movement of the mixture to be digested, and which also removed all digested products. By this ingenious device he was able to show that at least 85 per cent. of the starch was converted into sugar, and he affirmed that were the instrument sufficiently perfect all the starch would be converted into sugar.

In his own words he says "When the digestion of starch by saliva is carried out under conditions which ensure a very considerable removal of the products as they are formed, then

1 The rate at which digestion takes place is greatly increased

(1) Chem. Centralblatt - 1865.

2 The total amount of starch converted into sugar is much greater, and the residue of dextrin is much less than under conditions otherwise similar, when the products are not removed" etc. etc.

The form of sugar resulting from the activity of ptyalin was called by O'Sullivan(1) maltose, while Nasse(2) used the term ptyalose. The researches of others, however, prove that ptyalose and maltose are one and the same substance.

FOURTHLY: What is the action of amylopsin on starch?

The action on starch is precisely similar to that resulting from ptyalin, except that in the present case the action is much more powerful.

For example, ptyalin converts starch into maltose, and if the action be sufficiently prolonged, a

(1) Journ. Chem. Soc. London (1872) Vol. XXV p. 579
(2) Arch. f. d. ges. Physiol. Bonn (1877) Bd. XIV. S. 477.

small quantity of dextrose is formed. In the case of amylopsin the starch is quickly converted into maltose, and dextrose is formed in considerable quantity. V. Mering and Musculus[1] have found achroodextrin, maltose and dextrose after submitting starch to the action of amylopsin for a few hours.

The difference of action then between ptyalin and amylopsin is only one of degree. Sheridan Lea[2] made observations with his dialyser on the action of amylopsin on starch, but his results in this instance were not so satisfactory, as the other pancreatic enzymes interfered somewhat with the process.

Still, amylopsin is so powerful and does its work in such a short time, that probably the products which would interfere with its action, were

(1) Zeitschr. f. physiol. Chem. Vol. II p. 403
(2) Loc. cit.

it prolonged, would have barely time to do so.

FIFTHLY: What is the action of the fat splitting ferment or pialyn?

This ferment by a process of hydrolysis causes a decomposition of the fat. We may represent the result by the following formula:-

$C_{57}H_{104}O_6 + 3\ H_2O = 3\ (C_{18}H_{34}O_2) + C_3H_8O_3$

Olein Water Oleic Acid Glycerine

This equation[1] shows how a fat such as olein under the influence of the ferment unites with water and splits into the corresponding fatty acid and glycerine.

Let me take another instance![2]

$C_3H_5\ (O\ C_{16}H_{31}O_3)\ 3 + 3\ H_2O = C_3H_5(OH)\ 3 + 3\ C_{16}H_{31}OOH$

Palmatin Water Glycerol Palmitic Acid

(1) McKendrick's Physiology, Vol. I p. 184

(2) Halliburton's Physiology, p. 492

Here again we find how the fat combines with water, and finally decomposes with the formation of a fatty acid and glycerol.

Most of our knowledge on the action of this enzyme is described by an elaborate investigation by Rachford[1] on the influence of bile on the fat splitting properties of the pancreatic juice.

He first described shortly the nature of chemical emulsions, and subsequently referred generally to the fat splitting properties of the pancreatic juice. He obtained the pancreatic juice of a rabbit by making a temporary pancreatic fistula.

The fat which was used was neutral olive oil. By using the spontaneous emulsion method of Gad in whose laboratory he was then working, he was able approximately to estimate the amount of fatty acid

(1) Journal of Physiology (1891) Vol. XII. p. 72

produced, and to ascertain when all the fat was converted into the fatty acid and glycerine.

His method consisted in the knowledge of the fact that one gets the best spontaneous emulsion with a solution of carbonate of soda, when 5½ per cent. of fatty acid is formed. He tested with a carbonate of soda solution from time to time.

He found at first that no emulsion formed, but as he gradually approached the proper percentage, viz. 5½ per cent. the best emulsion resulted, but with the increased formation of acid the emulsive power grew less and less until 12 per cent. of acid formed, when no result occurred. His conclusions briefly were as follows:-

1 The pancreatic juice is alkaline, and remains so for some time after its removal.

2 If shaken with neutral olive oil, this oil rapidly takes on an acid reaction from the formation of a fatty acid.

3 All the oil is split up into fatty acid and glycerine in from one to two hours' action by the pancreatic juice.

4 The time required for the pancreatic juice to give its maximum spontaneous emulsion, i.e. to develop 5½ per cent. of fatty acid is about twenty minutes.

5 The action of pancreatic juice on most fat is rapid and complete.

The above are the results obtained almost in the words of the author.

I will not mention the investigation which followed on the action of bile on this ferment further than to say that rabbits' bile greatly hastens the fat splitting properties of the pancreatic juice, especially in the presence of ¼ per cent. H.Cl. solution. This is important as we know that in the duodenum both H.Cl. and bile are present, and so consequently the fat splitting ferment in this part will act more effectively.

SIXTHLY: What is the action of the intestinal ferments?

Three actions are produced by the intestinal enzymes. The first and most important is the inver-

sion of cane sugar into levulose and dextrose, as shewn by the following formula:-

$C_{12}H_{22}O_{11}$ H_2O = $C_6H_{12}O_6$ $C_6H_{12}O_6$

Saccharose Water Dextrose Levulose

The second action is the conversion of starch into maltose, in the same manner as that by ptyalin and amylopsin.

The third action is the power of converting maltose into grape sugar (Brown and Heron).

I have endeavoured shortly to describe the actions of the more important enzymes as the knowledge of the substances produced are of the greatest importance when we consider in what form the food stuffs are absorbed, and in what form proteids and carbohydrates are carried to the liver.

Many a doubtful physiological problem will be solved when we know precisely what are the actions of these and other enzymes.

CHAPTER V.

APART FROM THE DIGESTIVE JUICES DO ENZYMES EXIST IN OTHER TISSUES?

Zymolysis, one of the manifestations of the digestive process, occurs in plants as well as in animals. We know that plants digest their food as well as animals, although the process is performed in a slightly different way.

Bernard[1] considers that the digestion in plants is in most cases an interstitial one, while that in animals is an exterior one.

(1) Leçons sur les phénomènes de la vie T 2: 1879, Paris.

By interstitial digestion, he means the conversion that takes place in the tissues of the food which is stored up there, for purposes of nutrition. For example, the starch that exists in the tuber of the potato undergoes conversion into sugar at the period of its growth. In the same way in the seeds, bulbs, tubers and roots, starchy, sugary and albuminous substances are present, which at a stated time, and with proper light, warmth, and moisture, become converted into other substances which nourish the flowers, fruit, and blossoms that are about to spring up.

This is an interstitial digestion carried on in the intimate cell structure of the plant by enzymes of a nature probably identical with those that exist in the digestive juices of the animal.

We can speak then of the zymolysis of plant life as the process of the conversion of stored up food stuffs into new substances, which have been formed by the activity of the soluble inorganised enzymes.

By an exterior digestion Bernard means the digestion that takes place in food stuffs which have been introduced from without.

This form of digestion occurs in man.

These two classes of digestion, however, overlap somewhat in particular cases.

For example, in the sun-dew this plant seizes insects and pours out around them a juice which is capable of digesting them, so that in this case we have a plant performing an exterior digestion corresponding closely to that which occurs in man.

The enzymic action of plants has been investigated by Green(1), Hansen(2), Wortmann and others, and it is now generally believed that in most plants

(1) Science Progress, London, Vol. I, p. 342: Vol. II p. 109. Vol. III p. 68, 376. Vol. V. p. 60.

(2) Bot. Ztg. 1886. S. 137.

there are at work enzymes of proteolytic, amylolytic and inversive natures.

In Halliburton's article on the chemical constituents of the body which appears in Schäfer's text book of physiology, reference is made to the proteolytic enzyme papain[1] that exists in the papaw plant, and which is so similar in its action to trypsin[2]; mention is made also of bromelin[3] which exists in the pine apple juice.

The products of the actions of those enzymes compare favourably with those of all proteolytic ferments.

(1) Named by Wurtz - Compt. rend. Acad. d. Sc., Paris (1879) p. 425.

(2) S. Martin. Journal of Physiology, Vol. V, p. 213

(3) Named by Marcano. Chittenden - Trans - Comment. Acad. Arts and Sc. New Haven (1891) Vol. VIII.

Again, it is generally admitted that the conversion of starch is due to a soluble ferment or diastase that is liberated from the plant cell, and that the inversion of cane sugar, as, for example, beetroot into invert sugar, during its inflorescence, is due to an inversive enzyme.

In Roberts' work on digestion and diet he sums up Bernard's views as follows:-

1 "Digestion, or the process by which crude food is changed into available nutriment, is a function or faculty of capital importance in every form of active life.

2 This function is exercised partly on food brought into proximity with the surface of the organism (exterior, chiefly intestinal digestion), and partly on reserves of food laid up in the interior of the organism (interstitial digestion).

3 The agents concerned in this function and their mode of action are essentially the same, whether the organism be a plant or an animal - and whether the action take place in the interior of the tissues - or on the general interstitial surfaces."

We know then that substances of the nature of

pepsin, ptyalin and invertin exist in most plants. We have now to consider generally in what tissues these exist in animals.

Digestion goes on in unicellular organisms just as in animals of the higher scale. These organisms secrete their enzymes in a precisely similar fashion. As we approach the animals of the higher scale, we find that pepsin has been found to be present in the gastric juice of all of these, with the exception possibly of some fishes[1].

We find also that enzymes such as ptyalin, amylopsin and invertin etc. exist in most animals as well.

Investigators have examined the organs and tissues of human embryos and foetuses and have shewn roughly when the activities of enzymes first appear. Pepsin, rennin, and trypsin occur in the

(1) Hammersten - Lehrbuch der Physiol. Chem. Wiesbaden (1895): Aufl. III S. 234

human individual at birth, amylopsin about one month later, ptyalin can be obtained from the parotid gland at birth, but from the sub-maxillary two months later(1).

It is not known when steapsin first appears.

The question now arises - since we have observed that in plants an interstitial digestion is constantly at work by the process of zymolysis, is it not possible that in the human tissues as well, enzymes are in action, of the same nature, or it may be of a different nature, in these tissues.

It is quite clear that in the human subject, so well provided with a digestive canal, it is not so necessary for an interstitial digestion to take place. But just as in plants where we have seen that at a particular period of their growth the

(1) Zweifel - "Untersuch ueber. den. Verdauungsapparat. der. Neugeborrnen" Berlin 1874.

constituents of the cell are transformed into new substances, in the same way in man, starchy food in the form of glycogen is being stored up in certain organs, and this substance at a particular time may also be converted into other substances.

Glycogen exists in many tissues. Is the conversion of this glycogen into sugar the result of the activity of the soluble enzyme liberated, it may be from the zymogen that exists in the protoplasm of the hepatic cell?

If this were so it would correspond in its action to the diastase of plants, and to the ptyalin and amylopsin that exist respectively in the saliva and pancreatic juices.

Again, may there not be in the cells of different tissues, or in the secretion from these cells, enzymes or soluble ferments which are proteolytic in nature, and have to do with the conversion of

the nitrogenous or albuminous substances that exist in their protoplasm.

We may say that owing to the arrangement of our digestive canal we do not require to consider the question as to the presence or absence of enzymes in the tissues, for digestion takes place in the alimentary tract, and the products merely are carried by the blood to the tissues for purposes of nutrition. Still, admitting this, it does not follow that such enzymes do not exist, nor does it follow that provided they exist they may not be of infinite value in certain forms of disease. Again, is it not possible that when an abnormal sprouting of a parent tissue takes place, this may in part be increased by the activity of a certain enzyme in that tissue? For example, when a sarcoma or a carcinoma grows, is it not possible that an interstitial digestion is at work altering the nutrition of the parent tissue, produced, it may, or may not be, by an organism?

And again, it seems to me to be quite clear that the reason why tumours grow more rapidly in certain tissues or organs than in others, depends upon such an interstitial process of digestion, and this by the enzymes that are present in the tissue or organ in which the growth occurs.

Without, however, theorising I was anxious to see whether by adopting a particular process for the extraction of tissues, enzymes (they might be organised or unorganised) existed in these tissues.

Enzymes, of course, of an entirely different nature from those which are present in the digestive juices might exist in the tissues, but my object was merely to find out whether enzymes comparable to those of ptyalin, amylopsin, pepsin, trypsin, invertin, or rennin existed in the tissues.

My investigations were performed, and my results recorded before noticing whether there was any literature on this subject.

Without finding all the actual passages from which the statements have been made, I have found one or two remarks in Halliburton and Sheridan Lea's text books bearing on this subject.

Halliburton says "Brücke has shewn that muscle in common with most of the tissues of the body contains a small quantity of pepsin," and again, "O Nasse shewed that muscle juice also contains an amylolytic ferment, which he supposes to act in the transformation of glycogen into sugar after death.

I have (Halliburton) made a few experiments on this subject, and can fully confirm Nasse's statement of the existence of this ferment: a watery extract of the dried alcoholic precipitate of muscle changes glycogen into a reducing sugar: it will also act upon starch in a similar way, and in both cases an intermediate product of the nature of dextrin is formed. The action on starch is, however, slow: at the temperature of 40° C.sugar is not discoverable by

Fehling's test until after the ferment has acted upon it for five to six hours." And again, he says "we have already seen that such a ferment (diastatic ferment) can be obtained from muscle, and it seems that diastatic activity is present in all living proteids."

Sheridan Lea when speaking of ptyalin says "while occurring chiefly and characteristically in saliva, a similar enzyme may be obtained in minute amount, but fairly consistently from almost any tissue or fluid of the body, more particularly in the case of the pig."

In an article by Brücke[1] entitled "Beirtrage zur Lehre von der Verdauung" there is a paragraph at the close of this contribution entitled "Die verdauende Substanz im Fleische."

This is the subject evidently referred to by

(1) Sitzung. Akad. der. Wissensch. Band XLIII, Abth. 2 (1861)

Halliburton, although Brücke may have described his results more fully in other papers. He shewed that the juice of flesh when treated with water, and subjected to the same ether and cholesterin process that he used in carrying out his experiments for the isolation of pepsin from the mucous membrane of the stomach, had decided digestive properties. The digestion was noticeable in from five to six hours, and in the course of the next day all fibrin had been completely digested.

He confirmed his results by a slightly different method. He obtained the juice from four lbs of ox beef, and treated this with phosphate of lime. The filtrate was dissolved in weak H.Cl. He obtained again a fluid which dissolves pieces of fibrin in the course of the same day.

The digestion was found to go on, not only at 38° C. but even in an ordinary atmosphere.

This experiment proves that Brücke had at least

found pepsin to be present in the juice of flesh. This flesh was probably muscle, but it may have consisted as well of skin, fat, arteries, veins and nerves, etc.

Although Brücke then obtained pepsin from a large piece of flesh, and references are made to the effect that in muscle as well as in most other tissues there is a diastatic enzyme of the nature of ptyalin or amylopsin, no one as far as I can find, has methodically taken up each tissue separately, and made a glycerine extract of it, to see whether any particular enzyme or enzymes exist in the different tissues.

The results of my experiments will now be described in Part II.

P A R T II.

CHAPTER VI.

DESCRIPTION OF METHOD ADOPTED IN CARRYING OUT RESEARCH. PROCESS OF EXTRACTION, AND TESTS SUBMITTED TO EACH EXTRACT.

In consideration of the fact that Von Wittich's methods of making glycerine extracts of tissues, rendered soluble, in most cases at least, the enzymes which were present in the tissues, I resolved to

adopt his method with slight modification.

My object was not to obtain the relative amount of the enzyme from the tissue, but to see if it were present at all. Otherwise, the task would have been a very laborious one, as various methods of extraction would have necessarily had to be made, in order to obtain the greatest activity of the different enzymes.

I subjected each tissue or a portion of each tissue, to the same process, so that one description will suffice for all.

The tissues were all fresh, except in the case of post mortem tissues when they had been exposed to the atmosphere for twenty-four hours. All the tissues, whether physiological or pathological, were macerated and put in alcohol before any putrefaction or other change could take place.

The only tissues where putrefaction might have occurred were the post mortem tissues. The greatest care was taken in having the vessels into which the tissues were placed thoroughly cleaned, so that no extraneous germs could have lodged there.

The tissues were minced in a fine mincing machine, and afterwards pounded in a mortar with powdered glass, until they were in a fine state of division. They were surrounded with absolute alcohol for 24 hours.

The alcohol was then allowed to evaporate at the ordinary temperature of the room, the evaporation occurring in a large bell jar in order to prevent any dust falling into the vessel.

The tissues were then frequently powdered again when dry, and were covered over with a strong solution of glycerine, the quantity of the glycerine being in excess of the quantity of the tissue.

The vessel was then sealed over with a glass lid, and the extraction allowed to go on for six or eight weeks. At the expiry of that time the contents were filtered through fine muslin, pressure being exerted to squeeze out any of the juice that remained in the tissue, and occasionally a little extra glycerine was added to increase the quantity of the solution.

The solution was now ready for examination purposes.

This method, as has been shewn by Von Wittich himself, is a satisfactory one for demonstration purposes, but it is by no means reliable for purely scientific objects, as the solutions contain enzymes in a far from pure state. Still, we know that most enzymes at least are soluble in glycerine, and, moreover, whether we are dealing with the pure enzyme or not, glycerine has the power of extracting

it in sufficient quantity to give satisfactory and trustworthy results.

The apparatus and material that were required were simple.

The apparatus consisted in an incubator with a heat regulator, so that any required temperature could be maintained: two dozen 2 oz. beakers; test tubes; graduated tubes up to 100 Cc.; pipettes; microscope and slides; Bunsen burner: scales and weights.

The material consisted in fresh fibrin: starch solution: solution of cane sugar: solution of .2 per cent. H.Cl.; solution of 1 per cent. of $Na_2C\ O_3$; and the following chemicals:-

Copper sulphate, caustic potash, Fehling's solution, ammonium sulphate, sodium acetate, phenyl hydrazine hydrochloride, and Millon's re-agent.

The starch solution was frequently renewed, and made as follows:-

The best obtainable rice starch was purchased and 1 gram. of it was dissolved in 50 Cc of boiling water.

The solution was a perfectly homogeneous one, and made by stirring vigorously, the temperature being gradually raised, and the period of boiling not allowed to exceed two or three minutes.

The solution of cane sugar was made by dissolving 1 gram. of cane sugar in 50 Cc. of water.

The fibrin was always perfectly fresh, and washed in running water for at least 12 hours before use.

Standard solutions of .2 per cent. H.Cl. and 1 per cent. $Na_2C\ O_3$ were kept in pint bottles.

The solution which contained the supposed enzyme, or the glycerine extract of the tissue under consideration we shall call X.

We shall enumerate the tests submitted to X in the same order as they occur in the tables which will afterwards be mentioned. X was divided into seven portions, a sufficient quantity being left behind for confirmatory tests. (I may state here that I have from one to two ounces of the surplus extract of almost every tissue that I have investigated, which will be useful for future research.)

A - 1st PORTION:

I took two drachms of starch solution in a test tube, and added about one drachm of X. The contents were thoroughly mixed; the mouth of the test tube plugged with cotton wool; this was placed in incubator at a temperature of 38° C.

B - 2nd PORTION:

One gram. of fresh fibrin was placed in a 2 oz. beaker: to this was added 10 Cc of X, diluted up to 40 cc of cold water; the beaker was covered with a glass lid and placed in the incubator.

C - 3rd PORTION:

One gram. of fibrin was placed in beaker: to this was added 10 cc of .2 per cent. H.Cl. solution: the beaker was covered with a glass lid and placed in the incubator.

D - 4th PORTION:

One gram. of fibrin was placed in beaker: to this was added 10 cc of X diluted up to 40 cc, with a 1 per cent. Na_2CO_3 solution: the beaker was covered with a glass lid, and placed in incubator.

E - 5th PORTION:

Two drachms of a solution of cane sugar was placed in a test tube, and to it was added about one drachm of X: the contents were thoroughly mixed: the mouth of the test tube was plugged with cotton wool: and this was placed in the incubator.

F - 6th PORTION:

To one half ounce of fresh milk in beaker, diluted to an ounce with water, was added one drachm of X: the mixture was stirred up: the beaker was covered with a glass lid and placed in incubator.

G - 7th PORTION:

One drachm of X was placed in a test tube and afterwards put into incubator: the mouth of test tube being plugged with cotton wool.

These mixtures were left in the incubator for 18 to 24 hours at a constant temperature of 38° C. On no occasion did the temperature fall below 37° C. or rise above 40° C. in making the experiments.

The mixtures were then tested as follows:-

(A) 10 cc of Fehling's solution were boiled in a test tube, and to this was added gradually the same quantity of A.

The two fluids were boiled at their points of juncture, and any reduction was noted.

If there was any reduction then the probability was that sugar had been formed in mixture A, and the fluid was submitted to further tests.

To 5 cc of A were added one decigramme of phenyl hydrazine hydrochloride and two decigrammes of sodium acetate.

The mixture was heated for half an hour, and

the deposit which formed on cooling, was examined microscopically for crystals of phenyl glucosazone and phenyl maltosazone.

I am not sure whether the strength of the supposed sugary solution A was not too strong, but at all events, I on no occasion observed the crystals which are shewn in text books as occurring in sheaths and bundles. I obtained crystals which were yellow in colour, but which were very small and almost amorphous in character.

(B) The appearance of the fibrin in B was noted, and to a portion of the filtered fluid was added an equal quantity of sulphate of ammonia, and the presence or absence of a precipitate was observed.

By this means we were able to see whether proteoses had been formed or not.

(C) The appearance of the fibrin in C was noted,

particular attention being taken to see whether there was any appearance of its digestion, and to what extent digestion had taken place. The fluid was then filtered, and to a portion of the filtrate one drop of a weak solution of copper sulphate, and an excess of caustic potash was added (as a rule a drop of copper sulphate was placed in the test tube which was afterwards shaken out before adding the fluid to be examined).

The rose pink colour was observed which shewed the presence or absence of peptones.

(D) The appearance of the fibrin in D also was noted to see whether any erosion of it had occurred.

A portion of the filtered fluid was examined by the biuret reaction, while another portion was exaporated down into a few drops and was examined microscopically to see whether any crystals of leucin

or tyrosin were present, or crystals approaching them in character.

On several occasions when the presence of leucin and tyrosin was suspected, a portion of the filtered fluid was put in a test tube and to it was added a small quantity of Millon's re-agent.

The precipitate which formed was filtered off, and the filtrate was evaporated down into small bulk. Any change in the colour of the solution was observed and a few drops of the concentrated liquid were examined microscopically.

(E) 10 cc of Fehling's solution was boiled in a test tube, and to it was added about the same quantity of E. The two fluids were boiled at their point of juncture and any reduction of Fehling's was observed. As in the case of A the phenyl hydrazine test was frequently applied.

(F) The contents of F were poured into another

vessel and any special curdling of the milk was observed.

(G) To 10 cc of Fehling's solution was added the same quantity of G.

On boiling the fluids at their points of juncture any reduction of Fehling's was noted.

In order to compare the results in the appearance of the fibrin in solutions C and D, with that of fibrin submitted to the action of H.Cl. and Na_2CO_3 alone, confirmatory tests of this kind were frequently performed. One gramme of fibrin was placed in a beaker with 10 cc of .2 per cent. H.Cl. diluted up to 40 cc, and also with 10 cc of 1 per cent. Na_2CO_3 diluted up to 40 cc. Both beakers were placed in incubator and left there for 18 to 24 hours as in the other cases.

By means of these tests one was able to ob-

serve whether;-

1 the starch solution had been converted by X into a fluid that could reduce Fehling (if such were the case, whether X did not contain an enzyme closely related to ptyalin or amylopsin).

2 the solution X plus water had, when heated, the power of converting fibrin into proteoses.

3 the fibrin had partially or wholly been digested by X plus .2 per cent. H.Cl. (if such were the case whether X contained an enzyme closely related to pepsin).

4 the fibrin had been eroded or completely digested by X plus 1 per cent. Na_2CO_3 (if such were the case whether X contained an enzyme closely related to trypsin).

5 the solution of cane sugar had been inverted by X into a fluid that reduced Fehling (if such were the case whether X contained an enzyme closely related to inversin).

6 the milk had curdled or not by X (if such were the case whether X contained an enzyme closely related to rennin

7 X itself was a reducing agent, in order not to confuse the results obtained from A and E, with those of X.

CHAPTER VII.

FALLACIES THAT MAY OCCUR IN THE EXPERIMENTS, AND HOW THESE MAY BE OVERCOME

Length of Time for Glycerine Extraction

Unless the tissues are allowed to remain in glycerine for a considerable time very little extraction of the enzyme may be obtained.

We know that in making glycerine extracts of organs which contain certain enzymes and are present in considerable amount, we have often to allow the extraction to go on for a week. Consequently, in tissues which probably contain an enzyme probably

in small amount, a longer interval must be given for a satisfactory extraction to take place.

I have in all cases subjected the tissues to the action of glycerine for at least six weeks.

The Temperature during Experiment

As we have seen in Chapter III enzymes act better at particular temperatures. All enzymic activity occurs best at 38° C. and it must be remembered that high temperatures prevent all action of enzymes. Hence, it is absolutely necessary to maintain a uniform temperature of about 38° C. This was done by means of a heat regulating apparatus attached to the incubator.

The Length of Time necessary for Enzymic Action to occur

It is of importance to subject the solutions containing the supposed enzyme to a temperature of 38° C. for a considerable time.

The conversion of starch by ptyalin or amylopsin is a fairly rapid process while the digestion of fibrin takes some hours.

The amount of change, however, depends upon the relative strengths of the enzymes in the tissue extract. It is as well to allow enzymic action to go on at this temperature for at least 12 hours before making any tests. While enzymes actually exist in tissues we may entirely overlook their presence by submitting the fluid for too short a time to the proper temperatures.

I invariably allowed the action to go on for 18 to 24 hours.

The Purity of the Solutions used

It is quite clear that the solutions of starch and cane sugar must be fresh, and possess no reducing powers. Starch must be of the purest kind. I used the best rice starch.

It must not be boiled too long as prolonged heat converts starch into sugar.

The cane sugar must be pure, and in making a solution of it the boiling must not exceed a few minutes. If the solutions are to be kept for any length of time a little thymol will prevent any change in their nature, but it is preferable to make small quantities of starch and cane sugar solutions, as they are required. In all cases the

solutions of starch and cane sugar must be tested before carrying on any experiments with them. If there be the slightest reduction of Fehling, fresh solutions must be made.

Again, the question has to be considered whether starch and cane sugar solutions when subjected to a temperature of 38° C for 24 hours, do not then possess reducing properties from a slight conversion or inversion into maltose or dextrose.

I have found that a pure starch or cane sugar solution when submitted to a temperature not exceeding 40° C. for 24 hours, provided that the vessel or test tube in which these solutions are contained is plugged, should possess no reducing properties at the end of that time.

Again, the Fehling's solution itself must be perfectly pure so that when it is boiled it should not alter in colour.

With these precautions we are able to say definitely, if the starch solution plus X reduces Fehling, that the extract itself has reducing properties, or else that the starch has been converted into a substance that can reduce Fehling.

The first possibility can be easily settled by testing the solution G, and if this has no reducing property, it follows that the starch solution has been altered by a body that is present in X, and consequently reduces Fehling.

The Presence of Organisms in Tissues

This question is bound to present itself in making experiments on tissues and organs, as we are aware that organisms and their ferments are capable of creating changes on starchy and proteid foods in a closely similar way to those by the un-

organised enzymes that exist in tissues. This question was pretty fully considered at the end of Chapter II, and the means of differentiation of the organised from the unorganised ferments were mentioned. Still, we are compelled to admit the possibilities of results depending on the existence of the organised ferments, to be confused with those of the unorganised, and we have hence to see that no organisms are allowed to enter during the preparation of the tissues, and no putrefactive change is present in the tissues which we investigate, or in the fibrin which we use.

All beakers and test tubes must be sterilized, and before submitting their contents to the action of heat they must be respectively sealed and plugged.

The organisms themselves are killed during the process of extraction, and immersion in alcohol, but we know that we have not to consider the or-

ganisms themselves merely, as they are capable of liberating a ferment which is taken up by a suitable extractive. By the use of antiseptics we can avoid this difficulty.

Although I never used antiseptics, as I intended to observe the results on the tissues as they existed, I hope in a future paper to compare the results I have obtained with those after using antiseptics, such as thymol or salicylic acid.

The only tissues where such a possibility exists are those of the intestines of the rabbit and child, certain of the pathological tissues such as sputum where pyogenic organisms are usually in abundance, and in the post mortem tissues.

In the latter case the tissues were removed in less than 24 hours after death, and were at once placed in absolute alcohol. The other tissues were perfectly fresh, and were removed, powdered and placed

in absolute alcohol in a few hours after their removal.

In the intestines putrefactive bacteria are always present amongst the food stuffs, but the greatest care was taken in stripping off the mucous membrane of the bowel and in washing it freely in running water before mincing and placing it in alcohol. The fibrin which was used was fresh, and contained no putrefactive organisms.

I admit that no means in the way of antiseptics have been used to distinguish whether the results depended on the action of the unorganised or organised ferments, but at the same time I consider that in most cases at least the results have not depended upon the organised, but upon the unorganised ferments or enzymes which play such an important part in the process of digestion.

The Cleavage of Proteids by Acids alone

Fibrin is unaltered by the action of pepsin alone, but in the presence of H.Cl. rapid digestion takes place. A weak solution of H.Cl. by itself has the power of causing the fibrin to swell up and become translucent, and to produce an acid albumin, or even albumoses and peptones. The schema of proteid cleavage by acids is given thus - Kühne.

Albumin

Anti-group	Hemi-group
Anti-albumid	
Anti-albumate	
Anti-albumose	Hemi-albumose
Anti-peptone	Hemi-peptone

Do we know then whether the peptones that are

produced in various experiments depend upon the activity of an enzyme in conjunction with H.Cl. or from H.Cl. itself?

The biuret reaction is a fairly distinctive test.

Drop into a test tube not more than one minim of a weak solution of copper sulphate, then shake this out as much as possible from the test tube. Add two drachms of the test fluid, and then an excess of caustic potash.

If pepsin has been at work then a beautiful rose pink colouration results, but if not a violet colouration is produced.

A delicate method of distinguishing the shades of colour occurred to me. If the test tube containing the fluid be held between the eye and a gas flame, so that the eye looks down the column

of fluid, rings are seen which are either rose pink or violet in colour.

Is it possible that Solution D contains the Enzyme Trypsin?

As we have already indicated trypsin has been declared to be insoluble in glycerine. We also know that the chief action of the organised ferment is to simulate this enzyme. Are we to conclude then that all results suggestive of tryptic action are dependent upon organisms? I think not, as it is still questionable whether trypsin is absolutely insoluble in glycerine, and moreover most solutions of glycerine contain water which in most cases is sufficient to dissolve a certain amount of trypsin.

In my experiments, however, I was seldom able to obtain tryptic action, but this in part might be

due to the difficulty that occurs in obtaining leucin and tyrosin in weak solutions.

The Coagulation of Milk

The coagulation of milk from the action of rennin is very marked. We must be careful not to confuse the slight clotting of milk that occurs from souring and exposure to heat.

In every case a certain amount of clotting takes place, but this is entirely different from the clotting that depends on enzymic activity.

For example, the clot that occurs after subjection of a glycerine extract of the mucous membrane of the stomach on milk is firm, and leaves above it a clear fluid. The milk was always well diluted, and sweet.

These then are a few of the difficulties that presented themselves in carrying out these experiments, which might have led to fallacies.

I have in Chapters VI and VII briefly mentioned the method of making glycerine extracts with the tests applied to each, and the difficulties to be overcome.

I shall enumerate the results of the experiments.

CHAPTER VIII.

PHYSIOLOGICAL TISSUES: ENUMERATION OF TABLES OF EXTRACTS WITH RESULTS OBTAINED AND A COMPARISON OF THESE RESULTS.

The physiological tissues were:-

1 Rabbit's Tissues

2 Child's Tissues (The child had breathed.)

3 Human Tissues removed by operation in the fresh state

4 Post Mortem Tissues - tissues which presented normal appearances, both microscopically and macroscopically.

The tissues will be taken up in the above order,-

and remarks of comparison etc. will be made after enumeration of the results obtained.

The table is divided into ten columns in all instances:-

1	First Column	-	Name of Tissue
2	Second "	-	The day the tissue was immersed in glycerine
3	Third "	-	The day the tests were applied to the extract
4	Fourth "	-	Whether starch solution is converted by X into a reducing agent - sugar
5	Fifth "	-	The appearance of fibrin and whether there is a reaction for proteoses - the fibrin being exposed to X plus water only
6	Sixth "	-	The appearance of fibrin, and whether there is a reaction for peptones (biuret) - the fibrin being exposed to X plus .2 per cent. H.Cl.
7	Seventh "	-	The appearance of fibrin and whether there is a reaction for peptones (biuret) and whether there are crystals of leucin and tyrosin - the fibrin being exposed to X plus 1 per cent. Na_2CO_3

8	Eighth Column	-	Whether a solution of cane sugar is inverted by X into a reducing sugar - dextrose and levulose
9	Ninth "	-	Whether the extract itself reduces Fehling
10	Tenth "	-	Whether milk is curdled (apart from the clotting from heat).

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Hind bones	5/12/98	25/2/99	None	No change. No proteoses
Small Intestine	5/12/98	25/2/99	Abundant Conversion	No change. No proteoses
Large Intestine	5/12/98	25/2/99	Abundant Conversion	No change. No proteoses
Blood	5/12/98	25/2/99	None	No change. No proteoses

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin. Tyrosin.	Inversion of Cane Sugar into Dextrose	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is swollen and gelatinous. Slight appearance of digestion. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin.	None	No	No
Fibrin is extremely gelatinous and partly digested. Considerable Biuret reaction	Fibrin is slightly corroded. Distinct Biuret reaction. Crystals of leucin and tyrosin and triple phosphates	None. (This is strange)	No	No
Fibrin is gelatinous and partly digested. Faint Biuret reaction	Fibrin not appreciably altered. A faint Biuret reaction, and microscopically crystals like tyrosin and Rhombic crystals like cystin	None. (This is strange)	No	No
Fibrin is highly gelatinous with appearances of slight digestion having taken place. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No crystals of leucin or tyrosin.	None	No	Faint?

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Stomach	5/12/98	25/2/99	Abundant conversion. Phenylhydrazine test showed presence of small yellow crystals	No appearance of change. No proteoses
Lungs	5/12/98	26/2/99	None	No change. No proteoses
Kidneys	5/12/98	26/2/99	Slight conversion	No change. No proteoses
Liver	5/12/98	26/2/99	Abundant Conversion	No appreciable change. No proteoses

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin and Tyrosin.	Inversion of Cane Sugar into Dextrose	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is totally dissolved. Marked Biuret reaction	No appreciable change in fibrin. No Biuret reaction. Microscopically a few needle shaped crystals	None	No	Distinct curdling
Fibrin is totally dissolved. (Confirmed on several occasions). Marked Biuret reaction	No change in appearance of fibrin. No Biuret reaction. No crystals of leucin or tyrosin	None	No	No
Fibrin is gelatinous and partly digested. Faint Biuret reaction	No change in fibrin. No Biuret reaction and no crystals of leucin or tyrosin. A few hexagonal crystals	None	No	Faint curdling
Fibrin is totally dissolved. Marked Biuret reaction	No change in fibrin. No Biuret reaction and no crystals of leucin or tyrosin. Hexagonal crystals found	Abundant inversion. (Probably to be accounted for by sugar in X).	Yes (markedly)	Distinct curdling

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Muscle	5/12/98	23/2/99	Abundant Conversion	No change. No proteoses
Pancreas	5/12/98	16/3/99	Abundant Conversion	No change No proteoses
Brain	5/12/98	16/3/99	None	No change No proteoses
Supra-renal Bodies	5/12/98	16/3/99	Considerable Conversion	No change No proteoses
Spleen	5/12/98	14/3/99	Abundant Conversion	No change No proteoses

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones Leucin and Tyrosin.	Inversion of Cane Sugar into Dextrose	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous and partly dissolved. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No crystals of leucin and tyrosin	None	No	No
Fibrin is gelatinous. No appearance of actual digestion, but distinct Biuret reaction	Fibrin is slightly corroded. Faint Biuret reaction. A few crystals like tyrosin but no leucin	None	No	Slight Curdling
Fibrin is gelatinous. Very faint Biuret reaction	Fibrin not appreciably changed. No Biuret reaction. No crystals of leucin or tyrosin	None	No	No?
Fibrin is gelatinous and partly digested. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No crystals of leucin or tyrosin	None	No	No
Fibrin is gelatinous and partly digested. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No crystals of leucin or tyrosin	None	No	No

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin, Proteoses
Heart	5/12/98	17/3/99	Slight Conversion	No change. No proteoses
Hair & Skin	5/12/98	17/3/99	None	No change. No proteoses
Eyes	5/12/98	18/3/99	None	No change. No proteoses
Spinal Cord	5/1/99	14/4/99	None	No change. No proteoses
Heart	5/1/99	18/4/99	Distinct Conversion	No change. No proteoses

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin and Tyrosin.	Inversion of Cane Sugar into Dextrose	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous and partly digested. Faint Biuret reaction	No change in fibrin. No Biuret reaction No leucin and tyrosin	None	No	No
Fibrin slightly gelatinous, but not appreciably altered. Faint Biuret reaction	No change in fibrin. No Biuret reaction No leucin or tyrosin	None	No	No
Fibrin is gelatinous, but not appreciably altered. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
CHITINOUS TISSUES				
Fibrin is gelatinous and swollen with no actual appearance of digestion. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin. (granular débris).	None	No	No
Fibrin is gelatinous and partly digested. Distinct Biuret reaction.	No change in fibrin. No Biuret reaction. No leucin or tyrosin.	None	No	No

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Muscle	5/1/99	18/4/99	Slight Conversion	No change. No Proteoses
Bone (partly ossified)	5/1/99	19/4/99	None	No change. No Proteoses
Liver	5/1/99	19/4/99	Abundant conversion. (more than accounted for by sugar in Extract) Brick Deposit	No change. No Proteoses
Thyroid	5/1/99	20/4/99	None	No change. No Proteoses
Large Intestine	5/1/99	20/4/99	Distinct conversion	No change. No Proteoses

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin and Tyrosin.	Inversion of Cane Sugar into Dextrose	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is highly gelatinous and partly digested. Distinct Biuret reaction	No change in fibrin. No Biuret reaction No leucin or tyrosin	None	No	No
Fibrin is gelatinous with no appreciable change. Very faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous and considerably digested. Marked Biuret reaction	Slight appearance of corrosion of fibrin. Faint Biuret reaction, but no leucin or tyrosin	Marked conversion (Probably from sugar in Extract) Ocre deposit	Distinct reduction (greenish-brown deposit)	No
Fibrin is gelatinous and considerably digested. Marked Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin, but needle-shaped hexagonal crystals (transparent)	None	No	No
Fibrin is gelatinous and partly digested. Marked Biuret reaction	Appearance of Corrosion of fibrin. Distinct Biuret reaction. No leucin or tyrosin	None (This is strange)	-	Faint curdling

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Skin	5/1/99	22/4/99	None	No change. No proteoses
Stomach	5/1/99	22/4/99	Faint Conversion	No change. No proteoses
Vermiform appendix	5/1/99	24/4/99	Very faint Conversion	No change. No proteoses
Lung	5/1/99	25/4/99	Considerable Conversion	No change. No proteoses
Spleen	5/1/99	25/4/99	Faint Conversion	No change. No proteoses

Appearance of Fibrin. Peptones	Appearance of Fibrin Peptones. Leucin and Tyrosin.	Inversion of Cane Sugar into Dextrose	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous with no other change	No change in fibrin. No Biuret reaction. No Leucin or tyrosin	None	No	No
Fibrin is totally digested. Extremely marked Biuret reaction	No appreciable change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	Distinct curdling
Fibrin is gelatinous and partly digested. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous and considerably digested. Marked Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyro sin	None	No	No.
Fibrin is gelatinous, but not otherwise altered. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Supra-renal Bodies	5/1/99	26/4/99	Considerable Conversion	No change. No proteoses
Brain	5/1/99	26/4/99	Distinct Conversion	No change. No proteoses
Kidneys	5/1/99	27/4/99	Considerable Conversion	No change. No proteoses
Small Intestine	5/1/99	29/4/99	Considerable Conversion	No change. No proteoses

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin and Tyrosin	Inversion of Cane Sugar into Dextrose	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous and partly dissolved. Distinct Biuret reaction	No change in fibrin. No Biuret reaction No leucin or tyrosin (amorphous matter)	None	No	No
Fibrin is gelatinous. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin (microscopically are seen globules like fat-globules)	None	No	Faint? (more than accounted for by heat alone
Fibrin is gelatinous and partly dissolved. Distinct Biuret reaction	No change in fibrin? Faint Biuret reaction. No leucin or tyrosin, but irregular crystals, and hexagonal crystals	None	No	No
Fibrin is gelatinous and partly digested. Distinct Biuret reaction	Slight appearance of corrosion of fibrin. Faint Biuret reaction. No crystals exactly like leucin or tyrosin	None	No	No

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Gall Bladder	5/1/99	29/4/99	None	No change. No proteoses
Thymus gland	5/1/99	1/5/99	Distinct Conversion	No change. No proteoses
Pancreas	5/1/99	1/5/99	Abundant Conversion	No change. No proteoses
Cartilage	5/1/99	2/5/99	Faint Conversion	No change. No proteoses
Fat	5/1/99	2/5/99	Faint Conversion (due probably to X itself	No change. No proteoses

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin and Tyrosin	Inversion of Cane Sugar into Dextrose etc.	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous, but not appreciably altered. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	Faint?
Fibrin is gelatinous and partly digested. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	Faint?
Fibrin is almost totally digested. Marked Biuret reaction	Fibrin is markedly corroded. Marked Biuret reaction. Characteristic crystals of tyrosin and leucin balls (Millon's reagent was used)	None	No	Distinct Curdling
No appreciable change in fibrin. Very faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	Faint reduction (due probably to X itself)	Faint reduction	No

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Tendo Achilles	9/12/98	3/4/99	Faint Conversion (Probably from Xitself)	No change. No proteoses
Fat	9/12/98	4/4/99	Distinct Conversion (Probably from X itself)	No change. No proteoses
Muscle	9/12/98	4/4/99	Abundant Conversion	No change. No proteoses
Cartilage	9/12/98	6/4/99	None	No change. No proteoses
Ligament & Synovial membrane	9/12/98	7/4/99	None	No change. No proteoses

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin and Tyrosin	Inversion of Cane Sugar into Dextrose etc.	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous, but not appreciably altered. Extremely faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	Faint Inversion (Probably from X itself	Faint reduction	No
Fibrin is gelatinous, and partly digested. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	Distinct Inversion (Probably from X itself	Distinct reduction	No
Fibrin is gelatinous and considerably digested. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous, but not otherwise altered. Very faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous, but not otherwise altered. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Bone	9/12/98	9/4/99	Faint Conversion (Probably from X itself)	No change. No proteoses
Skin	9/12/98	9/4/99	Faint Conversion	No change. No proteoses
Connective Tissue	9/12/98	10/4/99	Faint Conversion (Probably from X itself	No change. No proteoses
Nerve	9/12/98	10/4/99	None	No change. No proteoses

(The above 9 tissues were secure

sarcoma

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin and Tyrosin	Inversion of Cane Sugar into Dextrose etc.	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous but with no other change. Faint Biuret reaction	No change of fibrin. No Biuret reaction. No leucin or tyrosin	Faint inversion (Probably from X itself)	Faint reduction	No
Fibrin is gelatinous but with no appearance of digestion. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous but with no other change. Extremely faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	Faint inversion (Probably from X itself	Faint reduction	No
Fibrin is gelatinous but with no appearance of digestion. Very faint Biuret reaction (the quantity of tissue very small	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No

ured from a healthy leg, removed by operation for

ma of the upper end of the Femur).

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Placenta	7/12/98	5/5/99	Faint Conversion	No change. No proteoses
			HUMAN POSTMORTEM TISSUES	
Liver (No. 1) (The liver is of a paler colour than the liver below)	23/12/98	22/3/99	Considerable Conversion (more than accounted for by X)	No change. No proteoses
Liver (No.2)	21/2/98	29/3/99	Abundant Conversion (more than accounted for by X)	No change. No proteoses
Lung	21/12/98	22/3/99	Abundant Conversion (more than accounted for by X) Yellow crystals with Phenylhydrazine hydrochloride	No change. No proteoses

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin and Tyrosin	Inversion of Cane Sugar into Dextrose etc.	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous but with no other change. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No

(Microscopically and macroscopically) - Normal)

Fibrin is gelatinous and partly digested. Distinct Biuret reaction	No appreciable change in fibrin. No Biuret reaction. No Leucin or tyrosin. (Hexagonal crystals in abundance	Distinct Inversion (probably from X)	Distinct reduction	Distinct curdling
Fibrin is gelatinous and partly digested. Distinct Biuret reaction	No appreciable change in fibrin. No Biuret reaction. No leucin or tyrosin	Faint Inversion (probably from X)	Faint reduction	Slight curdling
Fibrin is gelatinous and partly digested. Very distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	Faint Inversion (Probably from X)	Faint reduction	Faint curdling

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Skin	21/12/98	24/3/99	None	No change. No proteoses
Large Intestine	21/12/98	25/3/99	Abundant Conversion (more than from X)	No change. No proteoses
Kidneys	21/12/98	25/3/99	Considerable Conversion	No change. No proteoses
Spleen	21/12/98	26/3/99	Abundant Conversion	No change. No proteoses
Muscle	21/12/98	29/3/99	Abundant Conversion (more than from X)	No change. No proteoses

Appearance of Fibrin. Peptones	Appearance of Fibrin Peptones. Leucin and Tyrosin	Inversion of Cane Sugar into Dextrose etc.	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous, but with no other change. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous and partly dissolved. Distinct Biuret reaction	Appearance of Corrosion of fibrin. Distinct Biuret reaction. Crystals of leucin and tyrosin	Distinct Inversion (partly perhaps from X itself	Distinct reduction	No
Fibrin is gelatinous and partly digested. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous and partly dissolved. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin.	None	No	No
Fibrin is gelatinous and partly digested. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	Faint inversion (Probably from X)	Faint reduction	No

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Small Intestine	21/12/98	30/3/99	Abundant Conversion (more than from X)	No change. No proteoses
Fat	21/12/98	2/4/99	Slight Conversion (Probably from X)	No change. No proteoses

	Appearance of Fibrin. Peptones	Appearance of Fibrin Peptones. Leucin and Tyrosin	Inversion of Cane Sugar into Dextrose etc.	Does Extract reduce Fehling?	Is there curdling of milk?
	Fibrin is gelatinous and partly digested. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	Faint Inversion (Probably from X)	Faint reduction	No
	Fibrin is gelatinous but with no other change. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin (There are small crystals like oxalic acid to be seen on evaporation)	Slight Inversion (Probably from X)	Slight reduction	No

I do not intend here to discuss why the results obtained in these columns have occurred, as that subject will be dealt with in Chapters X and XI, but merely to group the results together, and to see how these compare in the different classes of tissues that have been examined.

It was impossible to obtain the corresponding tissues in all cases so that I must for that reason only compare the tissues whose extracts I have obtained.

One would have liked to be able to examine portions of the human organs removed fresh from the body, but this was impossible, and consequently the tissues that have been analysed are those which naturally contain only a small quantity of enzymes.

I have used the terms abundant, considerable, distinct and faint as a means of comparing the

relative densities of the precipitate formed in the reduction of Fehling. These terms would therefore represent the relative powers of activity of the enzymes in the glycerine extract.

We shall consider the results in the order in which they appear in the columns.

A. TABLE SHOWING COMPARISON OF RESULT OF

	I Rabbit's Tissues	II Child's Tissues
Abundant Conversion	Small Intestine[x] Large Intestine[x] Stomach Liver[x] Muscle Pancreas Spleen	Liver[x] Pancreas
Considerable Conversion	Suprarenal Bodies	Lungs Suprarenal Bodies Kidneys Small Intestine
Distinct Conversion		Heart Muscle Large Intestine Brain Thymus Gland
Slight Conversion	Kidneys Heart Muscle	Muscle Stomach Veriform Appendix Spleen Cartilage Fat[x]
No Conversion	Bones Blood Lungs Brain Hair and skin Eyes	Bones Thyroid Spinal Cord Skin Gall Bladder

III Human Adult Tissues	IV Postmortem Tissues
	Small Intestine[x]
	Large Intestine
	Liver (No.2)[x]
Muscle	Muscle[x]
Placenta	Lung[x]
	Spleen
	Liver (No.1)[x]
	Kidneys
Fat[x]	
Bone[x]	
Skin[x]	
Connective Tissue	
Tendon[x]	
	Fat[x]
Cartilage	
Ligament	
Nerve	
	Skin

In some cases a larger quantity of the tissue was used than in others, so that the extract sometimes was considerably stronger than others, hence the grouping of tissues under the headings of abundant, considerable etc. does not necessarily mean that the enzyme was present in the tissue in the same proportion. Still, a hasty glance at this table shews that most of the succulent organs and tissues have the power of yielding an extract which rapidly converts starch into sugar, while the dried tissues such as bone cartilage etc. yield extracts which have no such power.

In comparing the various tissues obtained from the rabbit, child, and adult, there is on the whole a relationship in their action.

Those tissues which I have marked by an X produced extracts which had the power of reducing Fehling.

As we shall see afterwards many tissues contain-

ing glycogen yield a sugar after their death. This fact may account for these extracts reducing Fehling, but in most cases the reduction of Fehling by the extract was slight as compared with the reduction by the starch solution previously acted on by X. Consequently, I believe that in most cases at least there is at work an enzyme of the nature of ptyalin or amylopsin in the tissues themselves.

It is in these cases where an extraction has been made from a tissue which is not fresh, such as in the post mortem tissues, that the extract itself has this reducing power most markedly.

(B) All tissues behave alike in yielding extracts which with water alone cause no change on fibrin, and when the solution is filtered and tested with ammonium sulphate, there is no precipitate which shews the presence of proteoses.

(C) All the physiological tissues that were

obtained from the rabbit or child, or from the adult during life or after death, have the power of more or less dissolving fibrin in a .2 per cent. solution of H.Cl. and of yielding a solution of peptones which give the biuret reaction.

This result cannot be due to the conversion of proteids into albumoses etc. by the acid itself, as fibrin subjected to the action of 40 cc of .2 per cent. H.Cl. causes it to swell up, but not to be dissolved.

In no cases could a biuret reaction be obtained from such a solution.

Some of the tissues have the power of dissolving fibrin more markedly than others. The chief of these are:-

Rabbit	Child	Adult	Post Mortem
Small Intestine	Large Intestine		
Stomach	Stomach		
Lungs	Lungs		Lung
Liver	Liver		Liver
Muscle	Muscle	Muscle	Muscle
	Thyroid		
	Pancreas		

It will be seen that those tissues which have the greatest power of digesting fibrin correspond pretty closely in the different groups, and moreover they correspond in great part to those tissues which yielded an extract which caused abundant conversion of starch into sugar.

(D) It will be observed how extremely seldom there was any digestion of fibrin when the extract was in an alkaline solution. The only cases in

which this occurred were:-

Rabbit	Child	Adult	Post Mortem
Small Intestine	Small Intestine		
Large do	Large do		Large Intestine
Pancreas	Pancreas		
	Liver		

These results open up two questions:-

1st As the reactions are so uniformly present in the intestines, and in no other tissues, except pancreas and liver, do the results depend on organisms with their liberated ferments or on an enzyme that is present in the tissues of a nature similar to trypsin of the pancreatic juice?

2nd Is the proteolytic ferment of the pancreatic juice soluble in glycerine, provided that the results do not depend upon organisms?

I do not intend to discuss these questions here

as they have been partly entered into already and are likely to be brought up again. Still, I feel bound to admit that with the exception of the large intestine obtained post mortem, in which tissue organisms are likely to be present, I do not see how the other results can depend on bacteria, as the tissues were in every instance cleansed in running water before extraction and were absolutely fresh.

The question would have been settled had antiseptics been used. In consequence, I am unable to oppose the views of Kühne and his school, or to agree with those of Hufner, although I feel that even with the use of antiseptics the same results would have probably occurred.

With regard to the second question there is not the slightest doubt that the glycerine which was used had the power of extracting a small quantity of trypsin, as the extract of the pancreas dissolved fibrin with the formation of peptones and

crystals which I take to be leucin and tyrosin.

Glycerine, however, extracts trypsin in small amount, and the solution obtained when placed with fibrin produces only a small quantity of peptones, and rarely crystals of leucin and tyrosin. To obtain a strong solution of trypsin one would have to adopt another method for its extraction, or to use a very watery solution of glycerine.

I do not think that it is at all likely that trypsin exists in many of the tissues, and the probability is that the proteolytic enzymes of the tissues is one which is similar in nature to pepsin.

(E) The only tissues where there appeared to be inversion of cane sugar into dextrose were:-

Rabbit	Child	Adult	Post Mortem
Liver[x]	Liver[x]		Liver Nos. 1[x] and 2[x]
Pancreas			Lung[x]
	Fat[x]	Fat[x]	Fat[x]
		Bone[x]	Large Intestine[x]
		Connective Tissue[x]	Muscle[x]
		Tendon[x]	Small Intestine[x]

(F) Paragraph E proves very little, for all tissues marked with a cross formed extracts which reduced Fehling.

I believe that in all cases, the results depended probably upon the sugar that was present in the extracts, and on no ferment action. The only extract that appeared to invert sugar without a doubt was that of the pancreas of the rabbit. The

pancreas is not supposed to have an invertive ferment, at least in the human subject, but this result obtained from the pancreas of the rabbit was most marked. In no case did I obtain reactions shewing the presence of inversin in the intestine.

(G) I endeavoured to see whether the tissues contained an enzyme similar to rennin.

One had to contend against the difficulty of mistaking true curdling for the clotting of milk which occurred on exposure to heat or from souring.

The only extracts which produced curdling of milk or a suspicion of it were:-

Rabbit	Child	Adult	Post Mortem
Blood?	Thymus?	Placenta	
Stomach	Stomach		
Kidneys?	Gall Bladder?		
Liver			Liver Nos. 1 & 2
Pancreas	Pancreas		Lung
	Large Intestine		
	Brain?		

The result was distinct in the cases of the stomach and pancreas of the rabbit and child: in the large intestine of the child: in the lung and liver (post mortem). The other results were doubtful.

CHAPTER IX.

PATHOLOGICAL TISSUES: ENUMERATION OF TABLES OF EXTRACTS, WITH THE RESULTS OBTAINED AND A COMPARISON OF THESE RESULTS.

I had hoped to have been able to make extractions of a large number of sarcomatous and carcinomatous tumours, but from the difficulty of obtaining these I have only been able to examine a few.

In some cases the portion of tissue was small, and consequently the extract had to be of small quantity, unless materially weakened by glycerine.

Besides the sarcomatous and carcinomatous

tumours, I have made extracts of some of the tissues obtained from a patient who died of eclamptic convulsions during pregnancy.

As so much doubt exists at present on the pathology of this disease, I considered it advisable to make extraction of the blood (during life), and of the various organs (post mortem), in order to see whether any light could be thrown upon the probable nature of the disease from this standpoint.

Extracts of varicose veins and tubercular sputum were also made.

The pathological tissues were:-

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses	
Carcinoma of skin (infected by cancer of Pylorus	5/12/98	5/5/99	Distinct Conversion	No change. No proteoses	
Scirrhus of Breast	8/12/98	7/5/99	Distinct Conversion	No change. No proteoses	
Sarcoma of Face	1/4/99	23/6/99	Faint Conversion	No change. No proteoses	
Angei-Sarcoma of leg	16/3/99	8/5/99	Distinct Conversion	No change. No proteoses	

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin and Tyrosin	Inversion of Cane Sugar into Dextrose etc.	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous with appearance of partial digestion. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin.	None	No	Faint curdling
Fibrin is gelatinous and partly digested. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous and partly digested. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	Distinct Inversion	No	No
Fibrin is gelatinous, but with no other change. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses
Blood (Eclampsia)	15/3/99	13/5/99	Abundant Conversion	No change. No proteoses
Liver (Eclampsia	15/3/99	13/5/99	Abundant Conversion	No change. No proteoses
Pancreas (Eclampsia	15/3/99	13/5/99	Abundant Conversion	No change. No proteoses

pearance of fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin and Tyrosin	Inversion of Cane Sugar into Dextrose etc.	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous and considerably digested. (It is difficult to test the Biuret reaction as the colour of the Hb. acid on by the Hb prevents this test)	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous and partly digested. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous and considerably digested. Marked Biuret reaction	Fibrin is totally dissolved. Marked Biuret reaction. Crystals of leucin and tyrosin	None	No	No

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses	
Spleen (Eclampsia)	15/3/99	16/5/99	Abundant Conversion	No change. No proteoses	Fib o B
Brain (Eclampsia)	15/3/99	16/5/99	Abundant Conversion	No change. No proteoses	Fib o o Fi a
Kidneys (Eclampsia)	15/3/99	17/5/99	Abundant Conversion	No change. No proteoses	Fib o t M a
Varicose Veins	7/12/98	6/5/99	Faint Conversion	No change. No proteoses	Fib o o Fi a

Appearance of Fibrin. Peptones	Appearance of Fibrin. Peptones. Leucin and Tyrosin	Inversion of Cane sugar into Dextrose etc.	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous and partly digested. Distinct Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous, but with no other change. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous and almost totally digested. Marked Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No
Fibrin is gelatinous, but with no other change. Faint Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	None	No	No

Name of Tissue	Preparation of Extract	Tests applied	Conversion of starch into sugar	Appearance of Fibrin. Proteoses	
Tubercular Sputum	7/12/98	7/5/99	Faint Conversion (Probably from Ptyalin in saliva)	No change. No proteoses	Fi

Appearance of Fibrin. Peptones	Appearance of Fibrin Peptones. Leucin and Tyrosin	Inversion of Cane Sugar into Dextrose etc.	Does Extract reduce Fehling?	Is there curdling of milk?
Fibrin is gelatinous and partly dissolved. Marked Biuret reaction	No change in fibrin. No Biuret reaction. No leucin or tyrosin	Abundant Inversion (with Phenylhydrazine hydrochloride are small yellow crystals not unlike those of the osazones)	No	No

In comparing the results obtained from the pathological tissues, I intend to follow the same order as was done with the physiological.

(A) Table shewing the comparison of results obtained in the conversion of starch into sugar.

Abundant - (Blood) (Liver) (Pancreas) (Spleen) (Brain) (Kidneys) Eclamptic Tissues

Considerable

Distinct - (Carcinoma of Skin (Scirrhus Cancer of Breast (Angeiosarcoma of the Leg

Slight - (Sarcoma of Face (Varicose Veins (Tubercular Sputum

No Reaction

All the pathological extracts have the power of converting starch into sugar.

Cancers and sarcomas do this pretty markedly, while the various extracts of tissues that were examined from the patient who suffered and died from eclampsia have a very powerful action in this respect.

One cannot say definitely that cancerous and sarcomatous tumours yield extracts which invariably convert starch into sugar, as a sufficient number has not been examined. The probability is, however, that this is so, and, moreover, soft or medullary carcinomata, and soft round or giant celled sarcomata will probably have a greater power in causing this conversion than the hard scirrhus cancer or spindle celled sarcoma.

Why should the tissues in eclampsia yield extracts which have such a powerful action in the conversion of starch?

It is not that they contain more glycogen, as the extract itself would have in that case re-

duced Fehling. Again, it is not probable that putrefactive organisms have had to do with this result, as in that case one would have expected something akin to tryptic digestion, which was always absent. Is there a special organism in this disease which has such a power, or, do the results depend upon the liberation of enzymes from the tissues in a greater abundance than exist normally? We shall return to this again. The tubercular sputum has a faint reaction in the conversion of starch. This result probably depends upon an organised ferment that is liberated after death from the pyogenic organisms which are present in such a sputum.

The result, of course, might depend upon the ptyalin in saliva.

(B) The extracts of all the pathological tissues had no reaction on fibrin in a solution of water alone.

(C) In a solution of .2 per cent. H.Cl. all the pathological extracts caused a partial digestion of fibrin with production of peptones which gave the biuret reaction. Certain of the extracts obtained from the tissues of eclamptic, as from the kidneys and pancreas, had this digestive power in a marked degree. The extracts of the tumours also dissolved fibrin, but in a less marked manner.

(D) With the exception of the pancreas the pathological tissues in no instance yielded extracts which had the least action on fibrin in a 1 per cent. solution of Na_2CO_3. This to some extent is a proof that putrefaction had not set in, and that the formation of peptones by X in a solution of H.Cl. was not due to bacteria.

The extract of the pancreas dissolved fibrin in a solution of 1 per cent. Na_2CO_3, with the formation of peptones, leucin and tyrosin, which is a proof that the proteolytic enzyme of the pancreas

was in this case at least partially soluble in the glycerine that was used.

(E) In only two cases was there inversion of sugar vizt. in the sarcoma of the face and in tubercular sputum.

One cannot readily account for the former result, but in the latter case this depends probably on an organised ferment.

The result is similar to that obtained from yeast. When the yeast is killed an organised ferment is liberated which is extracted by glycerine and which inverts cane sugar into dextrose as in the present instance.

(F) None of the pathological extracts reduced Fehling.

(G) The extract of the pancreas obtained from the eclamptic caused distinct curdling of milk. A doubtful reaction was obtained also in the case of

the carcinoma of the skin, but with these two exceptions there was no reaction obtained suggestive of enzymic activity.

CHAPTER X.

A COMPARISON OF THE RESULTS OBTAINED FROM THE ANALYSES OF PHYSIOLOGICAL AND PATHOLOGICAL EXTRACTS WITH REMARKS ON STRIKING REACTIONS

The best comparison can be made by careful study of the tables, but in order to appreciate these results more quickly, I shall briefly consider each reaction.

I. All the physiological tissues which were of a soft nature yielded extracts which converted

starch into sugar. This was most marked in the case of the intestines, pancreas, muscle, and post mortem tissues, while tissues such as brain, nerve, bone, cartilage, and ligament had seldom this power.

All the pathological tissues again, caused the conversion of starch into sugar in a considerable degree. This was most marked in the eclamptic tissues.

As a rule the same tissues whether in a physiological or pathological state had the same power, and almost the same degree of power in converting starch into sugar.

II. The extract itself in presence of water had no action on fibrin, whether it was made from a physiological or pathological tissue.

III. Every tissue that was examined, whether physiological or pathological, had the power of dis-

solving fibrin in some degree at least, in presence of .2 per cent. H.Cl.

This was very marked in the case of the stomach, liver and muscle, from the physiological series; and in that of the pancreas and kidneys from the pathological.

IV. Although results were obtained in several instances shewing that certain physiological extracts had the power of digesting fibrin in a solution of 1 per cent. Na_2CO_3, on no occasion, except as before stated in the case of the pancreas, was there any such result from pathological tissues.

Of the physiological tissues, however, which gave reactions, proving the probable presence of a proteolytic enzyme, the intestines almost alone gave such results, so that the probability is that had the intestines been examined in disease as well, there would have been results corresponding to tryp-

tic action in a similar way, and which were not necessarily dependent on organisms.

V. Although many of the physiological extracts, especially from the post mortem group, had the power of reducing Fehling themselves, this did not occur in a single instance from the pathological extracts.

No difficulty in the latter case existed in observing the inversion of cane sugar into dextrose and levulose. This inversion was extremely marked by the extract of the tubercular sputum.

VI. Several of the physiological extracts curdled milk, the chief of these being stomach, large intestine, pancreas and liver. The only pathological extracts that had this power were carcinoma of the skin and the pancreas from the eclamptic.

The general comparison is very similar in the two classes of tissues.

I shall now enumerate shortly some of the more striking results with remarks on each.

1. Glycerine extracts as we have seen were made from large and small intestine of rabbit, from the small and large intestine of child, and from the small and large intestine of the adult obtained post mortem. In none of these cases was there a reaction shewing the presence of inversin. Why was this?

It has been proved by Paschutin that this enzyme can be obtained more effectively from the mucous membrane of the intestine than from the juice itself.

Is it possible that such an enzyme is not present in rabbit or child's intestines?

In the extract of the intestine obtained post mortem it was impossible to say what the reduction

of Fehling was due to. It may have been due to the extract itself, and not to an inversion of the cane sugar.

Another possibility is that glycerine may not extract the enzyme inversin as it does most other ferments. In any case no reaction was obtained shewing the presence of inversin in the intestines of the rabbit or child.

All the intestines examined had the power of yielding extracts which with .2 per cent. H.Cl. had a marked action on fibrin.

The same extracts had no action or only doubtful action in alkaline solutions.

If we lay aside the action of organisms, which if they had been present would have caused digestion of fibrin in alkaline solutions, we have to conclude that the digestion is due to a ferment of the nature

of pepsin which acts in an acid medium.

Is it not probable then that a proteolytic ferment is secreted from the intestinal mucous membrane which is related closely to pepsin? Of course, in physiological conditions pepsin would not exert its influence in the process of digestion as the intestinal juice is alkaline.

We know that a juice is secreted from the upper part of the duodenum which contains pepsin. I think it quite probable that such an enzyme may exist along the whole intestinal mucous tract.

Again, it was extremely easy to obtain by glycerine extraction the enzyme corresponding to ptyalin or amylopsin.

In all cases this enzyme was extremely active. It is possible that in the child there is more use for this enzyme than in adults, as ptyalin of the saliva and amylopsin of the pancreatic juice may

not be present in sufficient abundance at such an early age, while in the case of the rabbit there is a greater necessity for such a ferment, as the diet is so starchy.

2. Glycerine extracts of the stomach of both rabbit and child not only gave reactions shewing presence of pepsin and rennin, but also ptyalin or amylopsin.

The conversion of starch into sugar in both these cases was very marked.

I have not noticed in any text book mention of ptyalin having been obtained by extraction of the mucous membrane of the stomach. This also may be a peculiarity of the stomach of the rabbit, and that of the child; but it will be important in future to see what effect a glycerine extract of a well wasted mucous membrane of an adult stomach has upon starch.

3. A very interesting result was obtained from the extract of the rabbit's lung. With 40 cc of .2 per cent. H.Cl. the extract caused 1 grm. of fibrin to become totally dissolved in a short time. The same result, although in a manner less marked, was obtained from the extract of the child's lung.

It seems strange that the lung of the rabbit should possess this power so markedly. Fibrin was digested by the extract of the lung as completely as by the extract of the stomach.

Does the lung then contain pepsin in almost as active a form as it exists in the stomach?

The exact significance of such a result, I cannot at present understand.

4. Glycerine extracts of the pancreas of rabbit and child, and also of the pancreas from the eclamptic, gave reaction which shewed the presence of pepsin in considerable amount.

The fibrin was always totally dissolved in the acid solution.

Does the pancreas then also contain pepsin?

The pancreatic juice destroys the action of pepsin as it is alkaline, and consequently even though pepsin be present, it has no influence in the digestion of food stuffs in the intestines. Still, it may be present in the pancreas all the same, and only exert its influence in certain forms of disease, or possibly when the intestinal juice becomes acid.

5. Glycerine extract of the liver invariably reduces Fehling, probably from the conversion of glycogen into a reducing sugar. In all cases, however, the reduction obtained by the starch solution, previously acted on by the extract was greater than that from the extract itself.

I believe then that in the liver there is present an enzyme that corresponds to ptyalin. There is also present an enzyme that corresponds to pepsin.

In two instances viz. liver of rabbit and liver post mortem, there was curdling of milk produced by the action of the extract. In no cases was there a reaction suggesting tryptic activity.

6. A glycerine extract of the blood of the rabbit, physiologically normal, had no reaction in the conversion of starch into sugar. On the other hand, the extract obtained from the blood of the eclamptic very rapidly converted starch into sugar. (This latter result we shall discuss in Chapter XII.)

7. All the extracts obtained from the tissues of the eclamptic had the power of converting starch into sugar very markedly, and also of partially digesting fibrin, while, with the exception of the

pancreas, they had no action in alkaline solutions. These reactions must depend upon an altered condition of tissues in this disease producing a greater quantity of active enzymes.

8. It is extremely interesting to note that a glycerine extract of tubercular sputum has a marked inversive action. It has a faint power in the conversion of starch into sugar (probably from ptyalin in saliva), and in the digestion of fibrin in an acid medium. Both these reactions are slight as compared with the inversive power.

As I have mentioned before the result is probably due to the liberation of an organised ferment from the pyogenic or other organisms which exist in sputum after their death.

9. Carcinomata and sarcomata yielded extracts which converted starch into sugar quite distinctly,

and also which digested fibrin slightly in an acid medium.

CHAPTER XI.

A DISCUSSION ON CERTAIN PROBLEMS THAT ARISE IN THIS RESEARCH

When a glycerine extract of a particular tissue has the power of converting starch into a reducing agent, I have always said that it converts starch into sugar.

Is this substance formed by the action of the extract upon starch really sugar?

The starch itself does not reduce Fehling, and if the Fehling is pure no reduction can take place

between the two fluids.

When the extract acts upon the starch, it frequently causes it to alter in character and to reduce Fehling.

Could starch be converted into any other substance that could reduce Fehling, and yet was not sugar? I know of no such substance, and the probability is that the reduction depends upon the formation of sugar.

In what form is the sugar likely to be in?

When ptyalin or amylopsin acts upon starch, we have seen that maltose is the chief substance formed, although a considerable quantity of dextrose is also formed after continued action. In the same way the presumption is that the starch has been converted into a sugar which is chiefly maltose. To prove the presence of sugar more satisfactorily

phenyl - hydrazine-hydrochloride and acetate of soda were added to the fluid: the mixture was boiled for half an hour and cooled gradually. By this process none of the characteristically formed crystals of phenyl maltosazone or phenyl glucosazone were formed, although small yellow crystals of an almost amorphous nature were invariably present.

It is quite probable that the small yellow crystals observed were those of phenyl maltosazone only partially crystallized, and the conclusion I have arrived at is that the extracts which have caused a starch solution to reduce Fehling have also caused the conversion of starch into maltose.

If this be so, what has existed in the tissue which has this power?

Just as saliva contains an enzyme which converts starch into maltose, in the same manner,

certain of the tissues contain a soluble ferment or enzyme which has this power. In every case where starch has been converted by the extract into a reducing sugar, provided that the result did not depend upon the presence of sugar in the extract itself, I consider that this result depended upon the activity in the tissues of an enzyme which was identical with ptyalin or amylopsin.

In an earlier chapter, I referred to the remark by Sheridan Lea which was that he believed that ptyalin was not only present in the saliva, but probably in every tissue or fluid in the body. This statement I can partly confirm.

More than half the tissues of the body yielded extracts which contained ptyalin or amylopsin in a greater or smaller amount.

In those cases, however, where the extract itself reduced Fehling the probability is that glyco-

gen existed in the tissues and became converted after death into sugar by the activity of the enzyme.

Glycogen exists in all embryonic tissues, and more especially in the liver, muscle and placenta.

In making the glycerine extract therefore, a portion of the glycogen or the already formed sugar is taken up, and when the extract is subjected to the action of heat the probability is that more of the glycogen is converted into sugar. Consequently such extracts reduced Fehling.

When the extract acted on fibrin in presence of water alone, why was there never any reaction for proteoses?

Proteoses are intermediate bodies in the conversion of proteids (fibrin) into peptones.

Had the extract contained organised ferments yielded from putrefactive bacteria, then the pro-

teid substance in all probability would have been dissolved into peptones, especially after 24 hours' subjection in incubator. In that case, little or no reaction would be obtained with ammonium sulphate, and yet there would be abundant evidence of peptones. In the same way, even though pepsin was present in the extracts, it would have little or no action on the fibrin in a neutral solution, and consequently the fibrin would remain unaltered. That is probably what has occurred in the present experiments.

Pepsin was always present in certain amount, and yet it had no appreciable action on fibrin in a neutral fluid.

In many cases, when there was no reaction for proteoses, the biuret test was applied and with no result.

As all the tissues were proved to have the

power of more or less digesting proteid in presence of a solution of .2 per cent H.Cl. we have to consider whether this depended upon the activity of an enzyme of the nature of pepsin.

Strong acids have the power of dissolving fibrin to a considerable extent, but a solution of .2 per cent. H.Cl. has little or no such action, and no biuret reaction is obtained on testing the filtered solution.

I have no hesitation in saying that the extracts which had the power of digesting fibrin in presence of .2 per cent. H.Cl. contained an enzyme similar to the pepsin of the gastric juice.

The rarity of the formation of the leucin and tyrosin when the extracts of the tissues acted on fibrin in an alkaline solution depended on three conditions:-

I The difficulty of obtaining leucin or tyrosin under any circumstances unless the enzyme trypsin is present in considerable abundance.

II The question of the solubility of the enzyme trypsin in glycerine.

III The absence of the action of organisms (putrefactive).

I believe that a proteolytic ferment is present in every tissue, which resembles pepsin, but not trypsin. Of course, in the pancreas, a proteolytic enzyme exists which acts in an alkaline medium.

The action of organisms closely simulates the action of trypsin, but as no results were obtained which approached the action of this ferment we may conclude that they were rarely or never present.

Peptones, leucin, and tyrosin, were obtained from a solution of fibrin that had been submitted to the pancreatic extract, so that consequently glycerine had the power of taking up this extract,

although the solubility of the enzyme as we have already stated, may have depended upon the water in the glycerine used.

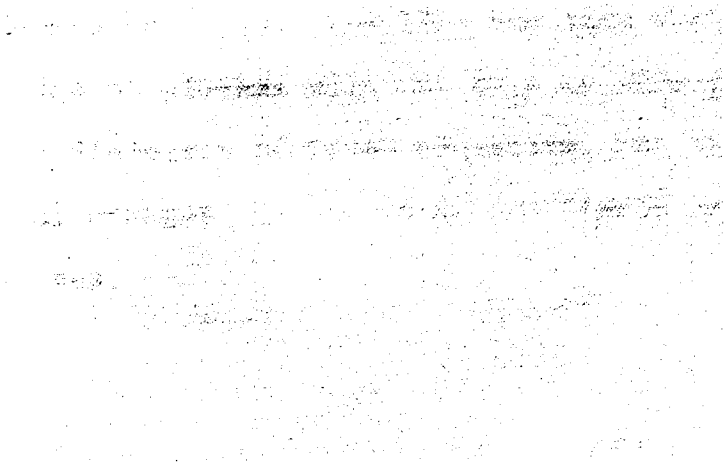
None of the extracts that were examined could be said definitely to yield a reaction which depended upon the activity of the enzyme inversin.

In many cases there was a reduction of Fehling produced by the action of the extract on the cane sugar solution, but this in most cases could be accounted for by the reducing power of the extract itself.

Tubercular sputum, however, had a distinct inversive power, but this may have been due to an organised ferment as has been already said, liberated from the organisms, that abounded in the sputum. The sputum contained the tubercle bacillus, the staphylococcus and streptococcus in large numbers.

Again, did any of the tissues contain an enzyme resembling rennin?

There was distinct curdling of milk in several instances, which, I believe, was due to rennin. One is apt to be confused with the clotting of milk that results from prolonged heat, but with comparative tests this difficulty can be overcome.



CHAPTER XII.

IS OUR KNOWLEDGE OF DISEASE HELPED IN ANY WAY BY THE ANALYSIS OF THE EXTRACTS OF PARTICULAR TISSUES?

It was indeed this question which first occurred to me in considering a subject for my thesis, as it seemed to me that the time has come when our knowledge of disease will not only be strengthened by the discovery of fresh organisms, but by the chemical changes that occur in the tissue cells themselves.

Bacteriology is a science which has sprung up practically within recent years, and the fact has now been proved that many diseased states depend upon the activity of particular germs, shewing of what infinite value this science has been in establishing our knowledge of disease on a strictly firm basis.

We can study the life history of these germs, and observe in what medium they grow best in; we get to know their rate and form of multiplication: and how they are affected by warmth, moisture, sunlight and various antiseptics.

With such a knowledge we are able to diagnose and treat specific ailments with a fresh light, and form a better judgment of the prognosis of the disease.

I do not say that the science of bacteriology

is now completely known. I believe that many bacteria will yet be observed which will throw fresh light on certain obscure diseases, but at the same time, I feel sure that our knowledge of disease will be greatly benefited by the investigations of chemical physiologists and pathologists.

Chemical physiology is a science that has advanced with rapid strides within recent years. The investigations, however, have always been more or less limited to the chemical changes that occur in the cells, and have to do with the processes of secretion, excretion, assimilation and metabolism. Disease is a deviation from health in the functions of the body, so that before one can study the chemistry of diseased tissues, he must have a thorough knowledge of the minute changes that occur in the physiological tissues, to have a comparison to go upon.

Even from our present knowledge of chemical physiology, however, there is now opened up to the pathological chemist a large field for original research in the study of disease. Our knowledge of the aetiology and pathology of many diseases is still very vague, and even with this, we are apt to spend much time in studying the nature and appearance of the diseased tissue, as it exists, without endeavouring to find out ab initio, what is the cause of the disease.

Histology shews us how a tissue appears when it is diseased. By the science of histology we can see the changes that are present in the cells, blood vessels and nerves, and also the sprouting of new tissues: we can discuss the significance of patches of inflammatory exudation, and by observing the relationship that exists between them and the bloodvessels, nerves and tissues, we can note the changes of degeneration, it may be, that are

present in the vessels, and any alteration that may have occurred in the organs and tissues.

New methods of staining shew us the course of nerve paths, lymph channels, arteries and veins, which assist us not only in considering the physiology of the tissue or organ, but also its anatomy. As a general rule, it may be said quite fairly that histology shews us how a tissue appears after it is once diseased, while it seldom proves definitely the pathological cause of the disease. Histological pathology then diagnoses, but rarely throws much light on the etiology of disease.

We see a tissue under the microscope, and we call it at once a cancerous tissue, but the question still remains as to what is the cause of the cancer. It is in such cases that so much can be done by chemical pathology.

Clinicians and scientific physicians are rapidly seeing the importance of making an accurate chemical analysis of the secretions and excretions during illness. In disorders of the stomach, analysis of the gastric contents are almost invariably made, so as to see whether H.Cl. is in proper proportion or is perhaps absent: whether there is the formation of lactic, butyric, or acetic acids: and whether pepsin exists in the juice in sufficient amount. In this way, any departure from the normal physiological state is observed, and treatment is suited accordingly. Similarly, analyses are made of the faeces and urine, and any deficiency or increase in certain elements, or the presence of entirely new substances which do not exist in health, can be observed from day to day. From such analyses, the abnormal condition can be frequently rectified by treatment. Physicians are thus able to obtain reliable records of what is probably tak-

ing place in the tissues and organs of the body.

Clinicians, however, cannot do all, and it remains for the chemical pathologist to investigate the juices and organs in diseased states, and, if necessary, to inject those juices or tissue extractions into animals and observe the results.

Halliburton and Mott[1] have recently been making observations in this way. They have shewn that the blood of patients suffering from general paralysis of the insane, when removed by vivisection during epileptform convulsions, contains the alkaloid choline, which is a derivative of lecithin.

They have also shewn that the alcoholic extract of the blood removed from a patient suffering from Beri-Beri, when injected into the external jugular vein of a cat caused fall of blood pressure. They

(1) Proceedings of the Royal Society - 1899. British Medical Journal, July 29th 1899.

considered that this blood contained a substance like choline.

It is by such experiments that our knowledge of disease will be increased.

In any obscure pathological disease, I consider that when it is possible a small quantity of the blood should be removed, and that a glycerine or alcoholic extract should be made from it.

The extract should be analysed in order to see what substances it contains, while a portion of it should be kept and divided into two equal parts. Of these parts the first should be examined chemically as I have done, while the second should be injected into a rabbit or other animal. One would thus know what substances were in the blood, the proportion of haemoglobin and cells (red and white), the presence of organisms, the specific gravity, the

reaction on starch solutions and fibrin, and lastly the actions produced on the respiratory, circulatory and other systems by its injections into a rabbit. One would know in what proportion sugar existed in the blood, and whether it contained soluble ferments, and if so whether these ferments were derived from the organisms that were present in the blood, or were merely due to the enzymic activity of certain of the tissues or of the leucocytes that were circulating in the blood serum.

The various toxaemias might be accounted for by the formation of a larger quantity of a particular enzyme that existed normally in the blood, or of a new enzyme which was formed in the particular disease. By injection into animals one could observe whether symptoms were produced in the animal of a similar nature, and whether there were signs proving that the injected fluid contained an active poison.

The analyses of the blood is merely one aspect of the question, but as so many pathological conditions are vaguely spoken of as toxaemic, it naturally is a very important one. Our knowledge of the toxicity of the blood will be materially helped by experimenting on such a line.

The toxines which exist in the blood irritate all the organs through which the blood passes.

Irritation of the brain causes symptoms to develop which can at once be recognised, hence it is that convulsions and coma so frequently arise as the result of a general toxaemia. In uraemia, an active poison exists in the blood which is not sufficiently rapidly eliminated from the system, and as a consequence there is the production of general convulsions of a marked type. The precise nature of this poison is not yet known.

Convulsions likewise occur as a result of

diseases of the stomach and intestines (tetany) from the absorption of poisons liberated either from the tissues as enzymes, or from organisms as ptomaines or organised ferments. Similarly, in eclampsia and in several of the acute fevers, not to speak of epilepsy, convulsions occur which are the result of brain irritation, probably arising from the toxines that are present in the blood.

Convulsions depending on actual organic lesions of the brain, as in the various forms of Jacksonian epilepsy, belong to a different class as the irritation depends upon the presence of the lesion quite apart from the blood conditions, although in many cases of Jacksonian epilepsy no such lesions can be found.[1] When no lesion is recognised post

(1) A case of Jacksonian Epilepsy is described by the author, in which no brain lesion could be found after death - Brain, Autumn number 1899.

mortem in cases of Jacksonian epilepsy two views have been mentioned as the probable cause of the brain irritation. Firstly that there is a chemical change occurring in the brain cells of the area affected, and secondly, that there is a vaso-motor disturbance of the motor zone.(1)

It seems to me that the aetiology of certain obscure diseases, and the probable pathology of several of the symptoms as they arose, might be more fully understood by making an analysis of the blood in all diseases.

An excessive ferment action in the blood, although our knowledge at the present time cannot quite see the significance of it, may be of the greatest importance in the causation of the toxicity of the blood. We do not necessarily require

(1) See references in the article by the Author on the writings of Hughlings Jackson.

then to find the organisms in these conditions, as it may not be present. We may prove, however, that a ferment exists which may have arisen from an organism in the blood or from the tissues and blood cells.

Again, when a portion of the glycerine extract is injected into an animal and produces symptoms quite comparable with those of the patient from whom the blood was removed, it is a positive proof that the actual disease is present in the substance injected. But, again, all organs should be examined in the same manner after death.

A minute chemical change occurring in the cells of a particular tissue, may cause the production of poisonous substances which if absorbed will produce definite symptoms. As we have already said, the destruction of brain tissue which contains lecithin yields choline which when inject-

ed into an animal causes a marked fall of pressure and has distinctly poisonous properties.

We know that cells are capable of yielding nuclein bodies which have most powerful poisonous actions. They are said to cause the coagulation of the blood almost instantaneously when injected into animals even in infinitesimal amount. If these nuclein bodies could exert their action on a localised portion of the blood column without affecting the general stream, they would be of exquisite value in the treatment of aneurism.

In diabetes, we know that carbohydrates undergo change and pass as sugar into the urine.

In some cases even after an entire cessation from starchy and sugary foods there is still elimination of sugar in the urine and sometimes in very large quantities.

This sugar does not depend merely upon the proteids that we consume, but on the conversion of the starchy and albuminous substances that exist in the cell tissue. In severe cases of diabetes destruction of tissues is constantly at work.

We notice the wasting of the patient's body, and the presence in the urine of an enormous increase in the quantities of urea and uric acid, resulting from the rapid destruction of the nitrogenous substances in the tissues. As a result of this disease the blood becomes toxic.

It acts on the brain centres and produces a condition of coma. It is impoverished and gives rise to ulcers and carbuncles on the skin, and it renders the tissues susceptible to the lodgment of various organisms, more especially for the tubercle bacillus.

But why does the blood become toxic in diabetes?

Is it the result of the increase in the blood of urea and uric acid, or is it possibly due to its being saturated with sugar?

Modern views are against both these possibilities. The prevalent idea is that the poison depends upon acetone whose formation in the blood as mentioned by Halliburton[1] depends upon the hydrolysis of sodium ethyl di-acetate. The formation of acetone is represented by the following chemical equation:-

$C_6H_9NaO_3$	$2\ H_2O$	=	C_3H_6O	C_2H_6O	$Na.H\ CO_3$
Sodium ethyl di-acetate	Water		Acetone	Alcohol	Sodium Hydrogen carbonate

Acetone, then, is considered to be the poison which produces the comatose state that so frequently

(1) Text Book of Chemical Physiology and Pathology p. 34

occurs towards the end of diabetes mellitus.

It is curious, however, that injections of acetone into animals rarely produce diabetes.

Diabetes is said to result from disorders of the pancreas and liver. The pancreas is supposed to contain a glycolytic ferment which prevents a too rapid formation of glycogen into sugar. This theory is supported by the fact that complete extirpation of the pancreas or complete destruction of its substance observed after death, destroys this property, and diabetes results. The liver is considered to convert its glycogen into dextrose in too great amount, depending upon some disorder of the nervous mechanism.

In this connection it is a curious fact that business men who are worried with anxiety, frequently suffer from glycosuria, and it is indeed a common

occurrence for disordered states of the brain, with or without convulsive phenomena, to be associated with sugar in the urine.

I mention these points with regard to diabetes as I consider that this also is a disease whose pathology is obscure, and where an examination of the extract of the blood and its injection into animals might lead to facts of immense importance.

Is it not possible that in cases of diabetes ferments of the nature of ptyalin are too actively at work, and that they may exist in the blood and in the tissues and there cause an alteration in the chemistry of the cell with the liberation of poisonous products?

This is merely one of those cases where the pathology of the disease is doubtful, and where such an examination might throw fresh light upon it.

I have not been able to procure the blood or the tissues after death in many diseased states, so that these remarks are chiefly intended to lay open paths for future investigation. I have been able, however, to make extractions from several malignant growths, as well as from the tissues and blood in a case of eclampsia, and as these two diseases depend upon pathological conditions which are by no means yet settled, I intend to enter into the question of the aetiology and pathology of tumours and eclampsia at some length.

Carcinomata Etc.

The aetiology and pathology of sarcomatous and carcinomatous tumours are far from being settled. Physiologists and pathologists on every part of the world's surface are engaged in ascertaining

the precise nature of cancer. After various fruitless attempts investigators cease their experimentations and there is a lull for a time in the pursuit of this difficult question.

Quite recently, owing to a series of articles that were written for the Practitioner, and owing to the recent advances that have been made by Plimmer and others, the world is again in a state of excitement as to the nature of cancer.

Various views have been expounded and various results have been obtained by different observers. Not one, however, has been able to bear the repetition of all observers, so that still we are doubtful of its pathology. Cohenheim considered that a portion of the embryonic tissue remained dormant in the tissues and at some time, owing to increased blood supply to the part, or owing to a fresh activity in the tissues themselves, this em-

bryonic tissue commenced to develop.

Adami[1] believed that the cause of the development of growths, malignant or otherwise, depended upon the habit of growth of the cells in a particular tissue - the habit of growth he considered depended upon increased nutrition, changed nerve mechanism, or possibly parasitic action.

The precise portion of tissue in which cancer first develops is a question that has also led to much discussion and controversy.

Ribbert believes that cancer grows from the sub-epithelial connective tissue, and not from the periphery, while others again state most emphatically that it originates in the extreme periphery.

McFarland inclines to the view that cancer is

(1) Mont. Med. Journal, 1896.

due to the inherent proliferating tendency of the epithelium, and he considers that cancer depends upon epitheliogenesis, and not parasitogenesis.

Other pathologists again, consider that cancer depends upon the activity of an organism. Russell[1] described certain bodies which he observed in the cancer tissue as coccidia or fuchsin bodies, and he believes that these were the cause of cancer. Kahne[2] also observed these bodies that were seen by Russell in malignant growths, and he considered that they were really blastomycetes, and identical in their nature with the saccharomyces cerevisiae.

Roncali[3] was of opinion that all coccidia and blastomycetes were identical. Only a short time ago Plimmer[4] observed in malignant growths

(1) B.M.J. Dec. 13th 1890

(2) Centralb. f. Allg. Path. und. Path.Anat. June 1896

(3) Centralb. f. Bakteriol. Parasitenk. v. Infektionskrank Nos. 9 and 10 (1897)

(4) Recent numbers of Lancet and Practitioner.

round bodies which he called plasmodia. These existed in the cancer cells, and he found that the insertion into an animal of a portion of the tissue which contained these organisms caused growths of a similar nature to develop in the various organs of the animal.

If we can depend entirely upon the accuracy of this experiment, this is indeed a great piece of work and may prove of vast importance in the true pathology of cancer. It is found, however, that only a limited number of carcinomata possess these bodies, so that this may not be the only cause of cancer. Pawlowski[1] mentions that he has found sporozoa in the sarcomata.

I think probably there is no doubt that these

(1) Virchow's Archiv. Vol. CXXXIII.

round, sometimes nucleated bodies, with granular protoplasmic substances, which have recently been observed in malignant growths, are probably true organisms and have to do with the growth of the tumour.

But are these bodies essential to the growth of a cancerous tumour? May they not merely act as irritating bodies and produce one form of cancer, while other bodies or other irritating substances may produce other forms?

I think Cohenheim's view is a good one, which is that an embryonic tissue lies dormant and sprouts forth under special conditions, while the view of Adami that the origin of cancer depends upon the activity of a particular tissue or upon its habit of growth seems to me also a quite probable explanation.

It is at this point I wish to make a few re-

marks on this subject. The cancerous tissues that I examined had the power of converting starch into sugar and also slightly of digesting fibrin.

Physiological tissues as we have seen also possessed these powers, but may not the results depend upon different causes?

The cancer tissue itself may liberate enzymes which cause the destruction of the surrounding tissue in which it starts. If cancer depend upon an organism it may also have the power of yielding a ferment which acts like ptyalin and also produces destruction of surrounding tissues. The rapidity of growth of cancers in certain tissues as compared with others would depend, from this point of view, upon the substances that were present in the particular organ or tissue in which the enzyme liberated from the cancer could act. Pathologists tell us that the reason why cancers grow so much more rapidly

in the liver than in other organs is on account of the distribution of its blood vessels. This will partly account for the fact, but it is difficult to say why other organs which are almost as well provided with capillaries should not be equally affected.

If an enzyme is liberated from a cancer which can convert starch into sugar, no matter what the origin of this enzyme may be, it is possible that it rapidly attacks the glycogen in the liver, and forms a sugar in which the cancer tissue can flourish. I have not noticed whether there is sugar in the urine in carcinomata of the liver.

With regard to the origin of cancer, whether it depend upon the chemical activity of a particular tissue resulting from an irritation, whether it depend upon the activity of an embryonic tissue that lies embedded in an organ or tissue, or whether it

depend upon an organism, it must be fed in order to flourish. I believe then that owing to irritation the cells of the tissue are stimulated to produce enzymes which exert particular actions in the stored up chemical substances in the cells, and form a suitable bed for their growth.

The origin of cancer then might resolve itself in some cases into a mere chemical alteration of the cell substance. A primary irritation must of necessity be present. The rapidity of growth would depend upon the nature of the tissue and the amount of enzyme liberated from it. On the other hand, even though cancer be always parasitic, the production of an enzyme in the tissues in which it is present, probably exerts very favourable influences on its activity. The parasite would multiply, and so would the parent tissue, the new growth itself possessing the same chemical properties as the tissue from which it originally sprang.

Three possible explanations could be advanced as to the aetiology of cancer.

1. That the tissues, stimulated to activity, secrete enzymes which act on the neighbouring cell substances. A chemical interchange takes place and with division of cells and growth. These cells now contain elements which will cause a similar sprouting of tissue in whatever organs or tissues they are carried.

2. That the organism causes cancer and liberates from it an organised ferment which acts on the surrounding cell substance and produces thereby nourishment for its proliferation.

3. That both the tissue cells and cancer bodies are essential to the process. That the tissues, stimulated to activity by irritation etc., yield enzymes which act on the surrounding cell substance, and produce nourishment for the proliferation of the organism and for the proliferation of the tissue in which it lies.

From these three possibilities, which closely overlap, one sees how very important it is to make a study of the chemical condition of new growths.

Much information could be obtained by injecting some of the glycerine extract of the cancer into rabbit, and observing the results that occurred. Or again, if cancer depended essentially on a chemical activity of a particular tissue, with the liberation of its enzymes, experiments could be performed, in which the outer skin of an animal was irritated, and injections of the glycerine extract of a tissue that had the power of converting starch into sugar, inserted into the irritated spots.

It is interesting in this connection why cancer so frequently develops on the side of the tongue, or on the lips. In both cases, there is the primary irritation produced from the edge of a sharp tooth, or from the adhesion of a clay pipe to the mucous membrane, while ptyalin in the saliva is constantly present.

Several physiologists and pathologists have ex-

perimented with the juice of cancer. Mayet believes that cancer can be inoculated from the juice, while others again doubt this statement.

Many have tried the injections of the serum of a horse, previously inoculated with the juices of the sarcomata and carcinomata, for the treatment of cancer, but with little success. Others have tried the injections of erysipelas[1] and various allied serums for the same purpose, but with no good result.

Even though one is yet in doubt as to the precise cause of cancer, yet I am convinced that the chemical analysis of the cancerous and neighbouring tissues, would help in proving the aetiology of cancer, and in offering a reason for its greater rapidity of growth in some tissues than in others.

(1) Emmerich and Schott - Deutsche Med. Wooh. April 1895.

These views are based on too few experiments to draw hasty conclusions, yet I mean to follow up this path of investigation, as little has yet been done in this direction.

Dr. Beatson tells me that he made a glycerine extract of several cancerous tumours, and found that in one case at least, he obtained an extract which converted starch into sugar. He has thus approached the subject in much the same way as I have done, but I do not know what method he performed for the tissue's extraction, other than that he used glycerine, nor do I know the actual results of his observations, as they have not been published.

The results of Dr. Beatson's experiments, however, in removing the ovaries for cancer of the breast, prove at least in part the fact, that

cancer in some cases depends upon the alteration in the chemical activity of the tissues with their enzymes.

Eclampsia

The pathology and aetiology of this disease, a series of symptoms that occur to the pregnant woman shortly before, during, or after her confinement, are still obscure, and have led to much theoretical speculation and investigation.

In the older text books one finds that the disease was reckoned as a kidney derangement, and that the convulsions which developed were due to the stagnation in the blood of urea and allied substances. We know, however, that in many cases of eclampsia, there is no kidney affection, and that

many patients who have been a victims to chronic Bright's disease, rarely develop puerperal convulsions[1]. The old view then does not hold ground, and the present opinion is that the disease depends primarily on a liver complaint, with a general toxaemia, the albuminuria resulting from the toxic process, and not the cause of the disease.

What is the toxaemia due to? Buchard[2] considers that in pregnancy poisons are always circulating in the blood, and that these are destroyed by the liver, and eliminated by the kidneys, lungs, and skin.

If the liver is then destroyed in any way, or the various pathways for the excretion of the toxic substances are blocked, then the accumulation

(1) This fact is emphasised by Boyd - Bull. Med. du Nord. 1895

(2) Leçons sur les Autointoxications dans les maladies.

of these substances in the blood causes the convulsions which become so violent.

The patient also is usually in a highly excitable state of mind, and consequently the poison acts reflexly with greater vigour than it would otherwise do.

The active poison has been ascribed to many causes. It may be the result of a specific microbe which has not yet been discovered[1], and the convulsions which ensue may depend upon the liberation of a ptomaine or organised ferment which is freed from the organism and circulates in the blood. Staphylococci have frequently been found in the blood of eclamptics, and Cué[2] believes

(1) Chambrelent - Nouvelles Archiv. d'obstétrique et de gynécologie, 1893.

(2) La Semaine Médicale. Paris, Jan. 22 1893.

that on this account eclampsia may be considered a manifestation of a puerperal infection. This view I think can be abandoned, as frequently no such organisms can be found in the blood, while many patients who have had eclamptic convulsions make an uninterrupted recovery, with little or no rise of temperature, or suggestion of a septic condition. Of the cases of eclampsia that I have personally seen in the Glasgow Maternity Hospital, two had no suggestion of a pyaemic process, while the third had. In the latter case, however, there was a laceration of the perineum and abrasion of the mucous membrane of the vagina, so that the septicaemia resulted probably from germs lodging in the injured surfaces, and which became rapidly absorbed.

I do think, however, that powerful convulsions or the eclamptic state render the body more susceptible to the entrance of infective organisms.

The poison, whatever it be, impoverishes the blood to such a degree that the tissues lose their recuperative power, and indeed often give rise to sloughs, bed sores and ulcers. There is thus a close analogy between this disease and diabetes: in both cases there is a general toxaemia, and as a result a damaged state of the general blood stream and of the tissues.

In this connection Clark and Skelton[1] supposed that eclampsia was due to the non-elimination of carbohydrate metamorphosis, and that those products underwent a degeneration with the development of convulsive poisons and acetone. Stumpt found also that the urine of eclamptics contained more sugar than existed normally in pregnant women.

I myself have never noticed the excessive increase of sugar in the urine in eclamptics, but, if this be so, I think the pathology of the disease

(1) Ann. Journ. Obstet. 1897.

is somewhat enlightened. Just as acetone is considered by some to be the poisonous product in cases of diabetes, depending upon derangement of the liver and with diabetic coma as a sequel, so in eclampsia, there is probably a liver disorder, and the presence of a large quantity of acetone which irritates the brain centres and produces convulsive storms.

The poison has been considered again to depend upon a diseased condition of placenta, or on the secretion from the placenta of active substances which poison the blood. Others believe that the poison originates in the foetal structures, while some have said that they have arisen in the intestines of the mother as a result of the putrefactive changes which occur there(1). These authorities

(1) Potter - Journ. Ann. Med. Assoc. 1897.

have endeavoured to stave off eclamptic convulsions by treating their patients with milk diet only, for two or three weeks before labour commences.

Just as Halliburton and Mott observed that the poisonous product choline was present in greatest amount during the convulsion, similarly in this disease Ludwig and Savor[1] have observed that the toxicity of the blood is most marked during the convulsion, while the urine is little affected.

Some authorities believe that in all pregnant women poisonous products are constantly circulating in the blood stream, as the result of the normal physiological activities of the tissues before labour commences, and they assert that if these are produced in too great amount, the kidneys and liver become disordered, which interferes

(1) Monatsk. f. Geburtsh. v. Gynäk 1895.

with their elimination and destruction, and which allows them to act as irritating substances on the brain and spinal centres.

It was with the view of throwing fresh light on the pathology of this disease that I endeavoured to procure the blood from a case of eclampsia, and to procure portions of the organs after death that were likely to be affected by the disease.

I have to thank Dr. Jardine of the Glasgow Maternity Hospital for procuring the blood and organs for me. He has been greatly interested in the pathology of eclampsia, and he was anxious that I should obtain the blood and tissues in the hope that I might throw some light on the subject, by dealing with it from a chemical point of view.

Although my investigation may not help materially in the knowledge of the pathology of eclampsia I hope that I may at least open up a new branch of

enquiry for further investigation. I have not been able to obtain any reference to any observations of a similar nature.

I made glycerine extracts of the fresh blood and of several of the organs. I found that although the extracts themselves did not reduce Fehling, each extract, including that from the blood itself, had the power of markedly converting starch into sugar, and to a less extent of changing proteids into peptones. These tissues then including the blood contained a ferment of some kind or other, which rapidly converted starch into sugar.

Is this ferment to be looked upon as belonging to the organised or the unorganised group? If eclampsia depend upon the activity of a germ, then possibly this result is due to the presence of an organised ferment or ptomaine.

Again, if eclampsia depend merely upon the over activity of the tissues themselves then this result is due to the action of an unorganised ferment similar to that of ptyalin or amylopsin.

I must confess that I lean somewhat to the latter view. I believe that owing to some primary nervous cause, there is a congestion of the various thoracic and abdominal organs, and that in consequence there is an increase in the activity of these tissues, a greater chemical metamorphosis, and a larger output of enzymes, chiefly of the nature of ptyalin.

These enzymes get absorbed into the blood and cause irritation and chemical changes in the liver and kidneys. The liver yields up its glycogen, and sugar is formed in too great abundance. Diabetes sets in. The dextrose that exists in the blood in too great abundance becomes converted

into acetone and other substances.

The kidneys become irritated and they are unable to eliminate in sufficient quantity the poisonous elements in the blood. The blood consequently becomes more and more toxic until it poisons the brain centres and convulsions supervene.

This naturally is merely a theory, but I believe that the presence in the blood of an enzyme which rapidly converts starch into sugar is one of immense scientific importance.

The blood in all cases of eclampsia should be analysed,and this test applied, as it is impossible to draw absolute conclusions from the observation and experimentation of one case. The injection of the blood extract should in all cases be performed into animals. This has been done by Chambrelent[1]

(1) Loc. cit. & Archiv. clin. de. Bordeaux 1894

who found that 4 cc of eclamptic blood killed a rabbit, while it required 10 cc of normal blood to do so.

I may state here that I got no result which shewed the presence of a diastatic ferment in the normal blood of a rabbit.

Bial and Röhmann stated in 1893 that normal blood contains such a ferment, and which is capable of converting starch into dextrose. If this observation be correct then eclampsia would probably depend on its increase.

If it exist normally in blood, one could see then why the sugar that is present in the blood is usually dextrose.

I have endeavoured to discuss these questions pretty fully, as I am convinced that much could be done in approaching these doubtful problems from

this point of view.

I am aware of the fragmentary nature of my remarks and theories, as my object has been all along to generalize on the subject as it is a new one, but one that will bear minute examination.

CHAPTER XIII.

SUMMARY OF THIS CONTRIBUTION WITH ENUMERATION OF THE POSSIBILITIES FOR FURTHER RESEARCH IN THIS DIRECTION

In the foregoing paper I have endeavoured briefly to give a general survey of the nature and action of enzymes, as they are known to exist in the digestive organs. I am aware that most text books deal very fully with this subject, but I have picked out those facts which would be of interest from a comparative point of view only, before commencing the second part of the paper.

Moreover, I have been able to add several references of more recent date, and to introduce certain facts chiefly from German literature, which I have not seen mentioned in any English text book. Throughout the first part also, I have expressed my views on various occasions, and have entered into the discussions of several scientific problems in regard to enzymic activity.

In the second part of the paper I have mentioned how the experiments were performed, and how certain difficulties which might lead to fallacies could be prevented, unless a thorough knowledge of these was understood. I traced the connection between enzymic activity of plant and animal life, and considered that probably in the animal, as well as in the plant, an interstitial digestion was constantly at work. Although our knowledge of this question is still doubtful and obscure I was of opinion that with the advancement of chemico-

physiological science such a result might be confirmed, and might throw fresh light on the pathology of many obscure diseases.

I then tabulated the results of experiments on upwards of sixty extracts obtained by the glycerine process from the tissues of the rabbit, child and the adult, both before and after death. Tables were next given of extracts of organs obtained in disease, and of tumours (sarcomata and carcinomata) and tubercular sputum. My results shewed:-

1. The presence of pepsin, or a substance analogous to it, in all the tissues, physiological and pathological.

2. The presence of a diastatic ferment in the larger proportion of the tissues examined - probably of the nature of ptyalin.

3. The absence of trypsin in the tissues, except in the pancreas. Reactions which may have depended upon trypsin occurred in the intestines and in certain of the organs obtained post mortem.

4. That tissues which normally contained much glycogen formed an extract which reduced Fehling.

5. That pepsin is present in a marked extent in the lung and liver of the rabbit, as well as in the stomach.

6. That the intestines contained a proteolytic ferment of the nature of pepsin. This result differs from that of most authorities.

7. That an inversive ferment was not obtained by the glycerine process of extraction from the intestines of the child or rabbit.

8. That an inversive ferment was rarely present in the tissues. It was distinctly present in the extract of tubercular sputum.

9. That a milk ferment, apart from those tissues in which it is known to exist, was rarely present.

10. That the cancerous and sarcomatous tissues which were examined had proteolytic and distinctly diastatic properties.

11. That rabbits' blood contained no diastatic enzyme, whereas eclamptic blood did.

12. That all the tissues from the case of eclampsia yielded extracts which had marked diastatic properties, although these themselves did not reduce Fehling.

I described briefly the significance of the results obtained, with my views on the more unusual and striking reactions. I then entered into a discussion on the possibility of obtaining much information on obscure pathological diseases by making glycerine or alcoholic extracts of the blood in all cases, and extractions of the tissues post mortem.

I discussed briefly the aetiology and pathology of cancer and eclampsia with results of my observations in these conditions, and my views as a possible explanation of their causation.

I should mention that I have been unable to obtain sufficient material to make comparisons, so the chief importance of the paper lies in the description of a method for investigation, which is new, at least, in the study of diseased states, and which even from my results proves well for future

research.

I will mention briefly the various pathways that have been opened up incidentally in experimenting on tissue extractions, and in writing this paper, which may prove useful for future investigations:-

1. Extracts of the tissues from many rabbits should be made, to see if all behave alike.

2. Extracts of sarcomata and carcinomata should be made to observe the diastatic properties that are present in its substance, and to observe if these be always present.

3. An endeavour to isolate the enzyme pepsin from the tissue - such as liver, muscle etc.

4. To isolate other ferments.

5. To attempt other methods of extraction of tissues in order to obtain ptyalin, pepsin etc. in greater amount.

6. To estimate the quantity of ferments secreted by different tissues.

7. To inject physiologically normal glycerine extracts into rabbits and observe results. If a tissue which contains much ptyalin be injected, to note whether symptoms like convulsions and coma develop and to observe the condition of the urine in such cases.

8. To inject eclamptic blood into a rabbit and note results, and subsequently to examine the glycerine extract of the rabbit's tissues, to observe whether there be an active diastatic enzyme in the tissues.

9. To make glycerine extracts of the blood in many diseases whose pathology is obscure, more especially in the toxaemias.

10. In making glycerine extractives to use antiseptics such as .1 per cent. salicylic acid or .5 per cent. thymol, in order to prevent the possibility of confusion between the action of the organised and unorganised ferment.

11. To irritate the skin or mucous membrane of a rabbit and place over the wound spittle or the glycerine extract of a tissue that contains much ptyalin, in order to see whether a cancer will grow by irritation in the presence of an enzyme like ptyalin.

12. To inject the glycerine extract of a soft cancer into the blood of a rabbit and to note the results, the extract being carefully filtered before the solution is used and being diluted possibly with a solution of soda.

13. To insert a piece of cancer tissue into the peritoneum of a rabbit, and to observe whether the cancer grows. If it does not to inject syringefuls of saliva or glycerine extractives containing much ptyalin, in order to see whether if cancer be due to an organism, the presence of such a ferment helps its development. If that be so, the organism would be killed secondarily by injecting substances into the tissues around the cancer, which would destroy ptyalin.

14. To inoculate into a rabbit the glycerine extract of tubercular sputum after it is filtered, and to observe the results.

These are merely a few of the lines for future investigation. When any subject is vague the probabilities and possibilities are enormous, and so it seems to be in this case. But in diseases such as cancer and eclampsia, which are so dire in their results, any new views on the subject should be tolerated, and even appreciated.

F I N I S