Experiment 3 (Fig. 2).

Time.	Ventilation per min. in litres.	O ₂ per min. in c.c.m.	CO ₂ per min. in c.c.m.	Respiratory Quotient.	Calories per hour.	Blood Sugar.	Temperature.	Pulse.	Respiration.
10 a.m. ...	5.04	222.0	172.8	0.778	63.1	—	99.4°	55	12
11.10 ...	5.47	213.3	164.6	0.772	61.0	0.130	99.6°	60	13
11.15 ...	Insulin—8 rabbit doses injected.								
12.15 p.m. ...	5.67	229.6	193.0	0.867	67.3	0.102	99.8°	73	13-14
2.15 ...	5.90	239.0	200.6	0.839	69.3	0.077	99.4°	70	16
3.30 ...	5.38	227.6	171.6	0.754	64.8	0.089	98.2°	72	13

FIG. 2.—The effect of eight rabbit doses of insulin on the same subject as Fig. 1. Calories per hour calculated as in Fig. 1.

At 11.15 a.m. eight rabbit doses of insulin were given. There were no symptoms, though the pulse and respiration rate rose slightly, except a subjective sensation of warmth soon after the injection, and a tendency to perspire at about 2 p.m.

explanation of the pronounced rise in the respiratory quotient. We are therefore confined in our consideration to the first and last possibilities. Let us examine the first of these and assume that the glucose is consumed under the influence of insulin.

The combustion of 2.9 grams of glucose would require 2.158 litres of oxygen, and would result in the liberation of a corresponding amount of carbon dioxide, since 1 gram-molecule (= 180 grams) requires for its complete combustion 6 gram-molecules of oxygen, or 6×22.33 litres at standard temperature and pressure, according to the equation—

If the glucose lost from the blood were burnt the rise in oxygen consumption per minute should average 36 c.c.m. during the hour of the fall. Actually, the increased consumption of carbohydrate would raise the basal metabolic rate of a starving person and cause a still greater increase in the amount of oxygen used. In this experiment there was no significant rise in the amount of oxygen used, and this, together with the rise of over 30 c.c.m. in the output of carbon dioxide a minute, suggests that the combustion of glucose plays only a minor part in the production of the high respiratory quotient, and that the principal factor in causing this change is the transformation of carbohydrate to some other complex, with economy of oxygen and liberation of excess of carbon dioxide. The metabolism in calories per hour is calculated in these experiments on the assumption that the rise in respiratory quotient is due to the com-

buption of additional carbohydrate, allowing a larger production of heat from a given oxygen consumption at a higher respiratory quotient. Even on this assumption, which we believe to be unsound, the greatest increase in metabolism calculated from the observed oxygen consumption and respiratory quotient—namely, 1.3 calories an hour—does not correspond with that calculated from the combustion of the amount of sugar lost from the blood—namely, 2.9×4.1 calories = 11.89 calories.

In Experiment 3, in which we gave a smaller dose of insulin, the results do not indicate so clearly the nature of the increase in respiratory quotient. The sugar lost from the blood in two and three-quarter hours was $0.053 \times 76 \times 85 = 3.42$ grams. The amount of oxygen

necessary for the combustion of this amount of sugar is 2.530 litres, or an average of 24 c.c.m. a minute. Although the observed increase in the oxygen consumption does not attain to this calculated value, it affords definite evidence of the combustion of glucose. The disproportion between the observed increase in oxygen used—that is, 2,120 c.c.m. (q.p.)—and that in carbon dioxide given out—that is, 3,193 c.c.m. in two and three-quarter hours—supports our contention that the rise in respiratory quotient is due in part at least to transformation of glucose into some complex poorer in oxygen, and that the metabolic rate as calculated from the oxygen used and the respiratory quotient is much above its real value.

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SOME PROBLEMS OF DIABETES MELLITUS.*

BY

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NEARLY forty years ago Mering and Minkowski showed that complete removal of the pancreas resulted in a condition of diabetes. Numerous attempts have since been made to prevent the diabetes following on depancreatization by injection of extracts of pancreas. No real success resulted until the great advance made by Banting and Best. It was found that if the acinous tissue of the pancreas was allowed to degenerate after tying of the duct, an aqueous extract of the gland would diminish the excretion of sugar when injected into depancreatized dogs. This tended to explain the failure of previous workers. Presumably the active principle of the islets of Langerhans, which they termed "insulin," was destroyed by some secretion of the acinous tissue.

The discovery, due to Collip,¹ that an alcoholic extract of normal pancreas is effective, has made possible an extensive trial of the effect of insulin on diabetes in man.² These workers have treated a large number of cases, with successful results. They establish beyond doubt that injection of insulin, in the majority of cases, stops the excretion of sugar and acetone, and enables the patient to utilize an increased amount of carbohydrate.

The action of the pancreas on carbohydrate metabolism has been the subject of much study. The work of Colnheim suggested that the pancreatic secretion caused an increased consumption of glucose by the muscles. Levene and Mayer³ found, however, that the disappearance of glucose was due to the formation of a disaccharide, and that this formation took place only in concentrated solutions of glucose. If the solution was diluted the disaccharide was hydrolysed, and the reducing power showed that the glucose concentration was the same as at the beginning of the experiment. Though it is difficult to determine the precise bearing of these results on diabetes, they tend to show that the action of the pancreas is to activate some enzyme system—for the addition of pancreas or muscle extract alone to glucose was without effect; only when muscle and pancreas were acting together was any effect observed. Clark⁴ showed that the consumption of sugar by the heart was increased when the perfusing fluid was also passed through the pancreas. In a later paper⁵ he established the remarkable fact that perfusion of the pancreas

* Since this was written there has appeared an article by Hewitt (*BRITISH MEDICAL JOURNAL*, April 7th, 1923, pp. 590-591) criticizing some of this work. We propose to reply in detail in the near future.

with a solution containing glucose caused the optical rotation to be diminished, whereas the copper reducing value remained unaltered. He suggested that the pancreas contained an enzyme which was responsible for converting dextrose into another form of sugar, which then could be utilized by the body.

The precursor of lactic acid in muscle has been investigated during recent years by Embden and his co-workers.⁶ They have shown that liberation of phosphoric acid accompanies the setting free of lactic acid. We have shown that when muscle is put into rigor the increase of inorganic phosphate may be demonstrated histologically.⁷ Embden has isolated from muscle an osazone which is identical with that of the hexose-diphosphoric acid compound obtained from yeast by Harden and Young. While it is uncertain whether the lactic acid precursor or "lactacidogen" is a hexose-phosphate, they consider that such a compound is certainly a component of the molecule which gives rise to lactic acid. Added hexose-phosphate was readily broken down by muscle juice with liberation of lactic and phosphoric acids; added glucose was not converted into lactic acid. Harden showed that the hexose-diphosphate which he obtained from yeast yielded fructose on hydrolysis. Robison⁸ has recently isolated from the products of fermentation by yeast juice a hexose-monophosphoric acid compound which gives on hydrolysis a hexose with a lower rotatory power than glucose. The present evidence points to the possibility that a special sugar may be formed in muscle which may readily break down into lactic acid.

The Problem of a Reactive Sugar.

With the idea that diabetes might be due to decreased formation of a reactive sugar we began in 1921 an investigation into the nature of the normal blood sugar by a comparison of its copper reducing and polarimetric values. For a determination of the latter the blood filtrate must be clear and concentrated to a small bulk owing to the small sugar content of normal blood. It was soon apparent that precipitation of the proteins by the usual methods was insufficient by itself, for on concentration the fluid invariably became turbid, showing the presence of unprecipitated protein. The proteins were precipitated according to the method of Folin and Wu; the filtrate was evaporated to dryness by distillation *in vacuo* at 40° C. The sugar was then extracted with 85 per cent. alcohol; the alcohol was distilled off under reduced pressure and the sugar taken up in a small quantity of water. While this method does not give a pure sugar solution, nitrogen determinations showed that only minimal amounts of such substances as urea, creatinine, etc., were present and the biuret and protein colour tests were negative.

By this method⁹ we find that the blood sugar of man and animals has a lower rotatory power than would be given by α, β glucose in equilibrium, the copper reducing value being reckoned as glucose. The polarimeter readings gradually approach the copper value and usually reach it in three days. While the time which elapses between the drawing of the blood and the determination of the rotatory power of the sugar being made is at least three hours, the sugar is in weak acid solution during the whole of this time, and alteration is likely to be reduced to a minimum.)

Examination of the blood sugar of diabetics shows that the sugar has a higher polarimetric value than that of α, β glucose. Control experiments, however, showed that solutions of α, β glucose treated in the same manner as the blood filtrates gave slightly higher polarimeter values than those deduced from the copper reducing power. A possible reason for this was advanced, and it was suggested that in diabetes the blood sugar is mainly α, β glucose. In certain cases of diabetes the polarimetric value was so much higher than that which would be given by α, β glucose that we suggested that disaccharides or other substances with a higher polarimeter to copper reducing ratio than glucose might be present. We have obtained further evidence in the support of this view which will be discussed later.

As we have previously stated,⁹ the nature of the normal blood sugar must be left undecided, though we hope in the future to obtain more direct evidence of its chemical nature. The facts now available suggest that it is of a reactive nature. The rotatory power is initially low, but tends to increase. Decolorization of KMnO_4 is at first rapid, and then becomes slower as the rotation increases. The fact that the blood sugar in such a solution as we have obtained decolorizes KMnO_4 more quickly than a solution of glucose of similar

concentration is of no significance, since many substances, especially phosphates, accelerate the oxidation of glucose. It is only when the rate changes from day to day that it is permissible to suggest that a reactive sugar is present. Hewitt and Pryde¹⁰ have shown that a solution of α, β glucose in equilibrium introduced into the intestine of anaesthetized rabbits undergoes a rapid downward mutarotation. On withdrawal from the intestine the rotation increases and rapidly reaches the copper reducing value. Simple filtration was employed to obtain a clear solution. Fluids in contact with living tissues are apt to become contaminated with protein and other substances, especially if the tissues are injured. The differences, however, in the rotations observed are very striking. It was stated that KMnO_4 was decolorized more rapidly by the sugar solution on withdrawal from the intestine than by Ringer-Tyrode solution or washings from the intestine. This does not dispose of the possibility that there may have been in the sugar solution some substance derived from the intestine which catalytically accelerated oxidation of the sugar by KMnO_4 . That the phenomenon was not observed with sugar solution withdrawn from the peritoneal cavity does not invalidate this criticism. Indications were obtained that stereochemical changes took place in sugar solutions held in the mouth for a few minutes; the changes were slight, and difficulty in filtering the solution was caused by the saliva. The authors suggest that α, β glucose is converted into γ glucose preparatory to absorption by the intestine. The reaction of the fluid was stated to be slightly alkaline, and the sugar reached equilibrium about twenty minutes after withdrawal. We have found that the blood sugar does not reach equilibrium for two or three days in acid solution. The influence of the reaction of the fluid is being studied, but it must be remembered that if the precipitation is carefully carried out, all but a trace of the tungstic acid combines with the proteins.

Whatever the precise nature of the normal blood sugar may be, the hypothesis of a reactive sugar being formed in the body is gaining ground. In a recent paper Laquer¹¹ gives the results of an investigation into the problem of lactic acid formation in muscle. He finds that lactic acid arises from glycogen under conditions in which the muscle can form no lactic acid from glucose. He concludes, "Glucose is burnt by muscle not directly, but after conversion into a reactive form. This reactive sugar is produced directly by the breakdown of glycogen."

The Action of Tissue Extracts.

In an attempt to determine whether tissue extracts could effect a change in the rotatory powers of glucose or fructose *in vitro*, we first examined the blood itself. Hewitt and de Souza¹² and Cooper and Walker,¹³ using different methods, had previously stated that an enzyme of this nature is not present in blood. We could find no alteration in the rotatory power of α, β glucose when it had been incubated with defibrinated blood and extracted by the usual method. More recently we have repeated these experiments with the blood of rabbits which have been killed after large doses of insulin, sufficient to cause convulsions. In every case the polarimeter reading was slightly above that obtained from the copper value.

No change took place when extracts of intestinal mucous membrane were used, either with or without the addition of insulin. More successful results were obtained when an extract of liver was employed.¹⁴ When a solution of glucose or fructose was incubated at 37° C. in a jacketed polarimeter tube (2 Dm.) in the presence of liver extract and insulin, the rotation was changed in a downward and upward direction respectively. The presence of insulin was necessary; but insulin or liver extract by themselves were not capable of effecting any change. The influence of inorganic salts on the system was tested; phosphates appeared to have an accelerating effect. The very small quantity of liver extract used precludes the possibility of sufficient glycogen being present to cause error in the determinations. The copper reducing power of the sugar at the end of the reaction was unaltered. The fact that the liver factor is destroyed by boiling, while insulin is thermostable in acid solution, suggests that the function of the latter is to act as a co-enzyme or activator of some enzyme in the liver.

An Effect of Insulin on Diabetes.

On the supposition that diabetes might be due to diminished formation of a reactive sugar, it seemed interesting to test whether the nature of the blood sugar of diabetics might be

altered after injections of insulin. In conjunction with Dr. Devcreux Forrest,¹⁵ we have investigated this possibility. Six cases were examined—severe and moderately severe. A sample of blood was drawn before treatment and the ratio polarimetric to copper reducing value of the blood sugar was determined. In each case the polarimetric reading was considerably above the copper value. An injection of insulin was then given each day for two or three days, and a second sample of blood drawn. In one case no change was observed; in the others the ratio polarimetric to copper reducing value had been lowered, showing that the blood sugar was more like that of normal persons. We have observed a further very marked difference between the blood sugar of diabetic and normal persons. We find¹⁶ that when the blood sugar of diabetics is subjected to short hydrolysis by weak acid a marked increase takes place in the polarimetric value. The copper reducing power remains almost unaltered. On longer hydrolysis by stronger acid the polarimeter and copper values agree for α , β glucose in equilibrium. This phenomenon does not occur with the blood sugar of normal persons. We have not determined what is the sugar complex in diabetic blood which is responsible for this increase of polarimeter reading on hydrolysis. The facts would suggest that a polysaccharide is present which is readily broken down into strongly dextro-rotatory components. In preparing osazones from diabetic blood, we have in certain cases observed crystals other than those of glucosazone. On examining the blood sugar of diabetics after injections of insulin, we find in the case of the patients who have benefited from the insulin, that on hydrolysis the polarimeter reading does not increase above the copper value. In cases which have not had their ratio polarimeter to copper value altered by the insulin, we find that the polarimeter reading does increase, showing that this abnormal sugar is still present.

Though much more work is necessary to provide a satisfactory explanation, these facts are of significance in emphasizing the difference between the normal blood sugar and that of diabetics, and in showing that in most cases the blood sugar of diabetics is more normal after injections of insulin.

Action of Adrenaline.

It is well known that injections of adrenaline into animals raise the blood sugar. We have made some experiments to determine the ratio polarimeter to copper reducing value of the blood sugar after the concentration has been raised by injection of adrenaline. Under these conditions the nature of the blood sugar is considerably altered. The initial polarimeter reading is usually higher than the copper value, and it increases on short hydrolysis. The mixture of sugars would appear, therefore, to be similar to that in diabetic blood before treatment with insulin. On preparing phenyl-osazones, crystalline forms other than those of glucosazone have been observed. The amount of adrenaline injected into each animal varied from 0.5 to 1 c.cm. of a 1 in 1,000 solution. While the concentration of adrenaline in the blood must be momentarily far greater than any likely to occur normally, even in pathological conditions, the fact that it will cause a derangement of sugar production in such a way as to simulate the blood sugar of diabetics may not be without significance.

Action of Yeast Extracts.

We have made some experiments to determine how far an extract of yeast will lower the blood-sugar of normal animals and diminish the symptoms of diabetes in man. The results tend to show that a substance similar to insulin may be obtained from yeast.

The action of the yeast extract on the blood sugar of the fasting rabbit may be seen from the following examples:

Rabbit 1 (weight 2 kg.).			Rabbit 2 (weight 2.5 kg.).		
Time.	Yeast	Blood sugar.	Time.	Yeast	Blood sugar.
10.30	...	0.15 %	11.45	...	0.11 %
10.45	yeast 0.03 mg.		12.0	yeast 0.06 mg.	
	per gm. body			per gm. body	
	weight injected			weight injected	
12.0	...	0.11 %	2.45	...	0.08 %
2.15	...	0.06 %	5.0	...	0.06 %
13.45	...	0.05 %	6.0	...	0.05 %
5.45	...	0.07 %	7.0	...	0.05 %

The animal went into convulsions; 40 c.cm. of a 10 per cent. solution of glucose were injected subcutaneously. Complete recovery resulted.

Through the kindness of the consulting physicians and staff of Addenbrooke's Hospital, we have been able to test the effect of the yeast extract on diabetic cases. Two cases had

been rendered sugar free by diet (Graham) before treatment with yeast extract. The others were given injections soon after admission to hospital. An unusually large quantity of protein is allowed by the Graham diet during the early days of treatment; but when the twenty-four hours' urine contained sugar, it was found that the urine passed up to seven hours after the injection was sugar-free, even in severe cases. Of the seven cases examined, five showed marked improvement. Only one injection was given each day, as we had previously found with pancreatic insulin that this was sufficient to cause definite improvement.

In three cases a sample of blood was drawn before treatment with yeast extract and the ratio polarimeter to copper value determined. All had a high ratio; in one case it was unusually high. One of the cases was sugar-free by diet; this, we had previously found, alters only the amount and not the nature of the blood sugar. After several days of treatment a second sample was drawn and the ratio redetermined. In all three cases the ratio had improved; in two the polarimeter reading was definitely below the copper value. In each case hydrolysis showed that no trace of polysaccharide was present. The results appear to be identical with those which we have previously obtained after injection of pancreatic insulin into diabetics. Two cases of long-standing disease showed no improvement from injection of yeast extract or pancreatic insulin. A possible reason for this is advanced in the discussion.

Table of Clinical Experiments.

	Polarimetric Readings on Successive Days.	Copper Value.
L. B. W., normal (100 c.cm. blood) ...	0.07, 0.11, 0.13, 0.14	0.14
L. B. W., after 100 grm. glucose (55 c.cm. blood) ...	0.04, 0.05, 0.06, 0.09	0.03
L. B. W., after 150 grm. fructose (100 c.cm. blood) ...	0.06, 0.08, 0.10, 0.13	0.16
Diabetic before insulin (55 c.cm. blood) ...	0.15, 0.12, 0.11, 0.10	0.03
Diabetic after insulin (85 c.cm. blood) ...	0.05, 0.06, 0.05	0.06
J. S., before yeast extract (50 c.cm. blood) ...	0.19, 0.18	0.13
J. S., after yeast (72 c.cm. blood) ...	0.13, 0.14	0.15
S., before yeast (30 c.cm. blood) ...	0.11, 0.09, 0.03	0.05
S., after yeast (65 c.cm. blood) ...	0.09, 0.11, 0.12	0.13
J. M., before yeast (30 c.cm. blood) ...	0.21, 0.15	0.01
J. M., after yeast (40 c.cm. blood) ...	0.17, 0.10, 0.10	0.10

The following are the blood sugar determinations (quantitative) on C. R., aged 11 years. This case received yeast extract only. On one day no injection was given. The blood sugar remained high on this day.

First Day.	Second Day.	Third Day.
12.45 0.14%	12.30 0.13%	11.0 0.13%
1.0, 90 mg. yeast	12.45, 90 mg. yeast	12.0, 90 mg. yeast
3.30 0.1%	3.30 0.09%	3.15 0.12%
7.0 0.03%	6.15 0.14%	5.30 0.10%
9.0 0.11%		
Fourth Day.	Fifth Day.	Sixth Day.
12.0 0.22%	12.0 0.22%	12.0 0.11%
No injection	12.30, 100 mg. yeast	12.30, 100 mg. yeast
3.0 0.20%	3.0 0.13%	3.15 0.07%
6.0 0.19%	6.0 0.10%	5.30, 10 gm. cane sugar by mouth.
		6.15 0.12%

During the first two days of treatment with yeast extract he was given 1 oz. bread a day above diet. During the remaining four days this was increased to 1½ oz. On the last day cane sugar was given as a precautionary measure. Since this he has been on an adult diet (full Graham), with addition of bread and, as a rule, also potatoes. He has remained sugar-free.

R. This case reacted neither to yeast extract nor to pancreatic insulin. The ratio polarimeter to copper reduction was unchanged. Before treatment: Polarimeter, 0.10; copper value, 0.07. After treatment: Polarimeter, 0.10; copper value, 0.07.

First Day.	Second Day.	Third Day.
10.30 0.10%	11.0 0.14%	11.15 0.12%
(30 c.cm. blood drawn.)	11.20, 140 mg. yeast.	11.30, 140 mg. yeast.
11.0, 140 mg. yeast.	3.45 0.12%	1.45 0.12%
3.0 0.10%	6.15 0.12%	6.0 0.12%
Fourth Day.	Fifth Day.	
10.0 0.13%	10.30 0.14%	
10.30, 140 mg. yeast.	11.0, 50 mg. insulin.	
2.0 0.11%	2.45 0.13%	
(42 c.cm. blood drawn.)	6.0 0.12%	

S. The polarimeter and copper reducing values before and after treatment are given above.

First Day.			Second Day.		
9.31	...	0.15%	11.15	...	0.12%
(35 c.cm. blood drawn.)			11.50, 140 mg. yeast.		
11.30, 140 mg. yeast.			3.0	...	0.07%
3.0	...	0.11%	6.0	...	0.13%
Third Day.			Fourth Day.		
11.0	...	0.13%	10.0	...	0.13%
11.35, 140 mg. yeast.			10.45, 140 mg. yeast.		
3.0	...	0.16%	1.30	...	0.03%
			(35 c.cm. blood drawn.)		
			6.0	...	0.14%

GENERAL DISCUSSION OF RESULTS.

Two theories of diabetes have been suggested. The first attributes the disease to overproduction of sugar, regarding the pancreas solely as an inhibitory mechanism. The second theory holds that the disease is due to progressive inability of the tissues to utilize sugar; and that in some way the internal secretion of the pancreas enables the tissue cells to grip the sugar and oxidize it. The deficient utilization of sugar in diabetes, as shown by the respiratory quotient and wasting of the tissues, is ascribed by supporters of the first theory to the toxic effect on the cells of the high concentration of sugar, the very excess of sugar offered impairing the power of the cells to oxidize it.

Evidence pointing to a reactive sugar being formed in the body lends powerful support to the second theory. The sugar which reaches the cells contains a lessened amount of a reactive type; the high blood sugar may be explained by regarding the sugar as a mixture of normal and abnormal types. The normal blood sugar is removed by the cells and oxidized, the abnormal cannot be burnt, and is excreted. As the disease progresses the proportion of normal blood sugar becomes smaller, and the continued call of the tissues for an oxidizable sugar causes the blood sugar concentration and the excretion in the urine to become greater.

In a theory of diabetes put forward by Ringer¹⁷ the author suggests that failure to utilize fat and carbohydrate is due to failure to accomplish a glucosidic union between the two molecules, and that the excretion of acetone bodies and sugar may be explained by the fact that they have not been linked together prior to metabolism by the cell. This hypothesis fits in well with the view of a reactive sugar.

The difference in the nature of the blood sugar of normal and diabetic persons is against the overproduction theory; on this theory the diabetic blood sugar should differ from the normal only in quantity. Further, injection of insulin into diabetics alters the nature of the blood sugar, with certain exceptions shortly to be discussed.

That at least two factors are concerned in the change is probable from the experiments on the action of insulin and liver extract on glucose and fructose *in vitro*. The optical activity of the sugar solutions was altered with no change in the copper reducing value. This suggests that the differences observed could not have been due to oxidation of the sugar or removal by some other means. Further, after the maximum change was observed, the rotation tended to revert to its original value; probably some component of the system was being inactivated and destroyed. We hope to make a further investigation on this point with a view to determining the conditions of activity of the enzyme. These experiments afford no indication as to the purpose of the sugar which was formed. Is it a preliminary to the formation of glycogen, or, granting that α , β glucose is formed from glycogen in the body, is it in process of converting this sugar into a reactive form for utilization?

The evidence at present available would suggest that glycogen may yield different sugars under different conditions. While the normal blood sugar has a low rotatory power, the blood sugar of animals after injection of adrenaline approaches that of the diabetic in its nature. In examining the blood sugar of man, we found in one subject, who had no diabetic history, that the sugar had a rotatory power corresponding to the copper value. Possibly nervous influences, the result of the operation of drawing the blood, had affected the normal breakdown of glycogen. Macleod and his co-workers have shown, by a study of the respiratory quotient, that injection of insulin causes an increased utilization of sugar. They have also shown that insulin causes considerable formation of glycogen in the liver of depancreatized dogs. It is possible that the normal blood sugar is a mixture of α , β

glucose and a reactive sugar, and that insulin shifts the equilibrium in the direction of increased formation of the reactive sugar, which is then stored or utilized, the latter depending on the need of the tissues for sugar.

When a large dose of insulin is given to an animal the blood sugar falls to a point incompatible with life—the nervous system is affected and convulsions ensue. Banting and Best have shown that these symptoms can be relieved by injection of glucose. We have recently found that rabbits which are in convulsions as a result of injection of insulin (three times minimum lethal dose) may be restored to a normal condition in a short time by injection of adrenaline. A probable explanation is that glycogen is broken down by the liver and sent into the blood.

	Time.	Blood Sugar.
Rabbit, weight 1,800 grams	9.45	0.12 per cent.
Insulin (pancreatic) 0.03 mg.	10.0	
per gram body weight	11.40	0.07 per cent.
Convulsions occurred	12.45	0.05 "
1 c.cm. 1 in 1,000 adrenaline given intravenously	12.50	
Animal normal	1.30	0.09 per cent.
	3.0	0.16 "

In the case of an animal which did not recover following administration of adrenaline, the liver was examined; it was found that a new growth had greatly diminished the amount of normal liver tissue. It is possible that in certain cases of diabetes impairment of some organ other than the pancreas may have occurred. Certain cases of diabetes we have found not to react readily to insulin from the pancreas or to an extract of yeast. Two showed no improvement whatever. Both had had the disease for many years. It is suggested that the liver may have been affected. On the view that the liver contains an enzyme which is concerned with the production of a reactive sugar, gradual inactivation of the enzyme may have taken place to a considerable degree; possibly consequent on deficient formation of the pancreatic hormone.

A point of difficulty arises when it is remembered that the muscles of the diabetic contain glycogen, the heart muscle especially being often abnormally loaded with it. The liver, on the other hand, is usually almost free of glycogen. It is possible that carbohydrate metabolism in muscle may be of a special kind, or, as the powers of the body to metabolize sugar become diminished, that the essential organs are kept supplied with what little reactive sugar is available, to the exclusion of the call of the less essential tissues. On the other hand, the abnormal glycogen content of the heart muscle may point to deficient power of utilization of this sugar. Possibly there may be more than one internal secretion of the pancreas.

Evidence is accumulating to show the delicate balance which exists between the various internal secretions of the body. On these lines may come the explanation of the gradual deficiency of the pancreatic hormone in diabetes. Owing to the very delicacy of the adjustment between the glands, caution must be observed in interpreting the results of injection of large doses of the secretion of any one of them. It is inadvisable, therefore, at the present stage, to theorize too precisely on the manner in which the pancreas influences the carbohydrate metabolism of the body.

Summary.

1. The normal blood sugar of man and animals has a lower rotatory power than α , β glucose. It is probably of a reactive nature.
2. The blood sugar of diabetics is mainly α , β glucose.
3. A more complex sugar is also present in diabetic blood.
4. In the majority of diabetic cases injection of insulin causes a change in the nature of the blood sugar. The complex sugar disappears and the ratio polarimeter to copper reducing value is altered.
5. Insulin and liver extract together alter the rotatory powers of α , β glucose or *d*-fructose *in vitro*.
6. Injection of adrenaline into normal rabbits causes the blood sugar to be altered. It appears to become like that of diabetics.
7. An extract of yeast has an effect similar to that produced by insulin, when tested on animals and on diabetic persons.

8. Insulin convulsions in rabbits may be relieved, and the animals restored to normal, by injection of adrenaline.

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THE ADMINISTRATION OF INSULIN BY INUNCTION.

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DURING the course of recent experimental work in conjunction with Professor D. Noël Paton on the isolation and subsequent testing of insulin the possible methods of administering this preparation formed one of the subjects of consideration. The method of subcutaneous injection of sterilized aqueous solutions of known strength was employed in the determinations of "potency" of the insulin which was isolated from time to time in the laboratory. Hypodermic medication has also been employed habitually in the clinical use of this material. It is obvious, however, that if the drug is to be employed for long periods this method of administration might, in many cases, prove to be a serious drawback.

In considering the possible methods of administration, it occurred to me that the skin surface might be utilized for the absorption of the drug by inunction. The following experiments on rabbits were accordingly carried out, to ascertain primarily if the inunction of a paste in which insulin was incorporated would produce the effects obtained by direct injection of an insulin solution.

Simple ointments of varying strength were made by mixing weighed quantities of crude insulin, isolated from ox pancreas in the laboratory (by the method communicated by Dr. Dale of the National Institute for Medical Research) with small quantities of lanoline and with lard. The abdomen of each rabbit, which had been starved for seventeen hours, was shaved, care being taken to avoid injury to the skin surface, and the insulin paste gently rubbed in over the bare area.

TABLE I.—Inunction of Insulin in Rabbits.

	Weight of Crude Insulin used.	Blood Sugar.		Remarks.
		Time Intervals.	Glucose per cent.	
Experiment 1. Mar. 30, 1923.	0.3 gm. in 1 gms. hydrous lanoline	10.50 p.m. 12 noon 2.30 p.m. 5.0 p.m. 10.0 p.m.	0.152 0.59 0.045 0.056 0.084	Slight twitching of head at 2 p.m., but no convulsive movements.
Experiment 2. Apr. 4, 1923.	0.07 gm. in 2 gms. hydrous lanoline	11.5 a.m. 12.5 p.m. 2.50 p.m. 8.10 p.m.	0.108 0.091 0.091 0.11	No signs evident.
Experiment 3. Apr. 10, 1923.	0.3 gm. in 3 gms. "adepts preparatus"	11.35 a.m. 12.35 p.m. 2.58 p.m. 4.30 p.m. 8.10 p.m.	0.108 0.035 0.052 0.075 0.098	No convulsions.
Experiment 4. Apr. 10, 1923.	1 gm. in 5 gms. "adepts preparatus"	11.45 a.m. 12.45 p.m. 3.8 p.m. 4.10 p.m.	0.112 0.034 0.034 0.02	Animal somewhat collapsed at 3.30 p.m. Convulsive seizures at 4.10 p.m. Recovery. 1 gm. glucose injected. Animal ate green food, but at 6.40 p.m. convulsions occurred again. Recovery after injection of 2 gms. glucose.

TABLE II.—Subcutaneous Injection of Insulin in Rabbits.

	Weight of Crude Insulin injected.	Blood Sugar.		Remarks.
		Time Intervals.	Glucose per cent.	
Experiment 5. Mar. 1, 1923.	0.16 gm. (at 11.15 a.m.)	11.11 a.m. 11.45 a.m. 12.16 p.m. 12.46 p.m. 1.17 p.m. 2.30 p.m. 11.10 a.m.	0.107 0.09 0.077 0.031 0.038 0.018 0.094	Convulsions at 2.30 p.m. 1 gm. glucose injected. Convulsions at 3.1 p.m. 2 gms. glucose injected; recovery.
Experiment 6. Mar. 6, 1923.	0.075 gm. (at 11.25 a.m.)	11.23 a.m. 12 noon 1.0 p.m. 2.30 p.m.	0.162 0.075 0.064 0.051	No convulsions.
Experiment 7. Mar. 12, 1923.	0.067 gm. (at 11.15 a.m.)	11.12 a.m. 11.56 a.m. 12.50 p.m. 1.15 p.m.	0.127 0.073 0.053 0.041	Convulsions at 1.10 p.m. 1 gm. glucose injected; recovery.
Experiment 8. Mar. 16, 1923.	0.027 gm. (at 3.15 p.m.)	3.10 p.m. 4.20 p.m. 5.10 p.m.	0.133 0.08 0.031	No convulsions.

This procedure generally lasted about five minutes. The treated area was then covered with a piece of jaconet, held in position by a bandage. Samples of blood from the marginal vein of the ear were taken for estimations of blood sugar, immediately before and at intervals after the inunction of the paste. The results obtained are collected in Table I. For purposes of comparison the effects produced on the blood sugar of rabbits by subcutaneous injection of various quantities of crude insulin are given in Table II.

In the first experiment (No. 1, Table I) a relatively large amount of insulin was employed, to ascertain if any effects could be produced by inunction. As noted, there was a reduction of blood sugar to 0.045 per cent., though no convulsions were induced during this period. The hypoglycaemia was still pronounced twelve hours after administration. A much smaller amount of material was employed in Experiment 2. The fall in blood sugar was just observable. The hypodermic injection of the same quantity of insulin (Experiment 7, Table II) induced convulsions in another animal within two hours. In Experiment 3 the crude insulin was incorporated in lard. The inunction of the paste induced a very considerable fall in blood sugar within an hour, the period of hypoglycaemia lasting about ten hours. A large excess of insulin was employed in Experiment 4. Soon after inunction the animal became very lethargic, and within an hour its appearance suggested collapse, though there were no convulsions till 4.10 p.m., when glucose was injected subcutaneously. The animal remained in a collapsed condition for some time, but later seemed to be normal, and ate some green food. At 6.40 p.m. it was again in a strongly convulsed state; 2 grams of glucose were injected subcutaneously. At 10 p.m. it appeared to be recovering, but refused green food. On the following morning it had completely recovered and was actively running about. The reduction in the blood sugar was very pronounced in this experiment, and a considerable amount of insulin must have been rapidly absorbed through the skin.

An examination of the results obtained on the subcutaneous injection of crude insulin (Table II) shows that by this method of administration the maximum effects could be produced with very much smaller doses. In Experiment 8 the reduction of the blood sugar to 0.038 per cent. resulted about one hour after the injection of 0.027 gram, while in Experiment 7 convulsions occurred with the fall in blood sugar to 0.041 per cent. two hours after the injection of 0.067 gram.

In reviewing these results the chief conclusion appears to be that insulin can be introduced into the blood stream by means of inunction. This method of administration necessitates the use of much larger quantities of the drug than those required to produce comparable effects by subcutaneous injection. It is worthy of note, however, that in estimating the effects produced account must be taken chiefly of the duration of the hypoglycaemia. A sudden reduction, followed by an abrupt rise in the blood sugar, such as is indicated in Experiment 8, Table II, where a relatively small amount of the drug was injected, indicates a transient effect. The ideal method of utilization would appear to be indicated by a