On Hepatic Glycogenesis

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VI. *On Hepatic Glycogenesis.*

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Communicated by John G. McKendrick, *M.D., F.R.S.*

PRELIMINARY.

Although hepatic glycogenesis was undiscovered before 1854, a large number of investigators of animal metabolism have directed their attention to its elucidation, and the literature on the subject has thus become very extensive. In spite of this, our knowledge of the physiology of the process is far from complete or satisfactory.

The term "glycogenesis" is here used in the sense in which it was originally employed by Claude Bernard, *i.e.*, the "production of sugar," and not the mere formation of glycogen—a sense in which it is too commonly employed by many writers.

That one of the great functions of the liver is to produce sugar will not, at the present time, be denied by any physiologist.*

The question, therefore, resolves itself into—

I. From what, and how, is hepatic sugar produced? And this at once leads to the question of—What is the relationship of hepatic sugar to glycogen?

Bernard and the vast majority of subsequent investigators have been led to the conclusion that glycogen is the forerunner of the sugar. Seegen alone opposes this view, and bases his opposition on the following grounds† :—

1st. That, during starvation, sugar is produced in the liver up to the last hours of life, and long after all the glycogen has disappeared from the organ.

2nd. That, according to the researches of Kratschmer and himself, the production of sugar in the post-mortem liver is in excess of the disappearance of glycogen.

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* The theory of Pavy, repeated in nearly every text-book, that the liver is " a sugar destroying, and not a sugar forming organ," rests on so unsubstantial a basis and has been so completely refuted by the work of Seegen and other investigators that it need not be considered.

† Pavy's position, of course, necessitates the conception that hepatic glycogen has a fate other than sugar.

To draw such a conclusion from the former fact shows a want of appreciation of the relationship of glycogen to the sugar, so clearly recognized by BERNARD. It is when a supply of glycogenic (*i.e.*, sugar yielding) material exists in the liver that it is stored as glycogen, just as in a resting gland cell, when there is no call for mucin, or the special zymin, an accumulation of mucinogen or of zymogen occurs. If the demand for sugar in the organism is urgent, the liver is deprived of the material for the formation of this substance. To argue the non-relationship of sugar to glycogen for this reason is as absurd as to argue the non-relationship of mucin and the zymins to the mucinogen and the zymogens, because, in the prolonged activity of the cells, these latter bodies are not accumulated and stored.

Against the experimental basis of the second objection must be set the observations of BOEHM and HOFFMANN ('Pflüger's Archiv,' vol. 23) and of GIRARD ('Pflüger's Archiv,' vol. 41), which clearly indicate that the amount of sugar formed in the liver post-mortem is proportionate to the glycogen disappearing.

But even supposing that SEEGEN and KRATSCHMER's results are correct, they by no means justify the conclusions founded upon them. The fact that glycogen is formed, not only from carbohydrates, but also from proteids, and the possibility that the vital functions of the cells of the body are continued for some time after the death of the animal, might explain the post-mortem production of the slightly increased amount of carbohydrates found by these investigators.*

With such conclusive evidence, further experiments are needless.

Glycogen being the forerunner of sugar, the problem of glycogenesis resolves itself into :—

1st. From what and how is glycogen formed in the liver ?

2nd. How is it related to the protoplasm of the liver cells ?

3rd. Into what kind of sugar is it changed, and how is the conversion brought about ?

As a result of his investigations, BERNARD ('Leçons sur la Diabète,' p. 307) gives as his answer to the first and third of these questions, that the process of sugar formation in the liver, " s'accomplit en deux actes. . . . L'acte *chimique*, c'est la transformation du glycogène en sucre. L'acte *vital*, c'est la production du glycogène au sein du tissue vivant."

All subsequent work has but confirmed the latter part of this statement, and has fully demonstrated that the progenitors of glycogen probably first become part of the living protoplasm of the cells before being changed into that substance. The literature on this aspect of the question is very extensive. Among the most important communications are those of VOIT and his scholars, the main results of which are given in vol. 28 of the 'Ztsch. f. Biol.' (" Ueber die Glykogenbildung nach Aufnahme verschiedener Zuckerarten"); while the whole evidence is ably considered and con-

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* FRAENKEL's theory of the mode of storage of glycogen in the cell ('Pflüger's Archiv,' vol. 52) may also afford an explanation of such results.

densed by PFLÜGER ("Ueber die Synthetischen Processe und die Bildungsart des Glykogens im thierischen Organismus."—'Pflüger's Arch.,' 1888).

The results of more recent observations have not, however, confirmed the former part of the statement, that the conversion of glycogen to sugar is a purely *chemical* act; and at present there is a tendency to view the conversion of glycogen as due, not merely to a chemical process, the result of the action of a soluble ferment or zymin, but to some process connected with the metabolic changes in the liver-cells.

While BERNARD was undoubtedly influenced, in coming to his conclusion, by the then recent discovery of the amylolytic action of the *zymins*—diastase, ptyalin, and the pancreatic ferment—the physiologists of the present day, influenced by the discovery of the true nature of the activity of secreting epithelium, naturally tend to regard the processes in the liver-cells as identical with these.

The direct experimental evidence on this matter is, however, far from satisfactory; and the object of this paper is to attempt to give a definite answer to this important question.

In considering this question it is necessary to bear in mind the chemical relationships of the various carbohydrates.

The simplest members of the group formerly known as the glucoses, now better known as the monosaccharids, are the aldehyds (*e.g.*, dextrose) or ketones (*e.g.*, lævulose) of a hexatomic alcohol, occurring in at least three isomeric forms, having the formula $C_6H_8(OH)_6$.

By the polymerization of two molecules of such monosaccharids with dehydration, the group of disaccharids, having the formula $C_{12}H_{22}O_{11}$ is formed. Of this series, the members best known are maltose, formed by the union of two molecules of dextrose; saccharose, or cane sugar, formed by the union of a molecule of dextrose and of lævulose; and lactose, composed of a molecule of dextrose and one of galactose.

By further polymerization and dehydration a series of polysaccharids, consisting of the dextrins and starches, animal and vegetable, and the gums, is produced. The most complex have molecules of enormous size, the molecule of starch being possibly composed of no less than 120 dehydrated monosaccharid molecules, $120(C_6H_{12}O_6-H_2O)$. From such bodies to the disaccharids, the dextrins form a continuous chain of less and less complex substances.

The conversion of glycogen to sugar is thus a double one of disintegration and hydrolysis. It may be brought about by various agencies.

1st. By the action of dilute mineral acids at a high temperature.

2nd. By the action of such *zymins* as occur in the saliva and pancreatic juice.

3rd. By the action of various micro-organisms.

4th. By an unknown process in the liver during life and after death.

It is with the nature of this process that we have at present to deal.

The possibility of the process being due to the action of an acid in the *living* body may be at once dismissed. The post-mortem development of acid in the liver some-

what complicates the problem, and this part of the subject will be afterwards dealt with.   The question is, therefore, whether the change is due to a zymin developed in the liver, or whether it is dependent on the metabolic processes which constitute the life of the liver cells.

That an amylolytic ferment can be extracted from the dead liver there can be no doubt.   EVES (' Journ. of Phys.,' 1884, vol. 5) gives a *résumé* of the literature upon the existence of such a ferment, and points out that the evidence seems to show that " the liver can scarcely be regarded as a more prolific source of such ferment than are the other tissues of the body."

Her experiments conducted in the Physiological Laboratory of Cambridge showed that an amylolytic ferment may undoubtedly be isolated from the liver of the sheep, which brings about a slow and incomplete conversion of glycogen into a sugar, which she considered to be probably maltose.   The nature of the sugar produced we shall again have to refer to.

DASTRE (' Arch. de Physiol.,' 1888, p. 76) also considers the literature on the isolation of an hepatic amylolytic ferment, and mentions the researches of EPSTEIN and MÜLLER, and of ABELES, which are not quoted by EVES.   In his experiments he endeavours to show that the so-called ferment action is really due to the influence of micro-organisms, and not to a zymin.   (For the experiments on which he bases this conclusion, see *loc. cit.*, p. 81, *et seq.*).

As to the *intra vitam* conversion of glycogen he says, " la transformation du glycogène en sucre n'est pas le résultat de l'intervention d'une diastase, séparable, isolable.   Elle est le fait de l'activité vitale des cellules hépatiques."

To establish this thesis he endeavoured to show that all factors which slow or arrest the activity of cells, slow or arrest the transformation of glycogen ; while they do not act in the same way on the activity of diastase (p. 94).

He points out that LANGENDORFF in 1886 (' Arch. de Physiol.') expressed the same view, without giving any evidence.   A similar view is expressed, though also without experimental evidence, by EVES (*loc. cit.*), by RANSOME (' Journal of Physiol.,' 1887, p. 113), and by NEUMEISTER (' Lehrbuch der physiologischen Chemie,' 1893, p. 258). NEUMEISTER says, p. 259, " Die Leber, einem lebenden Tiere schnell entnommen und sogleich in siedendes Wasser verbracht, enthält in der That Zucker, dessen Menge 0.2–0.6 Proz. beträgt.   Diese Zuckermenge vermehrt sich allerdings schnell beim Liegenlassen des ausgeschnittenen Organs, aber keineswegs durch einen postmortalen Vorgang, sondern im Gegenteil, weil das überlebende Protoplasma der Leberzellen noch weiter umsetzend auf das Glykogen einwirkt, während der gebildete Zucker nicht durch die Cirkulation fortgeführt wird."

While such observations go far to throw doubt upon BERNARD's conclusion that the conversion of glycogen to sugar is a chemical act—the result of the action of a soluble ferment, and independent of the life of the liver cells, they cannot be considered to disprove it.   The recent researches of BIAL and ROHMANN (' Pflüger's Arch.,' vols. 52, 53

and 54) on the diastatic ferment of the blood, producing a true glucose from glycogen and starch, would rather favour the older view of BERNARD.

The question must still be considered as open and requiring further investigation.

In carrying out such investigation, the first question to be answered is—how far is the process of glycogen conversion dependent on, or independent of, the life of the liver cells? The enormous post-mortem production of sugar from glycogen would at first sight seem to indicate that the process is independent of the vitality of the liver. But the continued life of many tissues, *e.g.*, of muscles, &c., after the death of the animal, must be borne in mind in considering the changes in the liver after somatic death.

The first part of this research then concerns :—

I. THE RELATIONSHIP OF GLYCOGEN CONVERSION TO THE CONDITION OF THE LIVER CELLS.

For this purpose the excised liver was used. As the object of the experiment was not to maintain as long as possible the vital processes in the liver cells, but merely to *contrast their results* with the changes subsequently occurring, it was not considered necessary to maintain the circulation by the perfusion method.

The roughly-minced organ, from the animal just killed by a blow behind the head and then bled, was placed in 0·75 per cent. salt solution, and maintained at the temperature of the body in an incubator with or without a current of air.

This method of studying the chemical changes in various organs, with the use of diluted defibrinated blood instead of salt solution, was first used by PFLÜGER. In a paper, " Der lebendige Organbrei und die Topographie des physiologischen Chemismus " ('Pflüger's Arch.,' vol. 23, p. 172), he strenuously defends its use against the attacks of ANDEER and W. KOCHS.

In the hands of BUNGE and SCHMIEDEBERG, and of SCHMIDT and his Dorpat scholars, the method has also yielded valuable results.

In endeavouring to trace the connection of glycogen conversion to the condition of the liver cells, it is necessary, in the first instance, to have a knowledge of the rate at which the glycogen disappears, at various periods, in the special conditions under which the liver is being observed.

A. THE RATE OF CONVERSION OF GLYCOGEN.

This has not been investigated in the special conditions described above. On the excised liver simply kept at the temperature of the room two series of observations have been made bearing upon this question.

SEEGEN (" Studien über Stoffwechsel," p. 405, *et seq.*), in a series of observations, in which he endeavoured to disprove the connection of sugar formation with glycogen formation, estimated the glycogen and glucose in the liver at different periods *post-mortem*,

He gives a table of the amount of glucose found in the liver of five dogs, which shows that in the first hour there was a gain of 43·5, 39·4, 40·0, 44·3, 67·7 per cent., an average of nearly 47 per cent. During the next twenty-four hours the gain was 28·7, 41·4, 26·0, 40·1, and 32·0 per cent., an average of 33·6 during the whole period, or of 1·4 per cent. per hour.

DALTON ('Treatise on Human Physiology,' p. 196) gives the results of three observations on the *post-mortem* production of sugar in the liver, from which the following table has been constructed.

EXPERIMENT 15.

Five seconds after death, liver contained 0·181 per cent. glucose. If none was present during life, during this interval glucose must have been produced at the rate of 1·372 per cent. per minute.

Fifteen minutes after death, the liver contained 0·679 per cent. glucose, an increase of 0·498 per cent., or of 0·033 per cent. per minute. Sixty minutes after death, the liver contained 1·026 per cent. glucose, an increase of 0·347 per cent., or of 0·007 per cent. per minute.

EXPERIMENT 19.

Five seconds after death, liver contained 0·3854 per cent. glucose, equivalent to the production of 4·248 per cent. per minute.

After six hours, the liver contained 1·1458 per cent., an increase of 0·7604, or of 0·0021 per cent. per minute.

EXPERIMENT 20.

Four seconds after death, the liver contained 0·2675 per cent. glucose, a production of 4·012 per cent. per minute. One hour after death there was 1·1888 per cent. glucose, an increase of 0·9213, equal to 0·015 per cent. per minute.

Four hours after, the glucose was 1·3361 per cent., an increase of 0·1475 per cent., or 0·0008 per cent. per minute. Twelve hours after, the glucose was 1·5317 per cent., an increase of 0·199 per cent., or 0·0004 per cent. per minute.

Both these series of experiments indicate an enormously active production of sugar in the liver just after death, and a progressively slower production during a later period.

From these observations it is impossible to draw absolutely definite conclusions as to the actual production of glucose or the disappearance of glycogen, because it is quite possible that carbohydrates other than glucose may be produced, and that the sugar, after being produced, may be destroyed. The observations of LÉPINE and BARRAL ('Comptes Rendus,' vol. 112 and 113) on the glycolytic action of the blood go far to prove the possibility of this occurring.

For this reason it was considered desirable to investigate directly the changes in hepatic glycogen at different periods after death, the liver being kept in the condition above described.

Method.

In these experiments, rabbits in a good state of nutrition, usually fed on oats and

bran with some green food, were used. The animal was killed by a blow behind the ears, and the carotids were at once severed, and the animal thus thoroughly bled. The abdomen was opened, and the inferior cava and portal vein were found collapsed and containing little blood. The liver was excised and the gall bladder torn away from it. On squeezing little blood could be expressed. The organ was then rapidly minced with a sharp razor and divided into several parts.

One of these was at once thrown into actively boiling water. The other portions were placed in wide-mouthed bottles containing about 150 cub. centims. of 0·75 per cent. salt solution, at a temperature of 37° to 40° C., and kept at this temperature in an incubator with or without a current of air for definite periods. At the end of the period the bottle was removed, and the contents thrown into boiling water and boiled actively for five minutes.

For the extraction of glycogen Brücke's method was usually employed. In some cases Külz's potash method was used. Vintschgau and Dietl ('Pflüger's Arch.,' vol. 13, pp. 253, 187) have shown that boiling with caustic potash causes a disappearance of a considerable quantity, sometimes as much as 10 per cent. of glycogen. This observation is confirmed by Külz ('Ztsch. f. Biol.,' vol. 22, p. 178). Külz claims for his method in the case of the liver no greater accuracy than Brücke's. He says (p. 193): "Sie gibt bei der Leber mindestens ebenso gute und beim Muskel entschieden bessere Resultate."

Seeing that the results given are no better than those obtained by simply boiling, the latter method was preferred, because it was found that the enormous proteid precipitate thrown down by the mercuric potassic iodide in Külz's process required such prolonged and copious washing that the volume of the filtrate was inconveniently large, and that enormous quantities of alcohol were required for the subsequent precipitation of the glycogen.

To remove the glycogen as completely as possible, the proportion of water used was always large in relationship to the amount of liver substance   A piece of liver of from 5 to 10 grms., finely pounded after the initial boiling, was boiled in a vessel containing over a litre. The boiling was continued for many hours—usually, at least, twelve, the vessel being filled as the water evaporated. Three times in the course of the extraction the extract was passed through a linen filter and the pounded liver well squeezed. The residue, on being treated with caustic potash, and then precipitated with mercuric potassic iodide, gave only occasionally a faint trace of the glycogen reaction with iodine.

The voluminous filtrate was evaporated to about 200 cub. centims., treated with hydrochloric acid and mercuric potassic iodide, and filtered. The precipitate was well washed with water containing mercuric potassic iodide and acid. To the filtrate four times its volume of methylated spirits was added; and, after twenty-four hours, the precipitate was brought on a dried and weighed filter paper, and washed first with 60 per cent. alcohol, secondly with methylated spirit, thirdly with absolute

alcohol, then with ether, and finally with absolute alcohol. It was then dried at 110° C. The ash was not determined. FRAENKEL's ('Pflüger's Arch.,' vol. 52, p. 125) recently introduced method was tried, but was found to yield unsatisfactory results.

It may be urged that KÜLZ's observations point to so many fallacies in connection with any method of glycogen determination as to render the results of little value. But it must be remembered that, if the determinations are made under precisely the same conditions, the deficit will be fairly constant. The actual results obtained seem to indicate that the method is practically much more accurate than it appears on theoretical consideration. This is well shown by these experiments, in which both the glycogen and sugar were determined.

EXPERIMENT 18.

	Time after death.	
	2 minutes.	24 hours.
Glycogen	5·88	0·55
Glucose	trace of reduction	5·29
Total carbohydrates . . .	5·88	5·84

EXPERIMENT 19.

	Time after death.						
	2 minutes.	45 minutes.		135 minutes.		315 minutes.	
		Chloroform.	No chloroform.	Chloroform.	No chloroform.	Chloroform.	No chloroform.
Glycogen . . .	7·09	5·68	6·23	5·00	5·60	4·60	5·42
Glucose	0·23	1·39	0·98	1·96	1·66	2·53	1·88
Total carbohydrates	7·09 to 7·32	7·07	7·21	6·96	7·20	7·18	7·20

An objection to every method of determining glycogen by precipitating it with alcohol is that some of the dextrins may also be in part thrown down, and be reckoned along with the glycogen. It would, perhaps, therefore, be better to call the results obtained " carbohydrates precipitated by 60 per cent. of alcohol," instead of " glycogen," but with this proviso it is needless to use such a cumbrous phrase.

When the sugar was also determined, the following method was employed. One half of the aqueous extract of the liver was heated and to it perchloride of iron in

sufficient amount to precipitate both proteids, and glycogen was added along with acetate of soda. The mixture was then neutralized with a solution of carbonate of soda and the clear watery fluid tested for glycogen by acidifying and adding iodine. If glycogen was present, the process was repeated. The clear fluid was then filtered off, the precipitate being well washed with hot water and the washings united to the filtrate.

This was then evaporated to a convenient volume, and the sugar determined volumetrically *as glucose*, by means of FEHLING's solution, sometimes with the addition of ferrocyanide of potassium (CAUSSE, 'Journal de Pharmacie et de Chimie,' 1889. DASTRE, 'Arch. de Physiol.,' 1891).

The following experiments were performed on the rate of conversion of hepatic glycogen.

EXPERIMENT 1. 6.2.93.

Rabbit killed at 1.5 P.M. Liver divided into three large pieces,
A weighed 5·1 grms. placed in boiling water at 1.6 P.M.
B   „   11·1   „   „   1.10 P.M.—4 minutes later.
C   „   5·5 grms., placed in 0·75 per cent. NaCl solution, and kept at 35° to 39° C. till 5 P.M. (236 minutes), then boiled. Glycogen estimated by BRÜCKE's method.

> A. Glycogen = 0·195 grm. = 3·82 per cent.
> B.   „   = 0·333 „   = 3·00   „
> C.   „   = 0·119 „   = 2·16   „

In first 4 minutes 0·82 grm. of glycogen per 100 parts of liver disappeared, equal to 2·05 in 10 minutes.

In next 236 minutes 0·84 grm. of glycogen per 100 parts of liver disappeared, equal to 0·035 in 10 minutes.

EXPERIMENT 2. 10.2.93.

Rabbit killed at 1.15 P.M. Liver divided into several parts, A, B, C, D.
A weighed 8·4 grms., placed in boiling water at 1.17 P.M.
B   „   8·6 grms., placed in 1·5 per cent. NaCl.
C   „   10·1   „   „   „
D   „   8·7   „   „   „
B kept at 40° C. till 2 P.M., then boiled.
C   „   „   3.30 P.M., then boiled.
D   „   „   6.30 P.M.,   „

Glycogen estimated by BRÜCKE's method, one half of the aqueous extract of each being taken for the purpose. The other half was treated by SCHMIDT MÜLHEIM's method, and the glucose estimated by means of FEHLING's solution without the addition of ferrocyanide of potassium. The end reactions were not well marked.

Glycogen.

> A. Glycogen = 0·292 grm. = 7·00 per cent.
> B.   „   = 0·268 „   = 6·23   „
> C.   „   = 0·281 „   = 5·6   „
> D.   „   = 0·233 „   = 5·42   „

Glucose.

	Glucose in grms. in liver taken.	Per cent. of glucose in liver.
A.	less than 0·01	less than 0·23
B.	0·042	0·98
C.	0·083	1·66
D.	0·081	1·88

In first 45 minutes 0·86 grm. of glycogen per 100 parts of liver disappeared, equal to 0·19 in each 10 minutes.

In the next 90 minutes 0·63 grm. disappeared, equal to 0·07 per 10 minutes.

In the next 180 minutes 0·18 grm. disappeared, equal to 0·01 per 10 minutes.

EXPERIMENT 3.   13.2.93.

Rabbit killed at 1.23 P.M.   Liver cut up into A, B, and C.

A weighed 6·2 grms., placed in boiling water at 1.25 P.M.

B   „   4·0   „   „   „   1.35 P.M., 10 minutes later.

C   „   5·1   „   0·75 per cent. NaCl solution and kept at 37° C. till 3 P.M., then boiled.

Glycogen estimated by BRÜCKE's method.

$$A. \text{ Glycogen} = 0\cdot405 \text{ grm.} = 6\cdot53 \text{ per cent.}$$
$$B. \quad „ \quad = 0\cdot239 \quad „ \quad = 5\cdot97 \quad „$$
$$C. \quad „ \quad = 0\cdot279 \quad „ \quad = 5\cdot47 \quad „$$

In first 10 minutes 0·58 grm. of glycogen per 100 parts of liver disappeared.

In the next 85 minutes 0·5 grm. disappeared, equal to 0·058 per 10 minutes.

EXPERIMENT 4.   13.1.93.

Rabbit killed at 12.58 P.M.   Liver minced and divided into A, B, C, D, and E.

A weighed 10·6 grms., placed in boiling water at 1 P.M.

B   „   10   „   0·75 per cent. NaCl solution at 1.1 P.M.

C   „   11·2   „   „   . „   „   1.2 P.M.

D   „   11·9   „   „   „   „   1.3 P.M.

E   „   11·0   „   „   „   „   1.4 P.M.

B kept at 40° C. for 1 hour (60 minutes).

C   „   „   2 hours 35 minutes (155 minutes).

D   „   „   4   „   15   „   (255   „   )

E   „   „   6   „   15   „   (375   „   ).

Glycogen extracted by KÜLZ's method.

$$A. \text{ Glycogen} = 1\cdot164 \text{ grm.} = 10\cdot981 \text{ per cent.}$$
$$B. \quad „ \quad = 1\cdot025 \quad „ \quad = 10\cdot250 \quad „$$
$$C. \quad „ \quad = 1\cdot095 \quad „ \quad = 9\cdot776 \quad „$$
$$D. \quad „ \quad = 1\cdot155 \quad „ \quad = 9\cdot664 \quad „$$
$$E. \quad „ \quad = 1\cdot004 \quad „ \quad = 9\cdot127 \quad „$$

Cultures on agar-agar from each gave free growth of micro-organisms, E had very strong smell.

In first 60 minutes 0·730 grm. of glycogen per 100 parts liver disappeared, or 0·122 per 10 minutes.

In next 95 minutes 0·273 grm. disappeared, or 0·03 per 10 minutes.

In next 100 minutes 0·112 grm., or 0·0112 per 10 minutes.

In next 120 minutes ·537 grm., or 0·045 per 10 minutes.

EXPERIMENT 5. 3.5.93.

Rabbit killed at 12.35 P.M.   Liver divided in A, B, C.

A weighing 10 grms., was placed in boiling water at 12.29 P.M.

B   ,,   8   ,,   ,,   ,,   12.32 P.M.

C   ,,   9·3   ,,   ,,   ,,   12.42 P.M.

Glycogen by BRÜCKE's method.

> A. Glycogen $= 0·861$ grm. $= 8·61$ per cent.
> B.   ,, $= 0·669$,, $= 8·36$,,
> C.   ,, $= 0·689$,, $= 7·4$,,

In first 3 minutes 0·25 grm. of glycogen per 100 parts of liver disappeared, or 0·83 grm. per 10 minutes.

In next 10 minutes 0·90 grm. of glycogen per 100 parts of liver disappeared, or 0·9 grm. per 10 minutes.

	Number of experiment.	Time in minutes from commencement.	Loss of glycogen per 100 parts liver.	Loss of glycogen per 100 parts liver per 10 minutes.
1st hour	5	3	0·25	0·85
,,	1	4	0·85	2·05
,,	3	10	0·58	0·58
,,	5	13	1·21	0·93
,,	2	45	0·86	0·19
,,	4	60	0·731	0·122
2nd hour	3	95	0·5	0·05
3rd ,,	4	155	1·49	0·07
4th ,,	1	240	1·66	0·069
5th ,,	4	255	1·205	0·051
6th ,,	2	315	1·67	0·05
,, ,,	4	375	1·855	0·049

In the next experiment the increase in glucose as indicated by the reducing power of the aqueous extract of the liver was estimated.

EXPERIMENT 6. 23.1.93.

Rabbit killed at 12.55 P.M.   Liver divided into pieces A, B, C, and D.

A weighed 6·2 grms., placed in boiling water at 1 P.M.

B   ,,   6·1   ,,   ,,   0·75 per cent. NaCl solution, sterilized.

C   ,,   7·2   ,,   ,,   0·75   ,,   ,,   ,,

D   ,,   6·6   ,,   ,,   0·75   ,,   ,,   ,,

B kept at 40° C. till 2 P.M. (1 hour).

C   ,,   ,,   5 P.M. (4 hours).

D   ,,   ,,   10 P.M. (9   ,,   ).

Glucose extracted as in Experiment 2.

  Filtrate of A.   Glucose = 0·593 per cent.

      ,,   B.   ,,   = 0·175 grms. = 2·87 per cent. in liver.

      ,,   C.   ,,   = 0·26   ,,   = 3·61   ,,

      ,,   D. = 400 cub. centims. (underwent fermentation before glucose was estimated).

Glucose formed per 10 minutes :—

    During first hour . . . . 0·362 per 100 parts of liver.

    From first to fourth hour . . . . . 0·04   ,,   ,,

Cultures on glycerine agar from B and C gave no growth of micro-organisms.

These experiments clearly show that the great and active disappearance of glycogen in the liver, kept in normal saline at the body temperature, is during the first half-hour, that the rate of conversion steadily diminishes during the first hour, and that after two hours it goes on at a very slow rate indeed.

The next point to be investigated is the relationship of the liver cells to these changes.

This may be studied in two ways :—

1st. By observing the influence of the destruction of the structure of the liver cells on the amylolysis.*

2nd. By investigating the changes which take place in the cells of the liver kept under the conditions above described.

B. Influence of Destruction of the Morphological Structure of the Liver Cells on Hepatic Amylolysis.

To destroy the cells, without in any way interfering with the possible action of any soluble ferments, the method of thoroughly rubbing a piece of liver up with fine clean sand was employed. The rabbit was killed in the usual manner and then pieces of the liver were taken. One of these was rubbed with sand till the whole became of a fine uniform cream-like consistence, in which microscopic examination revealed no structure. This was placed in about 150 cub. centims. of ·75 per cent. salt solution, at from 37° to 40° C. Another portion, roughly minced, was at the same time put into an exactly similar solution, while the third part was at the same moment thrown into boiling water. The first two portions were kept in the incubator for varying periods, and were then boiled and the glycogen extracted, as above described.

  * Throughout this paper amylolysis is used as a convenient abbreviation for " conversion of glycogen to glucose."

EXPERIMENT 7.   13.2.93.

Rabbit killed at 1.22 P.M.   Liver divided into parts A, B, C.
A weighed 5·7 grms., rubbed with sand in mortar and placed in 0·75 salt solution at 37° C., at 1.35 P.M.
B weighed 6·3 grms., roughly minced and placed in salt solution as above at 1.35 P.M.
C weighed 4·0 grms., placed in boiling water at 1.35 P.M.
A and B kept at 37° C. till 3·10 P.M. = Glycogen by BRÜCKE'S method.

    A. Glycogen = 0·312 grm. = 5·47 per cent. (slight loss due to cracking of beaker from bumping caused by sand).

    B. Glycogen = 0·278 grm. = 4·41 per cent.
    C.   „   = 0·239 „ = 5·97   „

EXPERIMENT 8.   3.4.93.

Rabbit killed at 11.42 A.M.   Liver divided into parts A, B, and C.
A weighed 11·3 grms. pounded with sand as in last experiment, and placed in salt solution at 37° C., at 11.48 A.M.
B weighed 10·7 grms. minced and placed in salt solution, as above, at 11.48 A.M.
C weighed 8·6 grms. placed in boiling water at 11.48 A.M.
A and B kept at 37° to 40° C., till 3.50, and then boiled.

    A. Glycogen = 0·572 grm. = 5·061 per cent.
    B.   „   = 0·25   „ = 2·336   „
    C.   „   = 0·453 „ = 5·267   „

Experiment.	Check.	Minced.	Pounded.	Time.	
				hrs.	min.
VII.	5·97	4·41	5·47 +	1	48
VIII.	5·267	2·336	5·061	4	8

The results of these two experiments are so fully confirmatory of one another that it was not considered necessary to extend the series.   They show very clearly *the enormous diminution in the amylolysis induced by destroying the structural integrity of the liver cells.   But at the same time they show that, though retarded, the conversion of glycogen is not completely stopped.*

C. STRUCTURAL CHANGES TAKING PLACE IN CELLS OF LIVER KEPT IN 0·75 SALT SOLUTION AT THE BODY TEMPERATURE.

The cellular death of an organ must be a slow and gradual process, not only as regards the individual cells in which the vital chemical changes probably slowly diminish and disappear, but also as regards the cells in mass, since experience shows that certain cells cease to act sooner than others.

It is impossible, by the microscopic examination of the cells, to say when the chemical changes, which constitute their life, have ceased. But it appeared probable that as these chemical changes diminished, the reaction of the cell to certain re-agents might alter, or that soon after the chemical changes had ceased, disintegrative changes might manifest themselves in the cell.

I. EHRLICH's researches ('Cbt. f. d. med. Wissen.', 1885, p. 113) induced me to try if, in methyl blue, a re-agent might be found which would indicate the cessation of active chemical changes.

For this purpose the reactions of this substance with ciliated epithelial cells and with leucocytes—the metabolic activity of which is so clearly indicated by movements —were studied. (α) Scrapings from the palate of a recently killed frog were spread on cover glasses and mounted in a ·4 per cent. solution of methyl blue in 0·75 per cent. salt solution.

It was found that the cells exhibiting active ciliary movement remained unstained, while those in which the ciliary movement had ceased, and the connective tissue cells, were all markedly stained—the nucleus being of a deep blue, the protoplasm of a paler blue. As the ciliary movement diminished and stopped, the previously active and unstained cells became blue. (β) The leucocytes of the frog's blood mounted in this solution remained uncoloured for fifteen or twenty minutes and then stained in the same manner as the ciliated cells.

In the case of the liver, when scrapings of the organ just excised from frogs or rabbits were treated with methyl blue in 0·75 per cent. salt solution in the cold, the cells and nuclei stained at once and deeply.

When the cells from the rabbit were treated with methyl blue solution at a temperature of 37° C. and examined in the warm stage at 37° C. the staining of the cells occurred just as in the cold.

The metabolic processes in liver cells, therefore, seem too slow to be indicated by their reaction with this colouring matter.

II. Another manner in which the cessation of active chemical changes might be made manifest is the occurrence of structural changes in the cells. Of course the onset of such changes may be the result of alterations in the metabolic processes or it may mark the commencement of post-mortem disintegrative changes.

The examination of liver cells at different periods after the excision of the organ, whether it be kept under the conditions described on p. 239 or simply allowed to lie in the room, shows the development of marked and interesting modifications in the structure of the cells.

Methods.

1. A fresh section of the liver was scraped and cover-glass preparations of the scraping made in the usual manner. These were examined :—

α. Unstained in 0·75 per cent. normal saline solution.

β. Stained in 0·4 per cent. solution of methyl blue in 0·75 saline.

γ. Stained in LUGOL's iodine iodide of potassium solution.

2. Similar cover-glass preparations were fixed in absolute alcohol and then stained in hæmatoxylin and eosin, mounted in balsam.

3. Pieces of the liver were hardened in absolute alcohol, embedded in paraffin and sections prepared in the usual way, and stained with hæmatoxylin and eosin or with EHRLICH-BIONDI's solution.

The specimens were studied with a LEITZ 7 objective, a ZEISS F., and a ZEISS apochromatic oil immersion, 3 m.m.

The examination of fresh cover-glass preparations stained with methyl blue is of special value in demonstrating the changes in the cell-substances, while the nuclear changes are more clearly seen in specimens hardened in alcohol and stained with hæmatoxylin and eosin.

The structure of the liver cell has been described by various histologists. LANGLEY (' Roy. Soc. Proc.,' vol. 34, p. 20) gives some account of the previous work upon the subject, and, as the result of his own investigations, describes the cell protoplasm as consisting of a network, the meshes of which, throughout the cell, being of much the same size, and the outer part of the cell being formed by a thin layer of slightly modified protoplasm. The interfibrillar paraplasm he describes as consisting of— 1. Spherical granules, probably proteid in nature; 2. Spherical globules of fat; 3. Hyaline substance filling up spaces not occupied by granules. He does not state whether this description applies to the living cell or to cells after hardening by the methods described in the paper.

The description and figures of the structure of the liver cell given in vol. 4 of the 5th edition of FOSTER's 'Physiology' are founded upon these observations of LANGLEY.

DELÉPINE (' Roy. Soc. Proc.,' vol. 49, p. 64), describes LANGLEY's " protoplasm network " as the mytoma, and calls the fluid interstitial substance the paramytoma. The term paraplasm he reserves for the results of the cellular metabolism, whether these be dissolved in the paramytoma or suspended in it as granules, globules, crystals, &c.

Setting aside all theories, what can be observed in the fresh living liver cell stained with methyl blue is a fine close network of fibres, which takes up the re-agent, and an interstitial material which does not stain. The network in the living cell is so close and fine that the protoplasm appears to be almost uniformly stained of a pale grey-blue. In the interstitial material, fat globules may sometimes be seen, and in specimens from well-nourished animals, stained with iodine, this material, either throughout the whole cell, or round the nucleus, or towards one or other margin, becomes of a mahogany-brown colour.

In fresh living cells I have been unable to see the granules described by LANGLEY. These are well seen in hardened specimens.

Most commonly there are two nuclei in each cell. These are large and circular, with a well-defined nuclear membrane, and a marked chromatin network, and one or more nucleoli. I have never, in a healthy adult liver cell, seen a mytotic nucleus.

The rapidity with which changes occur in the cells of the excised liver, when simply kept in the room or placed in a moist atmosphere in the incubator at 40° C., or when kept in 0·75 per cent. saline solution at 40° C., varies enormously in different animals.

In the cells of the liver kept at the ordinary room temperature, 16° C., no marked changes occur for 12 or 24 hours. If the organ is placed in the incubator, changes become manifest much earlier—within three or four hours. When the tissue has been in salt solution in the incubator changes may sometimes be made out within an hour, though frequently they are delayed for two or more hours.

The changes which occur are the same in all cases.

The first alteration is in the intra-cellular network. This becomes more manifest, giving the cells a more granular appearance. After two hours, in specimens kept in salt solution, this change is usually very marked. At first it was thought that this was due to the imbibition of fluid from the surrounding solution. But the same changes occur in the cells of the liver when not placed in solution, but kept dry.

The change once commenced advances slowly. The network becomes coarser, and becomes broken up. It still stains intensely with methyl blue, and the cell now appears as a clear, colourless structure with masses of the blue-stained network in it. These tend to collect round the nucleus. The nuclear changes are usually later in occurring, though in one or two cases I have observed them fairly early. The nucleus loses its sharp outline, loses its distinct network, and stains more diffusely blue with hæmatoxylin. After some hours it begins to break down and to appear as masses of material throughout the cell, stained partly blue with hæmatoxylin, partly red with eosin.

Speaking generally, it may be said that these changes manifest themselves, in the liver cells kept at 40° C. in saline solution, sometimes within the first hour, and that they become very marked within three hours.

In specimens placed in salt solution rendered faintly alkaline with carbonate of soda the rapidity of the early changes is not as a rule markedly delayed, but the late changes may be postponed for a very considerable period.

In a subsequent part of this paper it will be shown that the presence of chloroform greatly accelerates the initial conversion of glycogen to glucose, but that it has no action on the slow secondary conversion. In this connection it is important to notice that the presence of chloroform enormously accelerates the changes just described as occurring in the liver cells. In a liver kept in saline solution at 40° C., through which a stream of chloroform vapour is passed, the changes in the liver cells are at the end of half an hour as far advanced as they are in a non-chloroform specimen

after three or four hours. In chloroform livers, at the end of three hours, the intra-cellular network and the nucleus are both in an advanced state of disintegration. *Chloroform markedly hastens these changes in the liver cells.*

These observations, taken in conjunction with the last, show that *the amylolysis may be divided into an early more rapid stage before and accompanying disintegration of the liver cells, and a late slower stage after disintegration of the cells.*

The difference between these two stages in the amylolysis is further supported by the influence of various other agents.

D. Influence of Various Factors on Hepatic Amylolysis.

1. *Temperature.*

At one time it was pretty universally held that by the influence of temperature the living action of cells might be distinguished from the chemical action of soluble ferments.

Hofmeister, in his researches on the changes of peptone in the intestinal mucous membrane, found that a temperature of 60° C. arrested these changes, and concluded that this indicated that they were dependent on the life of the cells, which he supposed was destroyed by such a temperature, and not to a zymin which should have acted even after exposure to a higher temperature.

While undoubtedly certain cells of lower organisms do resist for some time a temperature as high as 60° C., I believe Hofmeister is right in concluding that the life of the cells of the mammalian body is destroyed by such a temperature. But it must be remembered that many ferments, under certain conditions, have their action, in part or in whole, abolished by exposure to even a lower temperature than 60° C.

For these reasons, the influence of temperature on hepatic amylolysis is by no means conclusive as to its nature.

The following two experiments seem to show that the extensive early amylolysis is stopped by exposure of the liver to a temperature of 60° C. for an hour, while the later slower change is allowed to progress :—

EXPERIMENT 9.   22.5.91.

Rabbit killed at 2.24. Liver cut into A, B, and C.

A weighing 12·8 grms., was placed at once in boiling water.

B   „   13·5   „   „   in 0·75 per cent. NaCl at 60° C.

C   „   11·7   „   „   „   „   „   40° C.

B and C were kept at these temperatures till 3.25, when B was cooled to 40° C. and left at this temperature with C till 6.15, when both were boiled.

Glycogen by Brücke's method.

A. Glycogen = 0·103 grm. = 0·804 per cent.

B.   „   = 0·093   „   = 0·69   „

C.   „   = 0·023   „   = 0·196   „

EXPERIMENT 10.   3.6.91.

Rabbit killed at 11.37.   Liver cut into A, B, and C.

A weighing 12·7 grms., placed at once in boiling water.

B   „   11·3   „   „   in 0·75 per cent. NaCl at 60° C.

C   „   11·1   „   „   „   „   „   40° C.

B and C kept at these temperatures till 12.20 then cooled to 40° C. and kept with C at this till 4.30, when both were boiled.

Glycogen by BRÜCKE's method.

A. Glycogen = 0·104 grm. = 0·811 per cent.
B.   „   = 0·079   „   = 0·700   „
C.   „   = 0·050   „   = 0·450   „

The cells of the liver, subjected to a temperature of 60° C. for about an hour, show a peculiar change very different from that so rapidly produced by chloroform, and which occurs more slowly in livers kept at 40° C.   Here the protoplasm becomes filled with minute granules, apparently due to precipitation of proteids.

2. *Fluoride of Sodium.*

Within the last year ARTHUS and HUBER ('Arch. de Physiol.,' 5th series, vol. 4, p. 651) have maintained that a 1 per cent. solution of fluoride of sodium destroys the activity of living protoplasm, but does not interfere with the actions of enzymes. They give experiments on the influence of this substance on the post mortem production of sugar in the liver as estimated by the amount of sugar present after periods of twenty-four hours and more, which show that the production of sugar is not stopped.   They say " On peut donc conclure que la transformation du glycogène en sucre dans le foie séparé de l'organisme est un phénomène de fermentation par ferment soluble."

With this substance I have made two experiments which clearly show that the early rapid amylolysis is retarded, although the later slower conversion may be allowed to go on as shown by ARTHUS and HUBER.

EXPERIMENT 11.   15.4.93.

Rabbit killed at 11.38.   Liver divided into A, B, and C.

A weighed 3·0 grms., placed in boiling water at 11.40.

B   „   5·1   „   „   0·75 per cent. NaCl at 40° C. at 11.41.

C   „   5·6   „   „   1·0   „   fluoride of sodium solution at 40° C. at 11.42.

B and C kept at 40° C. till 1.38, then boiled.

Glycogen by BRÜCKE's method.

A. Glycogen weighed 0·246 grm. = 8·22 per cent.
B.   „   „   0·270   „   = 5·29   „
C.   „   „   0·459   „   = 8·19   „

This experiment is not quite satisfactory since fluoride of sodium is not fully soluble in 60 per cent. of alcohol.

For this reason the influence of the fluoride on the amount of sugar produced was studied.

EXPERIMENT 12. 7.6.93.

Rabbit killed at 10.45 A.M. Liver divided into A, B, and C.

A weighed 15 grms. in boiling water at 10.47.

B „ 10·48 grms. in 1 per cent. NaCl at 10.48.

C „ 10·49 „ „ „ fluoride of sodium at 10.49.

B and C kept at 37° to 40° C. till 11.50, then boiled. Glucose as in previous experiments.

	Glucose in extract.	Glucose per cent. in liver.
A.	0·068	0·43
B.	0·196	1·63
C.	0 138	0·86

This substance does not accelerate the disintegration changes in the liver cells described on p. 248.

3. *Chloroform.*

Early in the investigation, the use of chloroform water, to distinguish between the vital action of cells and the influence of a soluble ferment, was suggested by a first glance at SALKOWSKI's paper "Ueber Autodigestion der Organe" ('Ztsch. f. klin. Med., Sup. Jubelheft,' 1890, p. 77). In this he maintains that, while chloroform water entirely prevents the action of living cells by stopping their metabolism, it in no way interferes with the action of the soluble zymins.[*] He gives one experiment to show that the amylolytic ferment continues to act in chloroform.

EXPERIMENT 3. (p. 90.)

Liver divided into two parts of 23 grms.

A. in 400 cub. centims. of chloroform water.

B. sterilized by boiling, then put in 440 cub. centims. of chloroform water. Both were digested for 68 hours.

In solution.	A.	B.
Sugar	Abundant.	Trace.
Glycogen	None.	Abundant.

The quantitative determination of sugar gave in 1000 grm. liver,

A. 48·23

B. 3·65

[*] MÜNTZ ('Compt. Rend.,' 1875, p. 1251) had fifteen years previously stated that chloroform has this action.

He concludes this experiment by saying, "Bezüglich des Glycogens bestätigt also der Versuch die geläufige Anschauung, dass die Umwandlung desselben in Zucker von einem Enzym abhängt, im Gegensatz zu Dastre, welcher kürzlich zu dem Resultat gelangt ist, dass dieser Process von dem Protoplasma der Leberzellen abhängt."

Such an experiment in no way justifies his conclusions.

Further study of this method (FOKKER, 'Fortsch. d. Med.' 1890, No. 3), as admitted by SALKOWSKI (*ibid.*, 1890, No. 5), shows that chloroform greatly diminishes the activity of many unorganized ferments, and therefore cannot be considered of much value in the differentiation of zymin action from the vital action of protoplasm.

The curious and unexpected result obtained in a preliminary experiment, and the statement that chloroform administration is followed by the occurrence of glycosuria (HILTON FAGGE, 'Principles and Practice of Medicine,' vol. 2, p. 414), as well as the experience of DR. STOCKMAN, who tells me that he has frequently, after the administration of chloroform to rabbits and dogs, found a fermentable and reducing substance in the urine, induced me to study more fully the action of chloroform on hepatic amylolysis.

That glycæmia as well as glycosuria is induced by chloroform has been shown by OTTO ('Pflüger's Arch.,' vol. 35, p. 467). He estimated before and after the administration of morphin, chloral, and chloroform, the reducing substances in the blood before and after fermentation, and found that morphia causes a slight increase in the glucose, but a marked increase in the not fermentable reducing substances; that chloral has little action on the glucose, but markedly increases the other reducing substances, while chloroform has the same action as morphin, but more marked.

Method.

To test the action of this substance upon the changes in the liver, the animal was killed as before described, the liver excised, minced and divided into three portions, which were weighed; one was instantly plunged into boiling water. The other two were placed in bottles containing 0·75 per cent. salt solution at from 37° to 40° C. Through one bottle a stream of air and through the other a stream of chloroform vapour were conducted by means of a pump, both bottles being kept in the incubator. At the end of varying periods the contents of both bottles were boiled and the glycogen extracted.

EXPERIMENT 13.   28.5.91.

Rabbit killed at 2.28 P.M.   Liver minced and divided into three parts, A, B, and C.

A weighed 6·1 grms., placed in boiling water at 2.34.

B   „   6·2   „   „   150 cub. centim., 0·75 per cent. NaCl solution with stream of chloroform vapour at 2.35.

C weighed 5·9 grms., placed in 150 cub. centims., 0·75 per cent. NaCl solution with stream of air at 2.35.

B and C kept at 40° C. till 6.15 P.M. (3 hrs. 40 min.).

Glycogen extracted by BRÜCKE's method.

A.   Glycogen = 0·201 grm. = 3·295 per cent.
B.   „   = 0·060 „   = 0·096   „
C.   No precipitate with alcohol.
        Glycogen = 0·00 per cent.

EXPERIMENT 14.   3.6.91.

Rabbit killed at 11.37 A.M.   Liver minced and divided into three parts.

A weighed 12·7 grms., placed at once in boiling water.

B   „   10·3   „   „   in 150 cub. centim. of 0·75 per cent. NaCl solution with stream of chloroform.

C weighed 11·1 grms., placed in 150 cub. centim. of 0·75 per cent. NaCl solution with stream of air.

B and C kept at 40° C. till 4.30 P.M. (5 hours).

Glycogen extracted by BRÜCKE's method.

A.   Glycogen = 0·104 grm. = 0·811 per cent.
B.   „   = 0·017 „   = 0·165   „
C.   „   = 0·50   „   = 0·450   „

EXPERIMENT 15.   9.11.91.

Rabbit killed at 12.30 P.M.   Liver divided into two parts A and B.

A weighed 25·2 grms., placed in 0·75 per cent. NaCl. solution with stream of air.

B   „   23·1 „   „   „   „   „   „   „   „   chloroform vapour.

Kept at 40° C. till 3.30 (3 hours).

Glycogen extracted by BRÜCKE's method.

A.   Glycogen = 1·012 per cent.
B.   „   = 0·735   „

EXPERIMENT 16.   22.8.92.

Rabbit killed at 11.30 A.M.   Liver minced and divided into three parts.

A weighed 17·6 grms., placed in boiling water.

B   „   15·0   „   „   0·75 per cent. NaCl solution with stream of air.

C   „   15·8   „   „   „   „   „   „   „   chloroform vapour.

B and C kept at 32° to 42° C. till 3.30 (4 hours).

Glycogen extracted by BRÜCKE's method.

A.   Glycogen = 0·37 grm. = 2·102 per cent.
B.   „   = 0·291 „   = 1·94   „
C.   „   = 0·134 „   = 0·848   „

That such results are not peculiar to the rabbit, or to herbivorous animals, is shown by the following experiment on the cat :—

EXPERIMENT 17.   7.3.93.

A large fat cat was killed by chloroform and bleeding.   The liver was excised, minced, and divided into portions A, B, and C.

A weighed 9·5 grms. placed in boiling water at 3.35 P.M.

B   „   10   „   in 0·75 per cent. NaCl with chloroform.

C   „   11·4 „   „   „   „   without „

B and C kept at 37° to 41° C. till 9.10 P.M., then boiled.

$$
\begin{aligned}
&\text{A. Glycogen} = 0\text{·}197 \text{ grm.} = 2\text{·}07 \text{ per cent.}\\
&\text{B.}\qquad\text{„}\qquad = 0\text{·}073\quad\text{„}\quad = 0\text{·}73\quad\text{„}\\
&\text{C.}\qquad\text{„}\qquad = 0\text{·}111\quad\text{„}\quad = 0\text{·}97\quad\text{„}
\end{aligned}
$$

SUMMARY.

Experiment.	Check.	Chloroform.	No chloroform.	Remarks.
13	3·295	0·000	0·096	40° C. for 4 hours
14	0·811	0·165	0·450	„ 5 „
15	Lost	0·735	1·012	„ 3 „
16	2·102	0·848	1·94	„ 4 „
17	2·07	0·73	0·97	

This series of experiments clearly shows that *in the excised liver the amylolysis is enormously increased by the presence of chloroform.*

That the glycogen is converted almost entirely into glucose is shown by the following experiment.

EXPERIMENT 18.   28.11.92.

Rabbit killed at 12.20.   Liver minced and divided into two, A and B.

A weighed 12·5 grms.; placed in boiling water at 12.28.

B   „   22   „   placed in 0·75 per cent. solution of NaCl, with stream of chloroform vapour at intervals.

B kept at 40° C. till 12.30 on 12th (24 hours).

Glycogen extracted by boiling as in previous experiments.   Extract concentrated.   Proteids and glycogen precipitated with excess of alcohol, and precipitate well washed with 70 per cent. alcohol. Filtrate evaporated to drive off alcohol, and glucose estimated by FEHLING's method.   Filter paper and precipitate extracted for glycogen by BRÜCKE's method.

Glucose.

A. Filtrate = 300 cub. centims., with 1 centim. of FEHLING, only a trace of reduction.

B. Filtrate (α), First determination, = 1·22 grm. glucose.

$$\text{Glucose} = 5\text{·}54 \text{ per cent. of liver.}$$

(β) Second determination, = 1·11 grm. glucose.

$$
\begin{aligned}
&\text{Glucose} = 5\text{·}045 \text{ per cent. of liver.}\\
&\text{Mean}\quad = 5\text{·}292\qquad\text{„}\qquad\text{„}
\end{aligned}
$$

Glycogen.

A. Glycogen = 0·735 grm. = 5·8 per cent.
B.   „   = 0·121 „ = 0·55 „

	Glycogen.	Glucose.	Total carbo-hydrates.
Before chloroform digestion . .	5·8	0	5·8
After „ „ . .	0·55	5·292	5·842

It now became necessary to investigate whether it is the initial rapid, or the subsequent slower amylolysis, or both, which are accelerated by the presence of chloroform.

For this purpose the following experiment was undertaken :—

EXPERIMENT 19.   10.2.93.

Rabbit killed at 1.15 P.M., and liver minced and divided into seven portions, A, B, b, C, c, D, d.
A weighed 8·4 grm. placed in boiling water at 1.17 P.M.
B   „   10·2 „   „   1·5 per cent. NaCl, with Chloroform at 37° C.
b   „   8·6 „   „   „   „   without „   „
C   „   9·9 „   „   „   „   with „   „
c   „   10·1 „   „   „   „   without „   „
D   „   8·7 „   „   „   „   with „   „
d   „   8·7 „   „   „   „   without „   „
B and b kept at 37° C. till 2   P.M., then boiled.
C and c   „   „   „ 3.30 P.M.   „
D and d   „   „   „ 6.30 P.M.   „

Aqueous extract divided into equal parts; in one glycogen determined by BRÜCKE's method, and in the other glucose determined by SCHMIDT MÜLHEIM's and FEHLING's methods.

Glycogen.

A. Glycogen = 0·292 grm. = 7·09 per cent.
B.   „   = 0·290 „ = 5·68 „
b.   „   = 0·268 „ = 6·23 „
C.   „   = 0·245 „ = 5·00 „
c.   „   = 0·281 „ = 5·60 „
D.   „   = 0·198 „ = 4·60 „
d.   „   = 0·233 „ = 5·42 „

	Glucose contained.	Glucose per cent. Liver.
A	— 0·01	— 0·23
B	0·071	1·39
b	0·042	0·98
C	0·097	1·96
c	0·083	1·66
D	0·111	2·68
d	0·081	1·88

	Glucose.		Glycogen.		Time.
	Glucose, per cent.	Gain per ten minutes.	Glycogen, per cent.	Loss per ten minutes.	
A	— 0·23	—	7·09	—	
B	1·39	0·308	5·68	0·31	} 45 minutes
b	0·98	0·218	6·23	0·19	
C	1·96	0·06	5·00	0·07	} 45 + 90 minutes
c	1·66	0·07	5·60	0·07	
D	2·58	0·036	4·60	0·02	} 45+90+180 minutes
d	1·88	0·012	5·42	0·01	

This experiment shows that it is *the early amylolysis which is accelerated under the influence of chloroform.*

That *the amylolysis, subsequent to the destruction of the cells, is not materially influenced by chloroform is shown by the next two experiments.*

EXPERIMENT 20.   7.12.92.

Rabbit killed at 12.55 P.M.   30 grms. of liver pounded in mortar with washed sand, extracted with 0·75 per cent. NaCl solution, and squeezed through calico.   1 grm. glycogen dissolved in 0·75 per cent. NaCl solution was added to the extract, and the whole made up to 210 cub. centims.   This was divided into :—

A.   70 cub. centims. placed at once in boiling water.

B.   70 cub. centims. placed in 0·75 per cent. NaCl with stream of chloroform vapour.

C.   70 cub. centims. placed in 0·75 per cent. NaCl with stream of air.

B and C kept at 40° till 9 P.M. (8 hours).

Glycogen extracted by BRÜCKE's method.

A.   Glycogen = 0·656 grm.

B.   ,,   = 0·465   ,,

C.   ,,   = 0·499   ,,

EXPERIMENT 21. 28.11.92.

Rabbit killed at 1 P.M. 32·7 grms. of liver pounded in mortar extracted with 0·75 per cent. NaCl solution, and filtered through cotton cloth. 1 grm. of glycogen dissolved in 5·70 per cent. NaCl solution was added to this extract, and the whole made up to 250 cub. centims. Of this,

A. 80 cub. centims. were placed at once in boiling water.
B. 80 cub. centims. treated with stream of chloroform vapour.
C. 80 cub. centims. treated with stream of air.

B and C kept at 40° C. till 5 P.M. (4 hours).

Glycogen extracted by BRÜCKE's method.

A. Glycogen = 0·507 grm.
B.   ,,   = 0·480 ,,
C.   ,,   = 0·421 ,,

ACTION of Chloroform on Glycogen of Liver in Living Animal.

The practical importance of the glycæmia and glycosuria of chloroform poisoning induced me to undertake some observations on the effect of the prolonged administration of the drug on the glycogen of the liver of living animals.

To arrive at definite results from such experiments is by no means easy. In the first place, the glycæmia may be due to an increased conversion of glycogen, but this may be masked by a concomitant increased formation of the substance in the liver. Again, the impossibility of securing anything like an equality in the amount of glycogen in the liver of two or more animals, even when kept for long on the same diet and in the same conditions, diminishes the value of comparison between the liver of poisoned and unpoisoned animals.

ROHMANN ('Pflüger's Arch.,' vol. 39, p. 21), for his experiments on the influence of ammonia on the hepatic glycogen, used two rabbits kept on the same diet and under the same conditions for some time, to one of which ammonia was given while the other was used as a check; and these experiments indicate that with care the amount of glycogen in the livers of two animals may be made approximately the same.

My observations show that the glycogen in the liver of adult animals kept on the same diet and in the same conditions does not vary greatly in amount; provided always the animals are either maintaining their weight or gaining weight in the same proportion, and *that the period between death and boiling the liver is sufficiently short.*

This is indicated by the following experiments :—

EXPERIMENT 22. 6.5.87.

Two young brown doe rabbits, procured on 26.4.87, and placed in separate cages, on diet of oats and water.

	Weight in grms.	
	A.	B.
26.4	1063	1134
29.4	1105	1134
30.4	1105	1134
2.5	1105	1077
3.5	1105	1105
5.5	1134	1134
6.5	1134	1105

The rectal temperature of both, on the 6th, was 39° C.

Both killed in the usual manner.

A. Liver, without gall bladder, weighed 39·3 grms., of which 30 grms. were taken for analysis.

B. Liver   ,,   ,,   ,,   weighed 29·72 grms., of which all was taken for analysis.

Several more minutes elapsed between the death of the animal and the boiling of the liver in B than in A.

Glycogen estimated by LANDWEHR's method ('Ztsch. f. physiol. Chemie,' vol. 8, p. 170).

Glucose estimated by evaporating the filtrate from the iron precipitate, and titrating with FEHLING's solution.

$$A. \quad \text{Glycogen} = 0·24 \quad \text{grm.} = 0·8 \quad \text{per cent.}$$
$$\text{Glucose} = 0·0526 \quad ,, \quad = 0·175 \quad ,,$$
$$\text{Total carbohydrates} = 0·2926 \quad ,, \quad = 0·975 \quad ,,$$

$$B. \quad \text{Glycogen} = 0·15 \quad ,, \quad = 0·5 \quad ,,$$
$$\text{Glucose} = 0·14 \quad ,, \quad = 0·471 \quad ,,$$
$$\text{Total carbohydrates} = 0·29 \quad ,, \quad = 0·971 \quad ,,$$

EXPERIMENT 23.

Two young white doe rabbits, put on diet of oats and water, on May 25th, 1887.

Date.	Weight, in grms.		Remarks.
	A.	B.	
27.5	708·7	623·7	Each took same amount
28.5	680·4	595·3	of food.

On May 28th they were killed in the usual manner, the blood being caught in a known volume of water.

A yielded 18 cub. centims. of blood.

Liver weighed 20·5 grms.

B yielded 18 cub. centims. of blood.

Liver weighed 18·7 grms.

Sugar of blood estimated by SEEGEN's method.

Glycogen of liver estimated by LANDWEHR's method, and sugar estimated as in last experiment.

A. *Blood*   Glucose = 0·106 per cent.
    Liver   Glycogen = 0·53   ,,
           Glucose = 0·231   ,,
  Total carbohydrates = 0·761   ,,

B. *Blood*   Glucose = 0·105   ,,
    Liver   Glycogen = 0·48   ,,
           Glucose = 0·300   ,,
  Total carbohydrates = 0·780   ,,

The results of the succeeding experiments fully confirm these observations.

If, however, the animals are not kept on a similar diet, and if they are not either steadily and uniformly gaining, losing, or maintaining weight, or if the greatest precautions are not taken to make the times between killing the animal and boiling the liver of short and equal duration, the greatest variations in the percentage of hepatic glycogen will be found.

In the following experiment the fullest precautions were taken to observe the above conditions :—

EXPERIMENT 24.

From a litter of nine young rabbits, three which were gaining weight were selected.

WEIGHT in Grms.

	5.9.92.	6.9.92.	7.9.92.
A	113	112	116
B	150	150	155
C	122	120	123

A was used as a check.

B was kept under chloroform from 11.30 to 2.20 when it was allowed to recover.

C had 0·01 grm. of bimeconate of morphin at 11.30 and 0·02 grm. at 12, but was never markedly under the influence of the drug.

A was killed in the usual way at 2.56 and the liver excised and thrown into boiling water at 2.58. It weighed 3·2 grms.

B was killed at 2.31 and the liver thrown into boiling water at 2.33. It weighed 5·2 grms.

C was killed at 3.25 and the liver thrown into boiling water at 3.27. It weighed 3·4 grms.

Glycogen extracted by BRÜCKE's method.

A. Weight of glycogen = 0·046 grm. = 1·437 per cent.
B.   ,,   ,,   = 0·039   ,,   = 0·75   ,,
C.   ,,   ,,   = 0·047   ,,   = 1·385   ,,

EXPERIMENT 25.

From a litter of nine young rabbits, three which were losing weight were selected.

WEIGHT in Grms.

	5.9.92.	6.9.92.	7.9.92.
A.	120	117	114
B.	100	98	96
C.	157	151	150

A was used as check.

B was kept under chloroform from 11.30 to 2.20 when it was allowed to recover.

C had 0·01 grm. of bimeconate of morphin at 11.20 and at 12. At 2 P.M. it was in a deep sleep.

A was killed at 3 and the liver was thrown into boiling water at 3.1. It weighed 3·2 grms.

B was killed at 3.34 and the liver thrown into boiling water at 3.36. It weighed 2·8 grms.

C was killed at 3.28 and the liver thrown into boiling water at 3.30. It weighed 4 grms.

Glycogen estimated by BRÜCKE's method.

$$\text{A. Weight of glycogen} = 0·012 \text{ grm.} = 0·375 \text{ per cent.}$$
$$\text{B.} \quad ,, \quad ,, \quad = 0·003 \quad ,, \quad = 0·107 \quad ,,$$
$$\text{C.} \quad ,, \quad ,, \quad = 0·016 \quad ,, \quad = 0·40 \quad ,,$$

EXPERIMENT 26.

Two young rabbits were bought on October 16th, 1892, and were kept in same cage and on same food till November 9th, being weighed each day.

WEIGHT in Grms.

	17.10.	19.10.	22.10.	25.10.	28.10.	31.10.	3.11.	6.11.	9.11.
A . . .	1220	1300	1300	1413	1430	1490	1600	1550	1560
B . . .	970	1002	1070	1085	1097	1120	1230	1185	1200

No food was given after morning of 8th.

At 11.30 on the 9th, A was chloroformed and was kept under the drug till, at 3.30, death ensued. The abdomen was rapidly opened, and the hepatic vein cut to deplete the liver. The liver weighed 64 grm., of which 26·2 grm. were taken for analysis by BRÜCKE's method.

$$\text{Weight of glycogen} = 0·436 \text{ grm.} = 1·664 \text{ per cent.}$$

B was killed at 3.50. The liver weighed 54 grm., of which 25·7 grm. were taken for analysis by BRÜCKE's method.

$$\text{Weight of glycogen} = 1·005 \text{ grm.} = 3·91 \text{ per cent.}$$

URINE taken from Bladder *post mortem.*

	Yeast.	Fehling.
A . . .	Marked fermentation	Marked reduction
B . . .	No ,,	Slight ,,

EXPERIMENT 27.

From young rabbits of one brood, bought on October 16th, 1892, and kept upon the same diet, being weighed daily till November 9th.

WEIGHT in Grms.

	17.10.	19.10.	22.10.	25.10.	28.10.	31.10.	3.11.	6.11.	9.11.
A . . .	1410	1424	1330	1532	1497	1550	1712	1660	1550
B . . .	1243	1245	1350	1325	1360	1440	1480	1460	1450
C . . .	1410	1450	1425	1432	1492	1520	1648	1625	1630
D . . .	1261	1240	1297	1262	1285	1352	1405	1360	1360

No food was given after the morning of the 8th November.

On November 9th, at 11.30, B, C, and D were chloroformed; B died suddenly at 12.30, C. died at 12.45. Immediately after death in each case the hepatic vein was opened, and the liver bled. D was killed at 3.40, and A at 4 P.M.

Liver of A = 51·5 grm., of which 20 were taken for analysis.

,,   B = 50   ,,   ,,   all was   ,,   ,,

,,   C = 54·5   ,,   ,,   27·5 were taken for analysis.

,,   D = 40·2   ,,   ,,   18·5   ,,   ,,

Analysis of glycogen by BRÜCKE's method.

A. Glycogen = 0·133 grm. = 0·665 per cent.

B.   ,,   = 0·358   ,,   = 0·716   ,,

C.   ,,   = 0·16   ,,   = 0·582   ,,

D.   ,,   = 0·017   ,,   = 0·091   ,,

URINE taken from Bladder *post mortem.*

	Yeast.	Fehling.
A . . .	No fermentation	Slight reduction
B . . .	,, ,,	,, ,,
C . . .	Slight fermentation	,, ,,
D . . .	Marked ,,	,, ,,

EXPERIMENT 28. 4.10.92.

Four young pups of one litter were weighed on October 4th.

A = 443 grm.

B = 457 grm.

C = 501 grm.

D = 440 grm.

C was rejected as not corresponding to the others in weight.

D was put under chloroform at 11 P.M., and kept under till 3.6 P.M.   The liver weighed 18·4 grm., and all was taken for analysis.

A was killed at 3.12 P.M.   The liver weighed 18·1 grm., and was all analyzed.

B was killed at 3.21 P.M.   The liver weighed 18·3 grm., and was all taken for analysis.

Glycogen extracted by BRÜCKE's method.

A. Weight of glycogen = 0·258 grm. = 1·425 per cent.

B.   „   „   = 0·183   „   = 1·000   „

D.   „   „   = 0·203   „   = 1·103   „

URINE taken from Bladder *post mortem.*

	Yeast.	FEHLING.
A . . .	No fermentation	No reduction
B . . .	„ „	„ „
D . . .	Slight fermentation	Not enough to test

SUMMARY of Chloroform Experiments on Living Animals.

Experiment.	Kind of animal.	Check animal.	Chloroformed for 3 or 4 hours.
24	Rabbit	1·437	0·75
25	„	0·375	0·016
26	„	3·91	1·664
27	„	0·665	0·091
28	Dog	{ 1·425 { 1·000	1·103

These experiments, although they must be accepted with caution, seem to indicate that, *in the living animal under the influence of chloroform, glycæmia and glycosuria are produced by an increased conversion of glycogen to glucose.*

In Experiment 28, in which young dogs were employed, the result was negative.

NEBELTHAU (' Ztsch. f. Biol.,' vol. 28, p. 138) attempts to prove that chloroform, as well as chloral hydrate, chloralamid, paraldehyd, ether, alcohol, and sulphonal, increases the amount of hepatic glycogen, but his experiments disprove rather than prove his

contention. He employed hens starved for six days. Külz had previously shown that, in these conditions, the amount of hepatic glycogen does not exceed 0·95 per cent. While, after the administration of chloral the hepatic glycogen was enormously increased from 1·22 per cent. to 5·12 per cent., the following results with chloroform show a *diminution* rather than an increase.

Duration of fast.	Amount of chloroform.	Duration of life after the first administration of chloroform.	Per cent. of glycogen in liver.
days.	cub. centims.	Hours.	
6	1·5	24	Trace.
6	0·5	24	0·42
6	1·5	26	0·60
6	1·5	18	1·49
6	1·25	14	1·40
6	1·0	15	0·68

Average = 0·76.

To what is this increased conversion due? Chloroform may act directly upon the liver cells, or, by impairing the oxidative changes in the liver.

That diminished oxidation does cause glycæmia and glycosuria has been shown by Dastre and more recently by Araki ('Ztsch. f. physiol. Chem.,' vol. 15, 335 and 546) and by Zillesen (*ibid.*, p. 387).

According to Lépine and Barral ('Compt. Rend.,' 23 Juin, 1890) this is due to a diminution in the glycolytic power of the blood. They, however, made no experiments to exclude the possibility of there being, at the same time, an increase in the amylolysis in the liver.

That an imperfect supply of oxygen has no direct effect on the rate of hepatic amylolysis seems to be indicated by the following two experiments on the excised liver.

Experiment 29.

Rabbit killed at 11 A.M. on 28.4.92. Liver cut up into A, B and C.

    A. Weighed 17·5 grms.. placed in boiling water at once.

    B.   ,,   24·8   ,,   ,,   0·75 per cent. salt solution at 38° C.

    C.   ,,   24·5   ,,   ,,   ,,   ,,   ,,   ,,

A continuous stream of air was kept up through B, while C was left at rest. Both were kept at from 36° to 40° C. till 1 P.M. (2 hours), and then boiled.

Glycogen by Brücke's method.

    A.   Weight of glycogen = 1·639 grm. = 9·36 per cent.

    B.   ,,   ,,   = 1·175 ,, = 4·73 ,,

    C.   ,,   ,,   = 1·123 ,, = 4·58 ,,

EXPERIMENT 30.

Rabbit killed at 12.53 P.M. on 23.1.93.   Liver cut up.

A weighed 6·2 grms. (analysis lost).

B   „   7·3 grms.

C   „   7·2   „

D   „   8·0   „

Each placed in 150 cub. centims. of 0·75 per cent. salt solution at 37° C.   The salt solution of B had been previously sterilized.   Through D a stream of hydrogen was passed for ten minutes, and the tubes were then sealed.   All the bottles were placed in the incubator till 5 P.M. (4 hours) and then boiled.

B.   Weight of glycogen = 0·273 grm. = 3·73 per cent.

C.   „   „   = 0·26   „   = 3·61   „

D.   „   „   = 0·296   „   = 3·70   „

The idea that the increased conversion of glycogen under the influence of chloroform is due to an impeded oxidation in the liver is, therefore, without experimental basis.

A digestive action of chloroform on proteids has been described by DENYS and MARBAIS ("Sur les peptonisations provoquées par le chloroforme." 'La Cellule,' vol. 15, p. 197; 'Nouvelles recherches sur la digestion chloroformique,' *ibid.*, I., 16, p. 4.)   These experiments tend to show that the hæmoglobin and the fibrin of dog's blood, whether in the serum of that animal, or in chloride of sodium solution of about 7 per cent. upon the addition of chloroform, ether, alcohol, thymol, or phenol, undergo a process of peptonisation.   They conclude, "La digestion chloroformique peut s'expliquer de deux façons ou bien par une action directe du chloroforme, ou bien par une zymase développée par ce dernier.   C'est la première hypothèse qui paraît la plus probable."

A study of the experiments on the influence of temperature upon this process tends, however, to the view that it is not a simple direct action of chloroform.   The digestion of entire blood, or of the hæmoglobin in it, is retarded by a temperature of even 45° C., and arrested by a temperature of 60° C., while the digestion of fibrin, which had not been subjected to a high temperature in serum previously boiled or deprived of its proteid by heating, does not exclude a possible action upon some substance *held in the fibrin*.

Perhaps the most inexplicable part of these researches, on the view adopted by them, is, that dog's fibrin is not digested in the serum of the ox, sheep, pig, or horse, on the addition of chloroform, *unless the serum has been previously boiled—i.e.,* unless it has ceased to be the characteristic serum.   The whole series of experiments, though interesting, are highly inconclusive, and throw little light upon the present series of observations.

The above experiments, especially when taken in connection with the observations on the influence of chloroform on the morphological changes in the liver cells

(see p. 248), seem to indicate that the *chloroform acts by accelerating the katabolic changes which accompany or immediately precede the death of the cells, and that it is as the result of these katabolic changes that we find the enormous conversion of glycogen.* That chloroform has this action in increasing katabolic changes is borne out by its influence on the disintegration of proteids. TANIGUTI ('Virchow's Archiv,' vol. 120, p. 121), after giving a *résumé* of the previous work on this subject, records his own experiments, which clearly show that chloroform does increase the excretion of nitrogen.

This view receives support from the mode of action of other agents on the liver cells, and on hepatic amylolysis.

4. *Ether.*

Although I am not aware that the administration of ether has been observed to be followed by glycosuria, the similarity of its action to that of chloroform seemed to render it desirable that its influence on hepatic amylolysis should be tested. The following experiments were accordingly performed.

EXPERIMENT 31. 17.11.91.

Rabbit killed at 2.5 P.M. Liver minced and divided into three parts.
A weighed 16·4 grms. placed in boiling water.
B   „   27·2   „   0·75 per cent. NaCl solution with stream of air.
C   „   25·7   „   „   „   „   „   ether vapour.
B and C kept at 40° C. till 4.25 (2 hours 20 minutes).
Glycogen extracted by BRÜCKE's method.

$$A = 7·88 \text{ per cent.}$$
$$B = 6·40 \text{ „}$$
$$C = 5·87 \text{ „}$$

EXPERIMENT 32. 5.1.93.

Rabbit killed at 12.54 P.M. Liver minced and divided into three parts.
A weighed 10·0 grms. placed in boiling water at once.
B   „   11·9   „   „   0·75 per cent. solution of NaCl with stream of ether.
C   „   10·2   „   „   „   „   „   „   „   air.
B and C kept at 40° C. till 9 P.M. (eight hours).
Glycogen extracted by BRÜCKE's method.

$$A. \text{ Glycogen} = 0·371 \text{ grm.} = 3·71 \text{ per cent.}$$
$$B. \quad „ \quad = 0·118 \text{ „} \quad = 0·991 \text{ „}$$
$$C. \quad „ \quad = 0·271 \text{ „} \quad = 2·650 \text{ „}$$

Ether, like chloroform, increases the amylolysis, but its action is much less decided. Like chloroform, it causes the early development of the cell changes described on p. 248; but in this respect, too, it is less powerful than chloroform.

5. *Pyrogallic Acid and Salicylate of Soda.*

The well-known influence of pyrogallic acid in accelerating the proteid waste and increasing the output of nitrogen (NOËL PATON, 'Brit. Med. Jour.,' 1886), and its marked destructive influence on hæmocytes, led me to investigate whether its action on hepatic amylolysis and on the liver cells corresponds to that of chloroform.

Salicylic acid, which in some of its actions resembles pyrogallic acid, was tried at the same time.

EXPERIMENT 33.   12.6.93.

Rabbit killed at 1.10 P.M.   Liver divided into four, A, B, C, D.
A weighing 5 grms., was placed in boiling water at 1.11.

B „ 10 „ „ „ 0·25 per cent. pyrogallic acid dissolved in 0·75 per cent. NaCl.
C „ 8·5 „ „ „ 0·75 „ NaCl.
D „ 10 „ „ „ 0·5 „ salicylate of soda in 0·75 per cent. NaCl.

B, C, and D, kept at 40° C. till 2.30, then boiled.
Glycogen by BRÜCKE's method.

A Glycogen = 0·185 grm. = 3·70 per cent.
B „ = 0·165 „ = 1·65 „
C „ = 0·157 „ = 1·84 „
D „ = 0·216 „ = 2·16 „

Pyrogallic acid thus slightly increases hepatic amylolysis and at the same time it produces a slight acceleration of the changes in the protoplasm similar to those produced by chloroform. Salicylate of soda in 0·5 per cent. solution does not accelerate these changes, nor does it increase hepatic amylolysis.

Among the substances the administration of which causes glycosuria are morphin, nitrite of amyl, and curare.

The following experiments were made on the influence of these bodies on the amylolysis of the excised liver :—

6. *Morphin.*

EXPERIMENT 34.   26.4.92.

Rabbit killed at 11 A.M.   Liver minced and divided into parts A, B, and C.
A weighing 22·5 grms. was placed in boiling water at 11.3 A.M.
B „ 23·0 „ „ about 150 cub. centims. of 0·75 per cent. NaCl in which 10 mgs.
of bisuccinate of morphia was dissolved.
C weighing 21·8 grms. was placed in 0·75 per cent. NaCl.
B and C were kept at from 37° to 40° C. till 5 P.M. (6 hours).
Glycogen extracted by BRÜCKE's method.

A Glycogen weighed 0·861 grm. = 3·826 per cent.
B   ,,   ,,   0·310 ,, = 1·35   ,,
C   ,,   ,,   0·369 ,, = 1·69   ,,

EXPERIMENT 35. 16.5.92.

Rabbit killed at 11 A.M. Liver minced and divided into parts A, B, and C.

A weighing 16 grms. was placed at once in boiling water.

B   ,,   15·6   ,,   ,, in 0·75 per cent. NaCl in which was dissolved 50 mgs. of hydrochlorate of morphia.

C weighing 17·0 grms. was placed in 0·75 per cent. NaCl.

B and C kept at 40° C. till 4 P.M. (5 hours).

Glycogen extracted by BRÜCKE's method.

A Glycogen weighed 0·518 grm. = 3·231 per cent.
B   ,,   ,,   0·211 ,, = 1·352   ,,
C   ,,   ,,   0·202 ,, = 1·188   ,,

Morphia has no influence on hepatic amylolysis in the excised liver. Experiments 24 and 25 tend to show that it is also without action in the living animal.

7. *Nitrite of Amyl.*

With nitrite of amyl only one experiment was performed.

EXPERIMENT 36. 24.8.92.

Rabbit killed at 11.10 A.M. Liver divided into three parts A, B, C.

A weighing 14·5 grms. placed in boiling water at 11.12.

B   ,,   12   ,,   was placed in 0·75 per cent. NaCl. at 40° C.

C   ,,   12·5 ,,   ,,   ,,   ,,   ,,   ,,

A stream of air was passed through B and C, that through C being saturated with nitrite of amyl. At 3.15 both were boiled.

Glycogen by BRÜCKE's method.

A Glycogen = 1·324 per cent.
B   ,,   = 0·720   ,,
C   ,,   = 0·704   ,,

Nitrite of amyl is therefore also without action on hepatic amylolysis.

8. *Curare.*

With curare a single experiment also was performed.

2 M 2

EXPERIMENT 37. 12.9.92.

A very large, fat rabbit killed at 11.25 A.M. Three portions of liver taken.
A weighing 21 grms. was placed in boiling water at 11.28.
B   „   26   „   „   0·75 per cent. NaCl.
C   „   25   „   „   about 150 cub. centim. of 0·75 per cent. NaCl. containing about
2 mgs. of curare which was known to be active.
B and C kept at 35° C. till 5 P.M. (5½ hours), then boiled.
Glycogen extracted by BRÜCKE's method.

A   Glycogen weighed 3·42   grms. = 16·29 per cent.*
B   „   „   3·313   „   = 12·74   „
C   „   „   3·315   „   = 13·26   „

From these observations it would seem that *while chloroform and ether increase the amylolysis and thus produce glycæmia, nitrite of amyl, morphin, and curare act in some other way, probably, as suggested by* ARAKI, *by diminishing the oxidation changes in the tissues.*

In connection with this it may be mentioned that none of these bodies produce the rapid changes in the protoplasm of this liver cell which are produced by chloroform and ether.

II. IS THERE ANY DIFFERENCE IN THE PRODUCT OF HEPATIC AMYLOLYSIS IN THE EARLY AND IN THE LATER PERIODS?

NATURE OF THE PRODUCTS OF AMYLOLYSIS IN THE EARLY AND IN THE LATER PERIODS.

Upon the nature of the sugar found in the *post-mortem* liver, several investigations have been made.

NASSE (' Pflüger's Archiv,' vol. 14, p. 473) describes the sugar of the liver as one which, when boiled with sulphuric acid, acquires no increase in its reducing power, and thus resembles glucose.

MUSCULUS and VON MERING (' Ztsch. f. phys. Chem.,' vol. 2, p. 416) confirmed NASSE's observations. They, however, found maltose. Dextrin, however, was not discovered. The method they adopted was to extract the liver with water, evaporate the extract, and extract with alcohol, and then precipitate with ether.

SEEGEN ('Studien ü. d. Stoffwechsel,' p. 392) points out that the method of MUSCULUS and MERING does not exclude certain dextrins which are precipitated with ether, and gives experiments which tend to show that the maltose of these investigations is merely a mixture of dextrin and glucose. By dialysis he separates a sugar

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* This is the largest amount of glycogen I have ever found in the liver of a rabbit.

which gave the reaction of glucose, and he concludes " dass Leberzucker aus-schliesslich Traubenzucker ist."

In a previous paper (*loc. cit.*, p. 383) he, however, says : " Wenn das Dialysat auf eine kleine Menge eingeengt und jetzt soviel absoluter Alcohol zugefügt war, bis die ganze Flüssigkeit einen ca. 90 proc. Alcohol bildete, entstand ein reicher weisser Niederschlag," and it is therefore somewhat surprising to find him using this method to differentiate dextrin from maltose. MUSCULUS and MEYER (' Bull. Soc. Chim.,' vol. 35, p. 370) also state that dextrin is slightly diffusible.

LIMPRICHT (' LIEBIG's Annalen,' 133–293, quoted by NASSE) extracted from 200 lbs. of the liver of the horse as much as 400 grms. of dextrin.

KÜLZ (PFLÜGER's ' Arch.,' vol. 24, p. 52) severely criticizes SEEGEN's paper, and confirms NASSE's results. He says : " Ob Dextrin und Maltose in der todtenstarren Hundeleber vorkommen oder fehlen, wage ich vorläufig mit Sicherheit nach keiner Seite hin eine Behauptung auszusprechen, bemerke jedoch dass für mich nur die Darstellung dieser Körper in Substanz beweisskräftig ist."

MUSCULUS and VON MERING (' Ztsch. f. phys. Chem.,' vol. 4, p. 93) attack SEEGEN's results, and show that his methods are unsatisfactory, though they do not give any experimental evidence in support of their assertion as to the presence of maltose, nor attempt to answer directly SEEGEN's criticism on their methods.

The question stands thus :—All investigators admit that the chief sugar of the excised liver is glucose. LIMPRICHT has definitely shown that dextrin may be present. MUSCULUS and VON MERING hold that maltose is also to be found.

A general consideration of the relationships of the lower dextrins to such di-saccharids as maltose clearly shows that a complete separation is most difficult, if not impossible. Many dextrins are partly soluble even in alcohol, much over 90 per cent. ; maltose is less soluble in strong alcohol than glucose ; dextrin in alcoholic solution, as well as maltose, is precipitated by ether, and forms a similar potash com-bination ; the osazone of dextrin is soluble in water, while that of maltose is only *somewhat* less soluble ; lastly, dextrin, though less diffusible than maltose, does undoubtedly pass through a dialyzing membrane.

For these reasons, after several unsuccessful attempts, the endeavour to separate the lower dextrins from maltose was abandoned, and attention was concentrated on the question of whether, along with glucose, dextrin, and maltose, or one or other of these, are produced at all periods in the liver excised from the body.

Method.

The liver was extracted with boiling water exactly as in the estimation of glycogen ; the hot aqueous extract was then precipitated with chloride of iron and acetate of soda, carbonate of soda being added to the point of neutralization. In this way, not only the proteids, but also glycogen, as shown by LANDWEHR (' Ztsch. f. phys. Chem.,' vol. 8) are thrown down. The clear supernatant fluid containing the sugars and

dextrins (LANDWEHR, *loc. cit.*, p. 170*) was filtered off and, 1st, tested for dextrin, by the addition of absolute alcohol, to 90 per cent.; 2nd, tested for sugar as glucose, by FEHLING'S solution; and, 3rd, a given volume was boiled for some time with 2 per cent. H_2SO_4, then neutralized, made up to its original volume, and the glucose again estimated with FEHLING'S solution.

To determine if the process is the same, during the rapid initial and during the slow later amylolysis, livers kept in 0·75 per cent. salt solution at from 37 to 40° C. were tested during the first hour, and, at the end of eight or ten hours in the above manner :—

A marked difference was found.

During the first hour :—

No precipitate was given with alcohol; dextrins were absent.

The reduction was exactly the same before and after boiling with H_2SO_4.

After several hours :—

There was usually a marked precipitate with alcohol, though no reaction with iodine. An achroö-dextrin, but no erythro-dextrin, was present. The reduction was usually markedly increased after boiling with H_2SO_4. Dextrin and maltose, or dextrin alone, were present.

The increase in the reducing power after boiling with sulphuric acid is usually so great that it cannot be due to a conversion of maltose to glucose, but must arise from the change of dextrin to glucose.

This is well shown in Experiment 40 which was performed to elucidate the influence of the acid in the liver. One part of the liver had been digested for ten hours with a weak alkali, carbonate of soda 0·06 per cent., while the other part was kept in salt solution and the acid allowed to develop. In the former, the reduction before boiling was equivalent to that produced by 2·8 per cent. of glucose; after boiling with H_2SO_4, it was equal to 3·2 per cent. In the latter, before boiling, the reduction was equivalent to that produced by 2·0 per cent. glucose, after treatment with an acid to 2·3 per cent. glucose.

These observations show that during the early amylolysis the product is glucose, during the later amylolysis intermediate products such as dextrin and possibly maltose also are formed.

III. NATURE OF HEPATIC AMYLOLYSIS.

We are now in a position to consider the nature of the early and of the later amylolysis in the excised liver.

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* NASSE (PFLÜGER'S 'Archiv,' vol. 37, p. 582) says that achroö-dextrin is precipitated with iron; but his objections are met by LANDWEHR (PFLÜGER'S 'Archiv,' vol. 38, p. 321). My own observations on erythro-dextrin and the achroö-dextrin formed from glycogen confirm LANDWEHR'S results.

A. EARLY AMYLOLYSIS.

Is the early, rapid change the result of the action of a ferment, or is it due to changes in the liver cells?

The *rapidity* with which it occurs is entirely opposed to the idea of its being due to the amylolytic ferment which may be extracted from the liver as from other organs, and which, according to all observers, acts very slowly.

The fact that the mechanical destruction of the liver-cells so markedly inhibits the process is also strongly opposed to the idea that the essential agent is a soluble ferment.

The connection of the process with the condition of the liver cells favours the view that the activity of the cells is the agent causing the amylolysis.

The influence of temperature and of fluoride of sodium, the increased amylolysis under chloroform, ether, and pyrogallic acid which accelerate the katabolic changes in the liver cells, and the absence of increased amylolysis with morphin, curare, nitrite of amyl, and salicylate of soda, seem to point to intra-cellular changes being the important factor in this early period.

Finally, the direct production of glucose, without intermedial bodies, marks off the changes at this period from those occurring later.

This early change in the excised liver is simply a continuation of the vital process in the organ, though the katabolic side of the metabolism is exaggerated, and the anabolic in abeyance.

In fact, all the evidence is in favour of the view that *the conversion of glycogen to glucose is precisely the same as the conversion of mucinogen to mucin or of zymogens to zymin. And there is no more reason to invoke the agency of a ferment in the explanation of the former than of the latter processes.*

B. LATER AMYLOLYSIS.

But when we come to consider the later amylolytic changes, which do undoubtedly go on in the liver after the death and destruction of the cells, the question becomes more involved. Is the change due to the *acid* which makes its appearance, to the agency of *micro-organisms,* or to the development of a soluble ferment, or *zymin?*

1. *Action of Acid.*

As has been shown by SEEGEN and other observers, the post-mortem liver becomes more and more markedly acid. SEEGEN (*loc. cit.*, p. 40) shows that lactic acid is present.

This acidity is largely due to the action of micro-organisms, as is shown by the following experiment.

EXPERIMENT 38.

The liver of a rabbit freshly killed was excised and divided up, the instruments and hands having been washed in methylated spirit, and every precaution to prevent ingress of organisms taken.

A was placed in 0·75 per cent. salt solution.

B   „   salt solution, with chloroform.

C   „   salt solution, previously sterilized.

All were kept at 40° C. for eight hours.

At the end of this—

A was markedly acid.

B was neutral.

C   „

Gelatine tubes inoculated from—

A showed in twelve hours a most active growth of a liquefying organism.

B showed no growth after two weeks.

C showed a growth after three days of an organism resembling Bacillus subtilis in its characters, but forming a thicker and more wrinkled pellicle.

SEEGEN demonstrated that lactic acid, when boiled with glycogen, converts it to dextrin, but DASTRE (*loc. cit.*, p. 93) finds that glycogen in presence of lactic acid at 40° C. for twelve to sixteen hours is not changed to sugar.

I find that glycogen may be kept in the presence of lactic acid, of considerably greater strength than is found in the post-mortem liver, for two days without undergoing any change.

On the third day a slight reduction with FEHLING may occur. Tubes inoculated from the flask gave no growth of micro-organisms, so the action could only have been due to the acid. If the acid is boiled with glycogen, a similar slight reduction is got after twelve hours.

From these facts it seems highly improbable that the presence of the organic acids can have any action in the late hepatic amylolysis. To determine this point the following experiment was performed.

EXPERIMENT 39.   10.5.93.

A rabbit, which had on the previous day thrown a litter of young, was killed at 11.35 A.M. In excising the liver the animal's skin, the hands, instruments, scale pan, &c., were well washed with perchloride of mercury, and then with methylated spirit. The liver was not allowed to come in contact with the skin of the animal or with any body not sterilized. The flasks into which the organs were placed, had, with their contents, been previously sterilized.

A weighing 7·1 grms. was placed in boiling water at 11.37.

B   „   8·5   „   „   about 150 cub. centims. of 0·75 per cent. NaCl.

b   „   11·3   „   „   „   „   „   „   to which carbonate of soda had been added to 0·06 per cent.

C weighing 11·0 grms. was placed in about 150 cub. centims. 0·75 per cent. NaCl.

c   „   12·5   „   „   „   „   „   „   to which carbonate of soda had been added to 0·06 per cent.

B and b were kept in the incubator at 40° C. till 1.20 P.M., about 1 hour.

C and c were kept at 40° C. till 9 P.M., about $8\frac{1}{2}$ hours.

Before boiling, gelatine tubes were inoculated from each. All remained sterile except C, in which a slight growth of a coccus appeared after three days. C was markedly acid with a sour smell. c was neutral and devoid of putrefaction or a sour smell.

Glycogen was extracted by BRÜCKE's method.

A Glycogen weighed 0·181 gram. = 2·54 per cent.
B „ „ 0·106 „ = 1·24 „
b „ „ 0·14 „ = 1·23 „
C „ „ 0·11 „ = 1·00 „
c „ „ 0·149 „ = 1·19 „

EXPERIMENT 40. 29.5.93.

Rabbit killed at 12.11, and the liver divided in the usual manner.

A weighing 6·9 grms., was placed in boiling water at 12.13.

B „ 12·0 „ „ „ at 12.15 in 0·75 per cent. NaCl to which carbonate of soda to 0·06 per cent. was added.

C weighing 11·6 grms. was placed in 0·75 per cent. NaCl at 12.15.

B and C were kept at 40° C. in the incubator till 9 P.M., and then boiled. B had a strong odour of liver, and was neutral. C had a marked sour putrefaction odour, and was strongly acid. Gelatine tubes were inoculated from each. C gave a strong growth of organisms (Bacillus subtilis) in two days.

B gave a slight growth in four days.

The watery extract of each part of the liver after evaporation was divided into equal parts.

From one part glycogen was extracted by BRÜCKE's method. In the second part the glucose was estimated in the usual manner.

To 2 cub. centims. of the solution after the separation of glycogen, absolute alcohol was added to 90 per cent.

A gave no precipitate.

B and C gave slight precipitate on standing, indicating the presence of dextrins.

None gave any reaction with iodine.

A Glycogen weighed 0·147 = 4·3 per cent.
B „ „ 0·091 = 1·5 „
C „ „ 0·095 = 1·6 „

Sugar (as glucose) determined by FEHLING's solution. The suboxide precipitated very perfectly.

A 44·5 cub. centims. gave no complete reduction of 2 cub. centims. of FEHLING's solution.

Sugar as glucose less than 0·2 per cent.

B 5·8–6·0–5·8 = 5·8 cub. centims. gave complete reduction of 2 cub. centims. of FEHLING's solution.

Sugar as glucose = 2·8 per cent.

C 12·8–12·4 = 12·6 cub. centims. gave complete reduction of 2 cub. centims. of FEHLING's solution.

Sugar as glucose = 2·0 per cent.

To 40 cub. centims. of B and 50 cub. centims. of C a few drops of H_2SO_4 were added in flasks which were boiled in the water bath for $2\frac{1}{2}$ hours. The contents of each flask were then neutralized and made up to the original volume, and the glucose estimated by FEHLING's method.

Both gave a very perfect precipitation of the suboxide.

B 4·8–5·0–5·0 (5·0 cub. centims.) completely reduced 2 cub. centims. of FEHLING's solution.

Glucose = 3·2 per cent.

C 10·7–10·8 (10·8 cub. centims.) completely reduced 2 cub. centims. of FEHLING's solution.

Glucose = 2·3 per cent.

	Glycogen.	Sugar as glucose.	Glucose, after boiling with acid.
	per cent.	per cent.	
A	4·3	less than 0·2	—
B	1·5	2·8	3·2
C	1·6	2·0	2·3

The smaller amount of sugar in C is probably due to the action of micro-organisms in destroying it.

These two experiments very clearly show that both *the early and the later amylolysis are independent of the development of an acid in the liver.*

The latter further shows that the peculiar indirect amylolysis of the later period proceeds in the same manner whether the acid be allowed to develop or not.

2. *Action of Micro-organisms.*

DASTRE (*loc. cit.*) maintains that these *post mortem* changes are not due to a soluble ferment derived from the liver-tissue, but simply to the action of micro-organisms. He says : "Les fermentations glycosiques que l'on a obtenues avec la macération ou la décoction du foie sont le résultat de l'activité des microbes," and supports this statement by experiments to show that, not only after boiling and heating to 110° C. does the tissue lose all power of converting glycogen, but that, after sterilizing at 55° C.—a temperature which he concludes does not injure a soluble ferment—all amylolytic power is lost.

Undoubtedly, certain microbes have a slow amylolytic action. Several organisms change glycogen, in part at least, to a reducing sugar when kept at 40° C. for twelve hours.

The differentiation of those which act and those which do not act forms no part of the present inquiry. That they are the amylolytic agents in the liver is opposed by the careful experiments of EVES on the isolated ferment, and by the following experiments.

EXPERIMENT 41. 23.1.93.

Rabbit killed at 12.57 p.m. Liver cut into pieces A, B, and C.
A weighed 6·2 grms. placed in boiling water at 1 p.m.
B ,, 7·3 grms. ,, 0·75 per cent. NaCl solution unsterilized.
C weighed 7·2 grms. placed in 0·75 per cent. NaCl solution sterilized.
B and C kept at 50° C. till 5 p.m. (4 hours).
Glucose estimated as in previous experiments.

Glucose.

Filtrate of A = 0·037 grm. glucose = 0·596 per cent. glucose in liver.
,, B = 0·27 ,, ,, = 3·69 ,, in liver.
,, C = 0·26 ,, ,, = 3·61 ,, ,,

Glucose formed per 10 minutes from commencement.

B = 0·125 per 100 parts of liver.
C = ,, ,, ,,

Cultures on glycerine agar from B gave copious growth, from C gave no growth.

EXPERIMENT 42. 13.1.93.

Rabbit killed at 12.58 P.M. Liver minced and divided into A, B, and C.
A weighed 10·6 grms. placed at once in boiling water at 1 P.M.
B ,, 11·4 ,, ,, in sterilized solution of NaCl, 0·75 per cent.
C ,, 11·5 ,, ,, non-sterilized solution of NaCl, 0·75 per cent.
B and C kept at 40° C. till 4.15 (4 hours 15 minutes).
Glycogen extracted by KÜLZ's method.

A Glycogen = 1·164 grms. = 10·981 per cent.
B ,, = 1·034 ,, = 9·07 ,,
C ,, = 1·109 ,, = 9·64 ,,

Glycogen lost per 10 minutes from commencement.

B 0·051 per cent.
C 0·075 ,,

Cultures on agar-agar from B gave no growth, from C a copious growth of micro-organisms (see also Experiment 30, p. 264).

In addition to these we may refer to Experiments 13 and 17 on the influence of chloroform, an agent preventing the growth of organisms, on the late amylolysis. Such experiments seem to *exclude the action of micro-organisms as the main factors in this late amylolysis.*

3. *Action of Zymin.*

The action of both the acids and of micro-organisms having thus been excluded, and the existence of a true ferment having been demonstrated in the liver some time after death, we are forced to the conclusion that *it is by the action of a ferment, in some cases assisted by the influence of micro-organisms, that the amylolosis which proceeds in the liver for so long after death is carried on.*

I have made no definite series of experiments upon this ferment, but I have satisfied myself that it can be extracted from the liver, and that it acts very slowly and incompletely. The researches of Eves* and others render further work upon it needless.

When the production of sugar, the result of the intracellular or vital changes, ceases, and when the ferment action begins, it is impossible to say. Almost certainly the two processes greatly overlap one another. Nevertheless, they are of totally different nature and should be carefully distinguished.

The general results of these investigations may be summarized :—

1st. The great and active disappearance of glycogen in the excised liver, kept at the body temperature, is during the first half hour. The rate of conversion steadily diminishes during the remainder of the first hour, and after two hours goes on very slowly (Experiments 1 to 6).

2nd. An enormous diminution in the amylolysis is produced by destroying the structural integrity of the liver cells (Experiments 7 and 8).

3rd. The active early amylolysis goes on before and during the development of structural changes in the liver cell, while the slower amylolysis advances after the cell structure is completely destroyed.

4th. Exposure for an hour to a temperature of 60° C. greatly retards, but does not arrest, amylolysis (Experiments 9 and 10).

5th. Fluoride of sodium in 1 per cent. solution greatly retards or stops the early amylolysis, but does not arrest the later changes (Experiments 11 and 12). It does not accelerate the structural changes in the liver cells.

6th. In the excised liver the amylolysis is enormously increased in rate in the presence of chloroform (Experiments 13 to 17).

7th. In this amylolysis the glycogen is changed to glucose (Experiment 18).

8th. It is the early active amylolysis, and not the later slower conversion, which is accelerated by chloroform (Experiments 19 to 21).

9th. The structural changes in the cells are greatly hastened and increased under the influence of chloroform.

10th. In the living animal, as in the excised liver, chloroform hastens the conversion of glycogen to glucose (Experiments 24 to 28); and the glycæmia and glycosuria of chloroform poisoning are probably due to this.

* *Loc. cit.*

11th. The accelerated conversion is not due to diminished oxidation (Experiments 29 and 30), but, apparently, to a direct action on the liver cells hastening the katabolic changes.

12th. Ether has a similar, but less marked, action to chloroform (Experiments 31 and 32).

13th. Pyrogallic acid acts in the same manner (Experiment 33).

14th. Morphin (Experiments 34 and 35), curare (Experiment 37), nitrite of amyl (Experiment 36), and salicylate of soda (Experiment 33), neither hasten the structural changes in the liver cells nor modify the rate of amylolysis. The glycosuria caused by the first three of these substances is, therefore, not due to accelerated hepatic amylolysis.

15th. During the early amylolysis the product is glucose; during the later changes intermediate bodies, dextrins, and possibly maltose, are also formed.

16th. The early changes in the excised liver are simply a continuation of the vital processes in the organ—the katabolic side of the metabolism being exaggerated, the anabolic side in abeyance.

17th. The change of glycogen to glucose is thus precisely analogous to the change of mucinogen to mucin and of zymogens to zymins, and is dependent on the metabolism of the cell protoplasm, and not upon the action of a zymin.

18th. The later amylolytic changes in the excised liver are not due to the development of acid (Experiments 39 and 40), nor are they largely due to the action of micro-organisms (Experiments 41 and 42 and 13 to 17), but they are caused by the development of a zymin probably formed in the disintegration of the cells.

NOTE, January 24, 1894.—It has been suggested to me by Mr. HORACE T. BROWN, F.R.S., that the slowing of the amylolysis during the first hour may be due not to the death of the cells but to the accumulation of the products of activity inhibiting the process. The following experiment seems to negative such an explanation.

Rabbit killed at 1.15 on 18.1.94. Liver divided into A, B, and C.

A weighing 4 grms., was placed in boiling water.

B „ 10·2 grms., was placed in 0·75 per cent. NaCl solution in a beaker.

C „ 11·4 „ „ „ „ „ vegetable parchment sausage paper suspended in a large quantity of salt solution and frequency agitated.

B and C were kept at 41° C. for 4 hours and then boiled.

The fluid around C, on evaporation, gave a reduction with FEHLING's solution.

Glycogen by BRÜCKE's method.

A Glycogen weighed 0·225 grm. = 5·62 per cent.
B „ „ 0·225 „ = 2·22 „
C „ „ 0·280 „ = 2·45 „