THE DIASTATIC FERMENT IN THE TISSUES IN DIABETES MELLITUS

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A striking fact observed in experimental diabetes after removal of the pancreas is the great diminution in the hepatic glycogen, notwithstanding the hyperglycaemia. Observations on the amount of glycogen present in the livers of diabetic patients are difficult to obtain and few in number; but the quantity present appears to be less than normal. On Bernard's hypothesis, the conversion of glycogen into dextrose is carried out by a diastatic ferment normally present in the liver. It is possible that the conversion of dextrose into glycogen also is effected by an enzyme, which might be either the diastatic ferment acting in a reverse way, or a different ferment altogether. The apparent inability of the liver in diabetes to store glycogen might be due to the absence of the necessary ferment. We have investigated the livers, blood and muscles of diabetic patients and of depancreatised cats, in order to ascertain whether they possess the normal diastatic ferments; and this we have found to be the case. The future question, whether it can be shown that the normal liver possesses a ferment which can act synthetically upon dextrose, and if so, whether this ferment is present or absent in diabetes, is under investigation.

METHODS

Preparation of the tissue extracts. Pavy has shown that pieces of liver, kept under alcohol for months, still contain an enzyme capable of converting glycogen into sugar. Our method of obtaining the ferment is based upon this observation, and is a modification of that employed by Pick. Miss Tebb has shown that the enzyme acts not only upon glycogen but also upon starch; and in most of our

experiments we have used Kahlbaum's pure soluble starch, as being more convenient to work with. In a few experiments we have used solutions of Merck's pure glycogen and obtained the same results as when using soluble starch.

Portions of liver, obtained as soon as possible after death, were minced and placed under five times their volume of 95 per cent. alcohol for several weeks. In the animal experiments, the liver was washed out and freed from blood before being minced and placed under alcohol. The alcohol finally was filtered off, and the liver washed with alcohol until the washings were free from sugar. The liver then was dried at 37° C., powdered and rubbed up with sand and water; some toluol was added, and the extraction was continued at 37° C. for twenty-four hours, during which time the flask was frequently shaken. The extract was filtered and yielded a clear yellow filtrate, which gave no colour reaction with iodine, and which contained small quantities of protein and sugar. Known quantities of this filtrate were incubated with a solution of soluble starch for twenty-four hours; an abundance of toluol was added. The solutions were then boiled in order to coagulate the protein and drive off the toluol; they were cooled, made up to a known volume and filtered. The filtrate was tested with iodine, and the amount of copper reducing substance present in it was estimated by Pavy's method, and the result is expressed as dextrose.

The blood used for extraction was received, during life, direct into alcohol. The subsequent procedure was identical with that described above in the case of the liver.

The muscle extract was made in the following way:—

In the animal experiments the muscles were washed, directly after death, as free from blood as possible by means of normal salt solution injected through the aorta. In the case of diabetic patients the muscles were not freed from blood. Portions of muscle were minced and pounded, first with sand and a little water, and then with Kieselguhr, until a nearly dry powder was obtained. This was exposed to pressure in a Buchner's press, and the muscle juice collected. The juice was then examined for the presence of ferment.

The extract of pancreas added to the other extracts in certain experiments corresponded to that described by Cohnheim, and was essentially a boiled watery extract of pig's pancreas, which had previously been minced and pounded up with sand. The strength of the extract was such that 2 c.c. of the solution contained the extract of about 1 gr. pancreas.

EXPERIMENTS

The presence in the liver, under normal conditions, of an enzyme capable of converting starch or glycogen into dextrose is so well established, that we merely examined a few livers taken from normal animals and from the post mortem room in order to demonstrate, firstly the effectiveness of the method of extraction, and secondly the existence of the enzyme in the livers twenty-four hours or more after death.

Two of the post mortem cases are recorded here :—

Case I.—Liver, obtained twenty-four hours after death, from a patient who died two hours after an accident which fractured his vertebral column. 25 c.c. of the liver extract were added to 30 c.c. of 1 per cent. solution of soluble starch, toluol was added, and the whole was incubated at 37° C. for twenty-four hours. The control flask was boiled for several minutes before the toluol was added. At the end of twenty-four hours the flasks were boiled, cooled, made up to 100 c.c., and filtered. The following table gives the result obtained :—

		Iodine Test	Sugar by Pavy
Control ...	...	Blue	0·06 gr.
Unboiled flask ...	...	Violet	0·18 gr.

Case II.—Patient died of a cerebral tumour; liver obtained forty-eight hours after death. Experiment carried out in the same way as above.

		Iodine Test	Sugar by Pavy
Control ...	...	Blue	0·075 gr.
Unboiled flask (1)	...	Violet	0·18 gr.
„ „ (2)	...	Violet	0·20 gr.

As regards the blood of normal men and animals, it has been shown by Ascoli and Bonfanti that a diastatic ferment is often present; and we have confirmed this in the case of the dog by the above method.

The muscle juice of normal animals possesses a feeble diastatic power. An experiment is recorded later which shows this effect in the dog.

(a) *The liver extract in diabetes.* We have examined the livers of four diabetic patients and of four depancreatized cats. The full clinical histories of the patients have been given in a previous paper (p. 439); the cases are not numbered consecutively, but have received the same numbers in this paper as they had in the previous one. The cats had undergone almost complete removal of the pancreas, and showed in each case well marked symptoms of diabetes; their urine contained an abundance of sugar and gave a feeble reaction with ferric chloride.

The method employed was the same in all cases. Thirty c.c. of liver extract were added to 40 c.c. of 1 per cent. solution of soluble starch; toluol was added, and the mixture incubated for twenty-four hours at 37° C. In some experiments a second flask contained the same mixture, with the addition of 10 c.c. of pancreatic extract, which corresponded to about 5 grs. of pancreas. The control flask was boiled before the addition of toluol. In some cases a fourth flask, containing liver extract with water and toluol, was incubated. The following table gives the results obtained:—

Case	Liver extract and water	Boiled control	Liver extract and starch at 37°	Liver extract, starch, and pancreatic extract at 37°
II. Diabetic Coma				
Iodine	No colour	Blue	No colour	—
Sugar by Pavy	0.05 gr.	0.05 gr.	0.24 gr.	—
III. Diabetic Coma				
Iodine	No colour	Blue	Violet	—
Sugar by Pavy	0.06 gr.	0.06 gr.	0.26 gr.	—
V. Diabetes and Phthisis				
Iodine	—	Blue	Violet	Violet
Sugar by Pavy	—	0.09 gr.	0.21 gr.	0.20 gr.

Case			Liver extract and water	Boiled control	Liver extract and starch at 37°	Liver extract, starch, and pancreatic extract at 37°
VI. Diabetes and Septicaemia						
Iodine ...	...	...	No colour	Blue	Violet	—
Sugar by Pavy ...	...	...	Trace	Trace	0·18 gr.	—
Cat A						
Iodine ...	...	...	—	Blue	Violet	Violet
Sugar by Pavy ...	...	...	—	Trace	0·10 gr.	0·12 gr.
Cat B						
Iodine ...	...	...	—	Blue	Violet	Violet
Sugar by Pavy ...	...	...	—	o	0·18 gr.	0·18 gr.
Cat E						
Iodine ...	...	...	No colour	Blue	Violet	—
Sugar by Pavy ...	...	...	o	o	0·12 gr.	—
Cat F						
Iodine ...	...	...	No colour	Blue	Violet	Violet
Sugar by Pavy ...	...	...	o	o	0·11 gr.	0·10 gr.

The presence of sugar in some of the boiled controls indicates that the corresponding livers contained both glycogen and a ferment capable of converting glycogen into sugar.

(b) *The muscle juice in diabetes.* Muscle was obtained from two patients, Cases II and III in the previous table, and from cat E. The same method was used throughout. Eight c.c. of muscle juice were added to 20 c.c. of 1 per cent. solution of soluble starch; toluol was added, and the mixture incubated for thirty hours at 37° C. A second flask contained the same solutions together with 10 c.c. of pancreatic extract. The control flask was boiled for several minutes before the toluol was added. At the end of thirty hours the flasks were boiled, cooled, made up to 100 c.c., and filtered. The following table of results contains, also, those obtained with the muscle of a normal dog.

Case		Boiled control	Muscle juice and starch at 37°	Muscle juice, starch and pancreatic extract at 37°
II. Diabetic Coma				
	Iodine ...	Blue	Violet	Violet
	Sugar by Pavy ...	o	0.11 gr.	0.12 gr.
III. Diabetic Coma				
	Iodine ...	Blue	Violet	Violet
	Sugar by Pavy ...	o	0.05 gr.	0.05 gr.
Cat E				
	Iodine ...	Blue	Blue	Violet
	Sugar by Pavy ...	o	0.03 gr.	0.05 gr.
Normal Dog				
	Iodine ...	Blue	Violet	—
	Sugar by Pavy ...	o	0.04 gr.	—

(c) *The blood extract in diabetes.* Blood was obtained during life from three diabetic patients, and from one depancreatized cat. One of the patients was Case II in the previous tables, and his blood was obtained when he was in coma. The two other patients were suffering from severe diabetes. When their blood was taken they were being treated by secretin given by the mouth. Their full clinical histories have been given in a previous paper (p. 433), where they are referred to as Cases II and III. It may be mentioned that Case III died of diabetes in November, 1906, eight months after our observations upon him were made.

The blood was treated and its diastatic activity observed by the method already described, so that it is unnecessary to give more than a table showing the results obtained.

Case	Boiled control	Blood extract and Starch at 37°
(1) Diabetic coma.—Case II		
Iodine	Blue	No colour
Sugar by Pavy	Trace	0.11 gr.
(2) Severe diabetes.—Case II		
Iodine	Blue	Blue and Violet
Sugar by Pavy	Trace	Too little to estimate
(3) Severe diabetes.—Case III		
Iodine	Blue	Blue and Violet
Sugar by Pavy	Trace	Too little to estimate
(4) Cat E		
Iodine	Blue	Violet
Sugar by Pavy	o	0.15 gr.

The almost complete absence of a diastatic ferment seen in the two cases of severe diabetes, has also been observed by Ascoli and Bonfanti in some healthy human beings and animals.

CONCLUSIONS

The livers and muscles of diabetic patients and of depancreatized cats contain a diastatic ferment. No attempt was made to ascertain whether the amount of enzyme present was greater or less than that found in normal animals. In two cases a diastatic ferment was found in the blood, and in two others it was absent. No evidence was obtained that the activity of the diastatic ferment of liver or muscle is increased by the addition of a boiled extract of pancreas.

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