THE EFFECT OF PANCREATIC EXTRACT (INSULIN) ON NORMAL RABBITS

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Received for publication July 7, 1922

The successful demonstration of the presence in extracts of degenerated and fetal pancreas (1) of a substance capable of reducing the degree of hyperglycemia and of raising the carbohydrate tolerance of diabetic (depancreated) dogs, and the subsequent discovery that potent extracts may also be prepared from the adult gland, led to the question whether the blood sugar of normal animals would also be affected by the same substance. For both theoretical and practical reasons this question is of great importance; theoretically, because if the blood sugar of normal, as well as diabetic animals, should be affected it would indicate that the action of the active principle in the extract (insulin) is a fundamental one in the control of blood sugar; and practically, because it would place in our hands a ready method by which to determine the potency of the extracts and to investigate various modifications in the methods of their preparation.

Before we proceed to give the results of our investigation it may be well briefly to refer to some of the previous publications in which substances capable of lowering the blood sugar of normal and diabetic animals are discussed. The best-known of the substances is phlorhizin, the action of which was discovered in 1885 by v. Mering (2). The hypoglycemic action, in dogs at least, was also definitely shown by various investigators particularly by Minkowski (3). Not only does this drug lower the blood sugar of normal dogs but also of those rendered diabetic by removal of the pancreas (4), (5). The degree of hypoglycemia that can be caused by phlorhizin is however not very marked; indeed in some animals, such as the rabbit, it may cause a slight rise in blood sugar. Hypoglycemia is also a symptom of phosphorus poisoning and it becomes of extreme degree when phlorhizin is given to phosphorus-poisoned dogs (6). Underhill (7) found that decided

though variable degrees of hypoglycemia occurred in one to three days after the administration, subcutaneously to dogs, of 50 mgm. hydrazine sulphate per kilo body weight. No characteristic symptoms were observed except extreme weakness. Similar results were not so constantly obtained in rabbits. It was further observed that death occurred when dextrose was injected subcutaneously (5 grams per kgm.) into dogs treated two days previously with non-fatal doses of hydrazine. Further observations by the same worker (8) showed that hydrazine in the above dosage is capable of preventing glycosuria in depancreated dogs, the inhibiting effect lasting for between two and four days. Not only did glycosuria fail to appear at the usual time after pancreatectomy in hydrazine-treated dogs but the glycosuria caused by pancreatectomy in normal animals could be inhibited by subsequently injecting hydrazine. The blood sugar also failed to rise or did so only slightly when pancreatectomy was performed in dogs previously injected with hydrazine. Salts of uranium, etc., also cause hypoglycemia (cf. MacNider).

Interesting though these observations are from a scientific standpoint, the results offer nothing of practical value in the treatment of diabetes in man. In this connection the observations of Underhill (9) and his collaborators (10) and of Murlin (11) and Kramer (12) are important. Briefly stated, the work of the former group shows that administration of alkaline carbonates can bring about a decided reduction in the hyperglycemia and glycosuria caused by injections of adrenalin in rabbits, but has no effect on the percentage of blood sugar in normal rabbits. Underhill (13), however, found that marked reduction in the sugar elimination could be brought about in a severe case of diabetes in man by prolonged ingestion of large doses of sodium bicarbonate. Murlin and Kramer by observations on the respiratory quotient, found that alkali administration facilitates the combustion of sugar in the depancreated dog. Injections of sodium carbonate into the blood stream of such animals also caused lowering of the blood sugar. These results, taken along with the previously known fact that administration of acids lowers the tolerance for sugar, are interpreted as indicating that the regulation of the percentage of sugar in the blood is associated to a certain extent with changes in the acid-base equilibrium of the body (14). From the standpoint of the present investigation, the important point is that alkali administration can reduce the blood sugar and diminish the extent of glycosuria in certain forms of diabetes, both experimental and clinical.

Another interesting type of hypoglycemia is that which develops after removal of the liver from the circulation. One of us (J. J.R.M.) (15) in conjunction with R. G. Pearce has shown that the blood sugar rapidly declines in animals from which the liver has been removed, thus confirming those previously made by Bock and Hofmann, Pavy, etc. More recently Mann and Magath (16) have shown in similar experiments that when the percentage of the blood sugar falls to a certain level (0.06 or less), characteristic symptoms of muscular weakness and coma begin to develop, passing later into a final stage with convulsions. The symptoms usually appear in 5 to 8 hours after removal of the liver and that they are definitely related to the reduction in blood sugar is shown by the fact that they are instantly removed by the injection (intravenous?) of solutions of glucose, or even of galactose and maltose. The recovery may last some time and if the symptoms reappear they can again be removed by the injections. By repeated injections the animal may be kept alive for 15 to 30 hours.

The attention of several workers has recently been turned to a form of hypoglycemia which is possibly of much greater physiological significance than any hitherto studied, namely, that which develops following alimentary hyperglycemia, or even after the ingestion of non-carbohydrate foodstuffs. Folin and Berglund (17) have found, for example, that the percentage of blood sugar in man may fall as low as 0.054 to 0.058 immediately following the rise caused by ingestion of glucose. Maclean and Wesselow (18) have also recently investigated this form of hypoglycemia. This work recalls the observations made some years ago by Vosburg and Richards (19) on dogs and by Levie (20) on man in which it was found that hypoglycemia develops after the hyperglycemia due to adrenalin. A certain degree of it was also observed in rabbits following the hyperglycemia due to intravenous injections of glucose (Jacobson, Bang).

Finally, we must allude briefly to the researches which have more directly led up to the present, namely, those on the effects of pancreatic extracts. Recognizing, as previous investigators also did, that the failure to obtain extracts which could influence the metabolism of sugar might be due to destruction of the specific hormone by proteolytic enzymes, E. L. Scott (21), in 1912, used alcohol as the extracting agency, but the extracts did not decidedly lower the sugar excretion. He found, however, that watery extracts given intravenously did temporarily lower the D/N ratio. Murlin (loc. cit.) also observed reduction in the sugar excretion in diabetic dogs by injecting alkaline

extracts of pancreas and Kleiner (22) was able to bring about a definite lowering of the blood sugar in such animals by slow injections of unfiltered watery extracts of the gland. Paulesco (23) also briefly reports favorable results. The most definite and constant results on this aspect of the problem have been those recently published from this laboratory (24), (25), (26), and it is especially significant that the observations on diabetic dogs have been confirmed in the medical clinic of the University of Toronto by similar ones on several diabetic patients (27).

METHODS. In order to establish tolerable uniformity in the nutritive condition of the rabbits used in this investigation, the animals have been fed on a diet of oats and hay. The blood obtained from the marginal ear vein was allowed to drop into a small crucible containing a small amount of powdered oxalate (an excess must be avoided since it interferes with the precipitation of the proteins). When there was any difficulty in securing a free flow of blood, as in an almost moribund animal, we have found that a satisfactory flow could almost invariably be obtained by causing vasodilatation by the application of some xylol to the tip of the ear.

The sugar was determined in the blood samples by the Schaffer-Hartmann method which we have found to be extremely satisfactory. At the termination of the observations whenever possible the glycogen in the liver was determined by Pflüger's method using the Schaffer-Hartmann method to determine the sugar after suitable dilution of the hydrolyzed glycogen solution.

The usual procedure was to take a sample of normal blood and then inject the insulin in several places subcutaneously after which further samples of blood were taken at regular intervals. The animals were meanwhile kept under constant supervision in order to observe the time of onset of any symptoms.

RESULTS. During the course of several hours the blood sugar of a rabbit may vary somewhat, the first two or three samples being higher than those removed subsequently (cf. Bang). The following observation probably shows the maximum extent to which this may occur.

Rabbit (2.39 kgm.) fed oats and hay

10:15 Blood sugar 1.27 per cent
11:15 Blood sugar 1.12 per cent
12:15 Blood sugar 1.12 per cent
2:15 Blood sugar 1.04 per cent
3:15 Blood sugar 0.97 per cent
4:15 Blood sugar 0.94 per cent
5:15 Blood sugar 1.04 (animal struggling)

Since it is upon the behavior of the curve that the assay of various preparations of insulin depends it is obviously important that this spontaneous fall in blood sugar should be borne in mind. Usually, however, the fall with active extract is much more rapid and pronounced than is ever observed under normal conditions. The following are detailed protocols of typical experiments.

Experiment, February 9, 1.6 kgm.

11:15 a.m. Blood sugar 0.167 per cent.
11:30 a.m. Subcutaneous injection of 4 cc. insulin.
12:00 m. Blood sugar 0.137 per cent.
1:45 p.m. Blood sugar 0.065 per cent.
3:00 p.m. Rabbit lying on its side apparently more or less unconscious and with rapid shallow breathing of a periodic character. Conjunctival reflex sluggish, eyeballs protruding and pupil widely dilated. Mechanical stimulation of the skin caused convulsive movements in which the animal threw itself about violently and rolled over sideways in the same direction, with head retracted. Each seizure lasted 2 to 3 minutes. Rectal temperature 37°C. If left to itself convulsive seizures might supervene without apparent exciting cause. In these the animal was lying on its side with the head retracted and the limbs contracting and relaxing as in running. After each convulsion the animal seemed to be better and could hold his head up but in a few minutes it again became comatose, another convulsion being common in about 15 minutes.
3:50 p.m. Blood sugar 0.028 per cent.
5:30 p.m. 4.5 grams pure dextrose in 20 per cent solution injected in various places subcutaneously. Within 5 to 7 minutes the rabbit was perceptibly improved and soon sat up in the normal position and apparently became normal (jumped about the room, etc.). Kept near radiator over night.

February 10. Animal hyperexcitable, the jaw constantly moving as in chewing, but ran about in normal way and ate food.

February 13. Has been eating freely but is still decidedly hyperexcitable, the jaw still showing chewing movements. Shook head violently when lifted by the ears. Losing weight.

February 14. The same.

February 15. Animal still losing weight. At 3 p.m. fits similar to those of the 9th reappeared and these continued at about 15 minute intervals until 5 p.m. when 5 grams dextrose were given subcutaneously with the result that the animal immediately recovered.

February 16. The rabbit was found dead in the cage and on post-mortem examination the pancreas was observed to be greatly atrophied (possibly autolytic) and the subcutaneous tissues of the anterior abdominal wall (where no subcutaneous injections had been made) changed to a mass of muciginous material. At various places under the skin of the back where injections of sugar had been made there was discoloration and induration of the tissues but no trace of the mucin-like material.

Experiment, February 10, 1.6 kgm.

12:00 noon. 4.5 cc. insulin injected subcutaneously.
4:00 p.m. Symptoms like those described above. It was particularly noted that the general condition of the animal was improved immediately following each convulsive seizure. (These occurred about every 5 minutes.)
4:15 p.m. 5 grams dextrose subcutaneously. The animal quickly recovered and by
5:30 p.m. was apparently perfectly normal.

Experiment, April 24

8:10 a.m. Blood sugar 0.129 per cent; 5 cc. insulin given subcutaneously.
8:55 a.m. Blood sugar 0.077 per cent.
Animal in convulsions some time before 11:30.
11:40 a.m. Blood sugar 0.047 per cent; rectal temperature 37.1°C.
11:48 a.m. 2.5 cc. more insulin injected.
12:00 noon. Convulsions, rectal temperature 36.0°C.
12:10 p.m. Blood sugar 0.033 per cent rectal temperature 36.0°C.
12:15 p.m. Convulsions.
12:18 p.m. 5 grams dextrose in 40 cc. water injected in several places subcutaneously—rectal temperature 36.0°C.
12:23 p.m. Blood sugar 0.056 per cent; rectal temperature 36.5°C.; rabbit now sitting up and apparently normal.
12:43 p.m. Blood sugar 0.091 per cent; rectal temperature 36.5°C., rabbit normal.
1:05 p.m. Blood sugar 0.070 per cent; rectal temperature 37.6°C.; rabbit normal.
2:35 p.m. Blood sugar 0.043 per cent; rectal temperature 38.0°C.
3:35 p.m. Blood sugar 0.053; rectal temperature 37.6°C. Convulsions.
5:40 p.m. Blood sugar 0.024; rectal temperature 35.0°C. Convulsions.

The symptoms as described above are fairly characteristic, but slight variations may be observed in different rabbits. Very frequently the first symptom is one of hyperexcitability and of apparent fear so that the slightest stimulus, such as touching the animal, shaking the cage or floor or clapping the hands, causes the animal to rush wildly about in an incoördinate fashion with the vision apparently affected since objects in his path are not avoided. A still earlier symptom often observed consists in chewing movements as if the rabbit were hungry, and at this stage if food is available it will be voraciously eaten.

While there is no evidence of marked cardio-vascular disturbance in a typical convulsion (blood being usually readily obtained from the ear vein), this is sometimes present making it very difficult to obtain any blood. These cases are especially noticeable when impure preparations of insulin are used and the depression of blood pressure is probably due to peptone-like substances (histamine). A characteristic symptom

in this group of cases is paralysis of the hind limbs, due probably to anemia of the lower portion of the spinal cord.

In many rabbits typical convulsions may last only a few minutes after which the animal gradually recovers without sugar injections. Such animals have frequently been used several days later for testing the potency of extracts.

A most interesting *post-mortem* finding in animals that have died in a convulsive seizure and in those that have temporarily recovered as a result of sugar injections but died subsequently, is the extensive mucigi-nous change in the subcutaneous tissues of the abdominal wall. Sometimes this material forms a more or less circumscribed tumor and, particularly after sugar injections, a considerable amount of fluid may be present along with the mucin-like material. This fluid has strong reducing properties. Nowhere else in the body have we observed this peculiar change. It is not due to any local action of the insulin since this is always injected in the back.

We will now give in tabular form less detailed data of 100 rabbits injected with insulin. In Table 1 the percentage of blood sugar fell by 25 per cent or over within 2 hours of the injection.

There are observations on 32 animals in this group and convulsions were observed in eleven cases. The average percentage of blood sugar in these is 0.042. In computing this average there are several cases where the value is taken either somewhat before or after the 2-hour period but the deviation is about the same in either direction and the figure probably represents very closely the average percentage of blood sugar at which convulsions appear in rabbits.

In the next table 2, those observations are given in which the blood sugar fell between approximately 25 and 50 per cent within 2 hours of the injection.

There are observations on 29 animals in this group and convulsions were observed within 2 hours of giving insulin in only one case (ECN, March 16). In several other cases the percentage of blood sugar fell considerably below this level with no symptoms (FGB, April 5, April 13; JBC, February 23, April 19; CHB, Apri 20).

Table 3 gives the cases in which the blood sugar fell by at least 50 per cent during the period of observation.

In the twelve cases of table 3, convulsions occurred in three, the blood sugars being 0.03, 0.04 and 0.065 respectively. No convulsions were observed in six cases with approximately 0.05 per cent of sugar nor in one with 0.042 per cent.

TABLE 1

Case in which blood sugar fell by approximately 50 per cent within 2 hours of injection

EXPERIMENT	BLOOD SUGAR		TIME AFTER EXTRACT	SYMPTOMS
	Before insulin	After insulin		
ECN. Feb. 16	0.111	0.045	35 min.	None
		0.0151	4½ hrs.	
ECN. Apl. 22	0.139	0.075	45 min.	None
ECN. Apl. 22	0.129	0.056	1 hr.	None
ECN. Apl. 25	0.132	0.067	35 min.	None
FGB. Apl. 6	0.145	0.060	1 hr. 30 min.	None
		0.049	3 hrs.	None
FGB. Apl. 10	0.151	0.071	2 hrs.	None
FGB. Apl. 12	0.157	0.047	2 hrs.	Convulsions in 2 hrs. after extract
		0.037	4 hrs.	
		0.165	23 hrs.	Recovered
FGB. Apl. 20	0.147	0.063	1½ hrs.	None
		0.069	4½ hrs.	
FGB. Apl. 20	0.139	0.077	1½ hrs.	None
		0.051	4½ hrs.	
FGB. Apl. 20	0.135	0.045	1 hr. 10 min.	None
FGB. Apl. 25	0.135	0.071	1 hr.	Convulsions in 2 hrs. after extract
		0.031	2 hrs.	
		0.153	9 hrs.	Died next day
JBC. Jan. 17	0.120	0.047	1¼ hrs.	None
JBC. Feb. 14		0.045	1½ hrs.	Convulsions
JBC. Feb. 16	0.111	0.045	35 min.	None
		0.151	4½ hrs.	
JBC. Feb. 28		0.040	1 hrs.	Convulsions
JBC. Mar. 3		0.025	35 min.	Coma-like symptoms, recovery with sugar
JBC. Mar. 17		0.060	2 hrs.	None
JBC. Mar. 25		0.060	1 hr.	Convulsions in 1½ hrs.
JBC. Mar. 28		0.040	1 hr.	Convulsions
JBC. Apl. 11		0.045	1½ hrs.	
JBC. Apl. 16	0.110	0.064	1 hr.	None
CHB. Apl. 5	0.137	0.064	2½ hrs.	Convulsions in 2 hrs.
CHB. Apl. 5	0.130	0.064	2 hrs.	None
CHB. Apl. 17	0.149	0.040	1½ hrs.	Convulsions
CHB. Apl. 18	0.127	0.037	2 hrs. 45 min.	Convulsions
CHB. Apl. 19	0.125	0.064	2 hrs.	None
CHB. Apl. 19	0.127	0.045	2½ hrs.	None
CHB. Apl. 19	0.127	0.065	2 hrs.	None
CHB. Apl. 19	0.115	0.069	1 hr.	None
CHB. Apl. 21	0.140	0.075	1 hr.	None
CHB. Apl. 24	0.132	0.065	1¼ hrs.	None
CHB. Apl. 24	0.125	0.037	2 hrs.	Convulsions
		0.01	2¾ hrs.	Moribund

TABLE 2

Blood sugar fell between 25 and 50 per cent in 2 hours

EXPERIMENT	BLOOD SUGAR		TIME AFTER EXTRACT	SYMPTOMS
	Before insulin	After insulin		
ECN. Feb. 24	0.120	0.093	35 min.	None
		0.080	2 hrs.	
ECN. Mar. 16	0.108	0.063	35 min.	Convulsions
		0.067	1½ hrs.	
ECN. Mar. 17	0.135	0.114	30 min.	
		0.103	1¼ hrs.	
		0.083	2 hrs.	None
ECN. Mar. 29	0.117	0.080	35 min.	None
ECN. Mar. 27	0.137	0.082	30 min.	None
ECN. Mar. 30	0.124	0.089	35 min.	None
FGB. Apl. 5	0.123	0.090	1½ hrs.	
		0.069	2½ hrs.	
		0.037	3½ hrs.	None
		0.039	4½ hrs.	
FGB. Apl. 12	0.149	0.083	1½ hrs.	None
		0.095	3½ hrs.	
FGB. Apl. 13	0.111	0.069	1½ hrs.	None
		0.065	3½ hrs.	
FGB. Apl. 14	0.149	0.081	1½ hrs.	None
FGB. Apl. 18	0.135	0.071	1 hr. 25 min.	None
JBC. Dec. 22	0.126	0.087	2 hrs.	None
JBC. Dec. 16	0.119	0.101	½ hr.	None
		0.089	1¼ hr.	
JBC. Jan. 4	0.124	0.093	1 hr.	None
JBC. Jan. 10	0.117	0.073	1 hr.	None
		0.085	3 hrs.	
		0.085	6 hrs.	
JBC. Jan. 12	0.112	0.072	1 hr.	None
JBC. Jan. 16	0.125	0.075	1 hr.	None
JBC. Jan. 23	0.094	0.068	1 hr.	None
JBC. Feb. 23	0.094	0.046	3 hrs.	
		0.049	4 hrs.	
		0.057	5 hrs.	None
		0.058	6 hrs.	
		0.131	Next day	
JBC. Mar. 22	0.141	0.087	½ hr.	None
		0.078	1½ hrs.	
JBC. Mar. 28	0.120	0.095	40 min.	None
JBC. Mar. 31	0.130	0.088	1 hr.	None
JBC. Apl. 6	0.124	0.078	45 min.	

TABLE 2—*Concluded*

EXPERIMENT	BLOOD SUGAR		TIME AFTER EXTRACT	SYMPTOMS
	Before insulin	After insulin		
JBC. Apl. 7	0.142	0.092	50 min.	
		0.045	6 hrs.	Convulsions in 6 hrs.
JBC. Apl. 19		0.065	30 min.	None
JBC. Apl. 19		0.085	31 min.	None
		0.060	4 hrs.	
		0.053	5 hrs.	
CHB. Apl. 20	0.089	0.061	2 hrs.	None
CHB. Apl. 20	0.108	0.071	1½ hrs.	None
CHB. Apl. 23	0.150	0.100	1½ hrs.	None

TABLE 3

Cases not included in preceding tables in which the blood sugar fell by approximately 50 per cent during period of observation

EXPERIMENT	BLOOD SUGAR		TIME AFTER INSULIN	SYMPTOMS
	Before insulin	After insulin		
ECN. Apl. 24	0.095	0.073 (more insulin)	45 min.	
		0.057	1 hr. 40 min. after second injection	None
		0.052	2 hrs. 45 min. after second injection	
JBC. Apl. 19		0.085	½ hr.	
		0.060	4 hrs.	None
		0.053	5 hrs.	
CHB. Apl. 7	0.135	0.051	3 hrs.	None
CHB. Apl. 6	0.130	0.03	8 hrs.	Convulsions at 7 hrs.
CHB. Apl. 13	0.111	0.065	3½ hrs.	None
CHB. Apl. 13	0.173	0.081	3 hrs.	None
CHB. Apl. 19	0.113	0.050	3 hrs.	None
CHB. Apl. 21	0.109	0.053	4 hrs.	None
CHB. Apl. 3	0.119	0.083	4 hrs.	None
CHB. Apl. 17	0.165	0.04	3 hrs.	Convulsions
JBC. Apl. 19		0.065	2½ hrs.	Convulsions (5 gm. dextrose)
		0.030	19 hrs.	Convulsions
JBC. Apl. 19		0.042	3 hrs.	None

Taking the three tables together there are observations on 73 animals. The blood sugar fell below 0.045 in thirteen and of these convulsions were present in eleven. Of the two cases with blood sugar below 0.045 not showing convulsions, one (JBC, April 19) gave 0.042 in 3 hours, the other (FGB, April 5) 0.037 in ½ hours and 0.039 in 4½ hours. There are nine cases with blood sugars between 0.045 and 0.050 of which convulsions were observed in three. The highest percentage at which convulsions were observed was 0.064[1] (CHB, April 5) 0.063[2] (ECN, March 16) and 0.065 (JBC, April 19). The lowest percentage at which there were no symptoms as already mentioned was 0.037 (FGB, April 5).

The foregoing analysis shows that the average computed, from the results of table 1, as that at which convulsions most commonly appear, namely 0.042, must be very close to the correct one.

For purposes of physiological assay of insulin we consider that the most satisfactory basis at present is the number of cubic centimeters which lowers the percentage of blood sugar in normal rabbits to 0.045 in from 2 to 4 hours. There are several advantages in using this standard, among which the following may be mentioned.

1. Since 0.045 per cent is the level of blood sugar at which definite convulsions supervene one can tell in a general way from the premonitory symptoms of the injected animal (evidence of hunger and thirst, hyperexcitability and evidence of fear) whether or not this level is likely to be reached.

2. The effect of insulin in reducing the blood sugar of a diabetic (depancreated) dog is considerably more marked than one would expect from the effect on the normal rabbit. Just as a certain dose of an antipyretic may cause a marked lowering of hyperpyrexia in fever, but have only a slight effect on the normal temperature, so will insulin reduce the blood sugar relatively much more in hyperglycemic than in normal animals. This fact is illustrated in the following observation. Ten cubic centimeters of insulin lowered the percentage of blood sugar in a rabbit from 0.135 to 0.071 in 1½ hours; whereas 20 cc. given to a depancreated dog weighing 11 kgm. lowered the sugar from 0.375 to 0.030 in 19 hours. The rabbit weighed about 2 kgm. so that on a basis of body weight the dog received only about one-third the dose of the rabbit.

[1] Both of the rabbits from which these results were obtained were injected with crude extracts, containing much protein, and it is probable that the symptoms were of the paralytic type referred to on p. 167.

[2] The insulin used in this animal was evidently very potent since the blood sugar was 0.03 19 hours after it was given, dextrose being meanwhile administered. It is therefore possible that the blood sugar was really below the figure given when the convulsions occurred.

3. One unit as defined above has been used as the basis for use on man with sufficient frequency to warrant the assertion that while this dose markedly lowers the degree of hyperglycemia, it never causes any alarming symptoms, although the administration may be followed in an hour or so by certain subjective symptoms, such as hunger.

When the blood sugar does actually reach 0.045 per cent there is then no difficulty in stating the dosage of the preparation with tolerable accuracy. When it fails to reach this level however the assay is more or less unsatisfactory. There are several factors to be considered in this connection, the most important of which are:

1. The rate of physiological action of the insulin. There is no doubt that this varies among different preparations. It is decidedly slower in impure preparations than in those that give only faint protein reactions. With the former, the blood sugar may in 4 hours not yet have reached to its lowest level so that the preparation is assayed at too low a value; with the latter, the lowest point may have been reached before the 4 hours so that the assay is too high. At present we know of no method to remove these sources of error but we recommend, when the sugar is not lowered below 0.065 in 4 hours, that another specimen of blood should be taken in 5 or 6 hours and if the hypoglycemia is more pronounced the extent to which the action is delayed should be stated in the assay of the preparation. If, on the other hand, the blood sugar never reaches below 0.065 per cent with an injection of more than 4 cc. the preparation must be considered unsatisfactory. When the sugar falls to between 0.055 and 0.065 we arbitrarily label it a half rabbit dose and that this is a comparatively safe expedient is evidenced in the results of table 4 by the fact that there is only one case marked half a dose in which convulsions afterwards developed.

2. The same dose of the same preparation of insulin may not have the same effect on different rabbits. This is illustrated in the following results:

EXPERIMENT	AMOUNT INJECTED	BLOOD SUGAR		TIME AFTER INJECTION	SYMPTOMS
		Before	After		
	cc.	*per cent*	*per cent*	*hours*	
CHB. June 12..		0.14	0.09		
	2		0.08	2	None
June 12..	2	0.11	0.11	2	None
June 14..	2	0.13	0.06	2	None
June 14..	2	0.185	0.180	2	None
June 20..	2	0.12	0.065	2	Convulsions at 10 hours
June 20..	2	0.105	0.040	2	Convulsions at 3 hours

We have chosen observations which probably give the extreme degrees of variability likely to be met with, but the failure, as in the

TABLE 4

DATE	BLOOD SUGAR		AMOUNT	TIME AFTER INSULIN	SYMPTOMS	ASSAY (RABBIT DOSES)
	Before insulin	After insulin				
			cc.	*hours*		
June 5	0.148	0.038	4	4	Violent convulsions	1 plus
June 12	0.12	0.040	4	3	Violent convulsions	1 plus
June 14	0.13	0.062	2	2	None	½ plus
June 13	0.122	0.040	3½	2½	Convulsions	1 plus
June 13	0.125	0.040	3	2½	Convulsions	1 plus
June 15	0.146	0.054	2½	2	None	1
June 15	0.115	0.065	2½	2	None	½
June 15	0.155	0.062	3	2	None	½
June 15	0.120	0.066	2	2	None	½
June 16	0.130	0.055	2	2¼	None	½
June 19	0.130	0.060	2	2¼	Convulsions later	½
June 19	0.180	0.040	2	1¾	Convulsions	1 plus
June 19	0.140	0.060	2	2	None	½
June 19	0.120	0.060	2	2	Convulsions later	½
June 20	0.120	0.065	2	2	Convulsions later	½
June 20	0.130	0.065	1	1½	None	½
June 20	0.105	0.04	2	2	Convulsions	1 plus
May 8	0.135	0.056	2	1½	None	½
May 16	0.135	0.045	2½	2¼	Convulsions	1
May 16	0.120	0.045	1½	2¾	Convulsions	1
May 18	0.140	0.045	3	2½	Convulsions	1
May 1	0.11	0.045	1	2	Convulsions	1
May 10	0.11	0.062	3	1¼	None	½
May 2	0.165	0.045	10	2	Convulsions	1
May 3	0.116	0.062	2½	4	None	½
May 3	0.138	0.056	5	1	None	½
May 2	0.141	0.042	1½	4	Violent convulsions	1 plus

experiments of June 12 and 14, of preparations that were of considerable potency in one animal not to cause any hypoglycemia in others is obviously unsatisfactory. We are engaged in seeking for a more

dependable method of assay but at present must fall back on the above as the only one available.

In the following table 4, are given some of our most recent results (not included in the preceding tables) in which the above principles of assay are followed.

Further details will be given in a subsequent paper regarding the influence of injections of various sugars and related substances on the convulsions. For the present it is important to note that in practically every case in which we have injected dextrose (4 grams in 20 per cent solution) within half an hour of the first appearance of definite convulsive seizures, the animals have recovered as described in the experiment on p. 166. Even when the convulsions have been allowed to go on until the animal is evidently almost moribund dextrose injections usually have a most marked effect. For example, we have on several occasions succeeded in restoring to a tolerably normal condition an animal in which the breathing, after being extremely rapid or periodic in type, had at last ceased. Injections of equal quantities of saline solution have no effect. Frequently animals that have been restored to normal by dextrose pass a second time into convulsions which may again be antidoted by dextrose.

CONCLUSIONS

1. Purified alcoholic extracts of pancreas, for which we suggest the name *insulin*, when injected subcutaneously into normal rabbits cause the percentage of sugar in the blood to fall within a few hours.

2. As a tentative basis for the physiological assay of insulin we consider as one unit the number of cubic centimeters which causes the blood sugar of normal rabbits to fall to 0.045 per cent within 4 hours. This dose is decidedly active in lowering the blood sugar in diabetic patients.

3. As the blood sugar falls, as a result of insulin injections, the rabbit exhibits highly characteristic symptoms and earliest of which are signs of hunger and thirst, hyperexcitability and apparent fear.

4. The animal may recover from these earlier symptoms but frequently, with active preparations, the hyperexcitability becomes extreme and clonic convulsive seizures involving the entire body and lasting for several minutes supervene. Between the convulsive seizures the animal is lying on its side in a more or less comatose condition with shallow, rapid and frequently periodic breathing.

5. In the great majority of cases exhibiting convulsions the blood sugar has been found to be about 0.045 per cent.

6. Subcutaneous injections of dextrose solutions antidote the convulsions and other symptoms, so that the animal in a few minutes becomes restored to a tolerably normal condition. Similar symptoms may again develop but they also can be antidoted by dextrose.

7. In animals that die as a result of the symptoms, a peculiar mucoginous degeneration of the subcutaneous tissues of the abdominal wall is very commonly observed.

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