THE MECHANISM OF HIBERNATION

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By

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A Thesis Presented to the McGill University Graduate School in Partial Fulfillment of the Requirements for the Degree of

Master of Science

May, 1928.

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CONTENTS

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I. INTRODUCTORY

The hibernating mammal presents several unique features as regards metabolism. The change from the homoiothermic to the poikilothermic condition, and vice versa, is of considerable interest. Numerous theories have been advanced to explain these transitions, and frequent attempts have been made to induce artificial hibernation but, so far as I know, the essential problem is still unsolved. A good account of previous work on this subject is contained in an article by Rasmussen (1916). Lowering of external temperature, depression of respiration by cold, deficient food supply, cerebral anemia, auto-narcosis, acapnia, and altered function of the endocrine system have all been cited as primary causes, but experiment has amply shown that not one of these is, in itself, sufficient to produce hibernation. Thus, animals that normally hibernate have been known to become torpid even in mid-summer and in the presence of a rich food supply; and cerebral congestion has been reported in animals during hibernation .

Mere lowering of body temperature was regarded as sufficient to induce artificial hibernation by Simpson (1902), who used monkeys, and by Simpson and Herring (1905), who used cats. The last named workers claimed that, when the rectal temperature falls to 24° C., the heat-regulating mechanism does not assert itself and the temperature of the animal tends

erature of the animal tends to become the same as that of its surroundings. This conception has been disproved by Britton (1922) who showed that cats reduced to a deep rectal temperature of between 24° and 22° C. effect spontaneous recovery in six to seven hours, and that these animals can recover from a temperature of between 21° and 19° C. in ten to twelve hours. Tait and Britton using the woodchuck (Arctomys monax) have further established the fact that an animal which naturally hibernates can recover spontaneously and promptly after artificial cooling to temperatures varying between 12° and 3° C.

Dubois (1909 and 1901) produced a condition of inactivity by causing his animals (marmots and dogs) to breath air containing a large proportion of carbon dioxide. This condition was termed by him auto-narcosis. The animals did not, however, assume the characteristic poikilothermic state.

Finally, one might quote several observations which would indicate the existence of a relationship between some of the ductless glands and hibernation. Cushing and Goetsch (1912) noted that excision of the pituitary in man and in animals produces symptoms comparable to those of hibernation. These authors corroborated the findings of gemelli(1906), who observed marked changes in the chromophile cells in the anterior lobes of pituitary glands removed from marmots in the hibernating state. Mann (1916) also reported such changes in the gopher, but they were not constant. He also found gross, as well as microscopical, seasonal changes in the adrenals, and in the glands of actually hibernating animals

noted a general shrinkage and vacuolization of the cells, especially in the zona granulosa and upper part of the zona fasciculata. The only other changes of interest that have been noted are in the islets of Langerhans in the pancreas which resemblex those of a normal resting gland.

Some of the characteristics of the true hibernating state are: unconsciousness, inactivity, and a fall of body temperature to within a degree or two above that of the environment; the respiratory rate is slowed to about one per minute or even less; and the heart rate is likewise greatly decreased. Further, it has been shown by Dubois (1894a) and by Rasmussen (1915) that the blood of the dormant animal contains an abnormally high percentage of carbon dioxide. While the metabolic rate is greatly reduced, there is evidence of a remarkable change in the character of the nutrition. During hibernation there is a gain in weight, Pembrey (1901) reporting that three marmots gained during one hour 11, 8, and 17 centigrams respectively . Reiset and Regnault (1849) recorded a gain of 5.9 grams in five days, also in the marmot. This is accompanied by a decreased ratio of carbon dioxide output to oxygen intake, both of which are greatly decreased, and by diminished excretion of water as compared with that of the active animal. (Pembrey,1901). On the transition to the active state there is marked shivering and a sudden rise of the respiratory quotient; the animal's temperature rapidly rises, that of the dormouse mounting from 13.5° to 35° C. in one hour, and within a very short time the usual mammalian temperature level is reached. It was found

by Claude Bernard, by Voit (1878), and by Dubois (1899) that the liver of the hibernating animal contains much glycogen, which disappears on awakening. Pembrey (1901) correlating this observation with the fact that the respiratory quotient of the hibernating animal is very low (0.53), concluded that during torpidity fat is being continuously converted to glucose, which is stored as glycogen, and reconverted to glucose for utilization during awakening, when the respiratory quotient rises (0.71 to 0.89).

The only record of the blood sugar concentration in the hibernating mammal to be found in the literature is that of Dubois, who reported it as 0.009 per cent for the European marmot; in the active a animal it was 0.117 per cent. In the light of Pembrey's conclusions, this finding naturally suggests some correlation between hibernation and the concentration of the blood sugar. One might further quote Dubois (1894b) to the effect that extirpation of the ganglia of the solar plexus or ligature of the portal vein and of the inferior vena cava just above the liver prevents the remarkable rise in temperature seen in the awakening marmot. Additional evidence for some such relationship is found in the fact that Mills (1892) and Simpson (1912) could prevent woodchucks from hibernating by providing them with an adequate supply of food, a fact which has also been observed in this laboratory on numerous occasions. The blood sugar concentration of one of these animals was determined on two occasions and was found to be 0.361 and 0.235 per cent respectively (no anesthetic was used in removing the blood). This woodchuck was given insulin and developed typical convulsions within two hours. Blood taken immediately after a convulsion

contained 0.057 per cent glucose. One could conclude from this experiment that, if the figure of 0.009 per cent for the dormant animal is characteristic, some mechanism must come into play during hibernation to prevent the occurrence of hypoglycemic convulsions. With these facts in mind, a series of experiments was undertaken in collaboration with Drs. S. Dworkin and G. J. Cassidy in which it was proposed tp try the combined effect of lowered body temperature and of insulin hypoglycemia in order to see whether further data concerning the mechanism of hibernation could be obtained.

II. METHOD

Cats and dogs were used in the earlier experiments; since a sufficient number of woodchucks were not available at that time. The animals were kept on a diet of lean meat, milk, and cereals for a week or two prior to the experiments. The temperature of the animals was lowered by immersing them in a cold bath. An anesthetic was necessary to prevent struggling during cooling. Amytal, isoamylethylbarbituric acid, was used; since it neither causes hyperglycemia, nor interferes with the fall of blood sugar following insulin administration. Preliminary control experiments clearly demonstrated that, in doses used by us, amytal does not prevent insulin convulsions in normal animals. Some of the experiments were c carried out without anesthesia; these will be referred to later. Deep rectal temperature was recorded, the bulb of the thermometer being inserted to a depth of about 8cms. Blood was removed from the external jugular vein in 1 c.c. quantities for sugar determination by the method of Folin and Wu. After removal from the bath, the animals were completely dried by rubbing with towels and by the application of dry heat, in order to prevent loss of heat by evaporation. The average weight of the cats was 3kgm.; that of the dogs, 8 kgm. The insulin was given subcutaneously in most cases in doses of from 3 to 5 units per kilogram of body weight.

In a later series of experiments, American woodchucks (Arctomys monax) were used. These animals were trapped during the month of May, and for a month preceding the experiments were kept in large wire cages, their food consisting for the most part of

green vegetables, potatoes, and carrots. At the time of use the animals were all healthy and active. The method of carrying out the first few experiments with these animals was essentially similar to that employed in the case of dogs and cats. In the later experiments, the body temperature of the woodchucks was lowered by placing them in a specially designed cooling chamber, which will be subsequently described.

III. THE RATE OF ACTION OF INSULIN IN ARTIFICIALLY COOLED MAMMALS

It is well known that exposure of a normal animal to cold causes an increase in the blood sugar concentration, due, presumably, to stimulation of the sympathetic nervous system and augmented secretion of adrenaline. For this reason it was considered preferable to lower the body temperature of the animal first and then to administer insulin, instead of preceeding in the reverse manner. The feasibility of this mode of procedure depends upon whether, or not, insulin will lower the blood sugar of an animal at lowered body temperature with reasonable rapidity.

The metabolic rate of an animal at a body temperature of 25° C. is much less than the basal rate at normal body temperature. (Finney, Dworkin, and Cassidy, 1927). There are a number of observations on record as detailed below to indicate that insulin acts very much more slowly in cold blooded animals than in mammals. For these reasons, and from a consideration of Van't Hoff's law, it was to be expected that the fall of blood sugar following insulin administration to a dog or cat at a body temperature of 25° C. would take place more slowly than at normal body temperature. Hence, it was considered necessary to carry out a series of experiments with the object of determining the rate of action of insulin at lowered body temperature before commencing upon the investigation of the major problem.

Krogh found that under ordinary circumstances frogs

develop insulin convulsions only 4 to 5 days after the injection of massive doses of insulin. This observation has been confirmed by Macleod (1924), by Huxley and Fulton (1924), and by Olmstead (1924). Huxley and Fulton (1924) further established that this period was reduced to 24 hours in frogs kept at 25° C. They concluded that, "The activity of insulin itself is not essentially altered by temperature, but that its speed of action is dependent upon the metabolic rate of the animal itself." These conclusions have been corroborated by Olmstead, who reported that the onset of insulin convulsions in fishes is hastened by raising their temperature.

Olmstead (1924) also attempted to ascertain the relation between insulin convulsions and blood sugar in frogs. He found the blood sugar concentration of normal frogs at room temperature to be very low (about 0.012 per cent), and that it rose to 0.056 per cent when the frogs had been kept at 28° C. for 2 days. When convulsions occurred, the blood sugar concentration varied between wide limits, and was often higher than it was before the administration of insulin. In view of these results, it seems impossible to correlate the blood sugar level and the occurrence of convulsions in such animals.

Before proceeding with the experiments on the rate of action of insulin in mammals at 25° C., it was necessary to determine the effect of lowering the body temperature upon blood sugar. Two amytalized dogs were cooled to 25° C., blood samples being secured at intervals during the process. Under these conditions there

was a slight preliminary decrease in blood sugar concentration, probably due to the occurrence of vigorous shivering at this time. This was followed by a gradual rise, and , when the body temperature had reached 25° C., the blood sugar had practically returned to its original value. (Table I).

TABLE I

Dog 5		Dog 10	
Temperature	Blood Sugar	Temperature	Blood Sugar
°C.	Per Cent	°C.	Per Cent
38	.107	38	.111
36	.084	32	.094
33	.099	30	.106
25	.095	24	.113

All of the cats and dogs used for the following experiments were starved for the 24 hours previous to the injection of insulin.

Two cats and five dogs were cooled to 25° C. under amytal anesthesia and kept at this temperature thruout the rest of the experiment by exposing them to cold air or by applying dry heat as occasion demanded. Immediately after removal from the bath, insulin was given subcutaneously in doses of from 3 to 5 units per kilogram of body weight. Blood was withdrawn at frequent intervals. It was found that the blood sugar fell to 0.045 per cent in from 1 hour and 20 minutes to 3 hours--see Table II. Since similar doses of insulin

administered to normal dogs and cats after 24 hours starvation reduce their blood sugar concentrations to the convulsive level in from 1 to 4 hours, it would appear that the rate of fall of blood sugar is not affected by lowering of the body temperature to 25° C.

TABLE II

	Blood Sugar before Insulin	Units Insulin per kg.	Time to Reach 0.045 p.c.	Duration of Experiment	Blood Sugar at End of Experiment
	Per Cent		Hours	Hours	Per Cent
Dog 7	.095	4	3	5 5-6	.026
Dog 10	.113	5	2 1-3	13	.016
Dog 11	. 091	4	1 1-2	2	.043
Dog 18	.111	3	1 1-3	6 1-2	.033
Dog 23	.124	3	2 1-3	3 2-3	.038
Cat 1	----	4	1 1-2	4	.030
Cat 2	----	4	2 1-3	4 1-2	.033

In order to obtain a more precise comparison between the rates of action of insulin at normal and at reduced body temperatures, the following experiment was performed. An amytalized dog was given 3 units of insulin per kilogram subcutaneously . Blood samples were removed under aseptic conditions at 15 minute intervals for a period of 3 hours. Three grams of glucose were then injected subcutaneously for each kilogram of body weight in order to

insure the animal's recovery. Three days later this animal was cooled under amytal anesthesia to 25° C., and was given the same amount of insulin as before. Although the general form of the blood sugar curve on this occasion was similar to that at normal body temperature, the convulsive level was reached 1 hour sooner--see Fig. I.

The above experiment was repeated on another dog. The curves for this animal differed from each other in two particulars:(1) The preliminary rise, which was quite marked at normal body temperature, was greatly exaggerated at 25° C.; (2) whereas the fall of blood sugar at normal body temperature began about ten minutes after the insulin injection, the fall at 25° C. commenced only after 1 hour. From this point on the curves ran almost exactly parallel.

Since the efficiency of the circulation is greatly impaired at a body temperature of 25° C., it was thought that the preliminary delay in the fall of blood sugar after subcutaneous insulin injection might have been due to slow absorption of the insulin. For this reason, a third experiment was undertaken, in which the insulin was injected intravenously. The curve for this dog at 25° C. showed a delay of 40 minutes before the fall of blood sugar began; whereas the control curve showed no delay (Fig. II). There was no preliminary rise in either case. Just as in the previous experiment, the two curves are practically parallel, once the fall commences. From this experiment the conclusion can be drawn that the preliminary delay in the fall of blood sugar at 25° C. is due to causes other than the slow absorption of insulin. This delay occurred in about half of

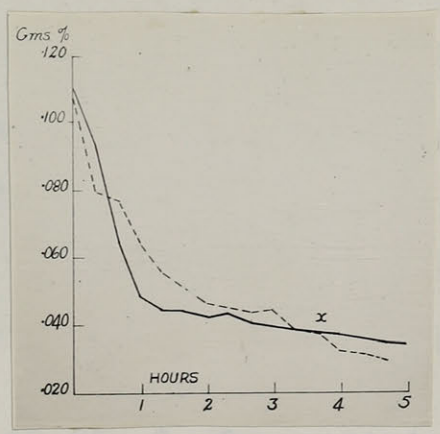

Fig. I. Insulin Curves.

Full Line--25° C.

Dotted Line--38° C.

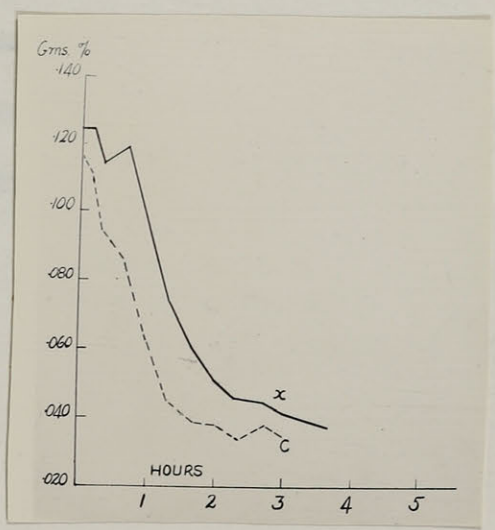

Fig. II. Insulin Curves.

Full Line--25° C.

Dotted Line--38° C.

C Convulsion.

the dogs and cats used.

Another characteristic of the curves for animals at 25° C. is that the portion of the curves (marked x in Figs. I and II) representing the secondary fall is smoother than the corresponding part of the controls. This might be caused by decreased activity of the adrenals brought about by the lowered body temperature.

Seven animals remained at 25° C. for periods up to 13 hours after the injection of insulin. In no case did the blood sugar show any tendency to return to normal. On the contrary, it continued to fall progressively until the end of the experiments. (Table II). A possible explanation of this fact can also be found in decreased adrenal secretion; since, as Cannon has shown, the sensitivity of an animal to insulin is greatly increased if the adrenals be extirpated or isolated from the nervous system.

IV. INHIBITION OF INSULIN CONVULSIONS BY LOWERED BODY TEMPERATURE.

None of the animals used in the experiments which have just been described developed insulin convulsions. although their blood sugar levels remained below 0.045% for hours. This would seem to indicate that lowered body temperature inhibits insulin convulsions. In order to investigate this point in greater detail, a special set of experiments was undertaken. In these experiments, after having been cooled to a temperature of 25°C. under amytal anesthesia, each animal was removed from the bath. Insulin was then injected and blood taken at frequent intervals in order to determine the moment at which the blood sugar reached the level of 0.045%. In the meantime artificial measures were taken to keep the body temperature between 24° and 25° C.

As shown in Table III these animals remained at a temperature of about 25° C. and at a blood sugar concentration considerably below 0.45% for periods ranging from 1 hour and 45 minutes to 10 hours and 30 minutes. No convulsions were observed in any instance under these conditions. The temperature of the first four animals was then raised by the application of dry heat. At or about normal body temperature all four showed well marked convulsions. These were in every case preceded by premonitory signs (jerky inspirations, tremors, and muscular twitchings). In one case these began at a temperature as low as 28° C. (Cat 2). The convulsions were sometimes tonic; sometimes tonic followed by clonic. The blood sugar fell progressively until the end of the experiments, reaching 0.014% in one case (Cat 2). The occurrence of premonitory signs at 28° C. suggests the possibility that

TABLE III.

	Blood sugar below .045%		Remarks
	Time kept at 25° C.	Time to raise temp.	
Cat 1	2 hrs. 25 mins.	2 hrs. 15 mins.	Animal warmed. Premonitory signs at 35.0° C. followed by tonic convulsions 60 mins. later. Blood sugar after convulsions - .020%.
Cat 2	2 hrs. 15 mins.	3 hrs. 25 mins.	Animal warmed. Premonitory signs at 28.5° C. Tonic convulsions at 37.3° C. Blood sugar after convulsion - .014%.
Cat 5	2 hrs. 25 mins.	2 hrs. 50 mins.	Animal warmed. Premonitory signs at 32° C. Weak convulsion at 37° C. followed by several tonic convulsions at 38.5° C. Blood sugar after convulsions .023%.
Dog 7	2 hrs. 50 mins.	3 hrs. 5 mins.	Animal warmed. Premonitory signs at 38o C. followed by tonic and clonic convulsions 10 mins.later. Blood sugar after convulsions - .026%.
Dog.10	10 hrs. 30 mins.	Not warmed.	No.convulsions. Blood sugar at death - .016%.
Dog 17	8 hrs.	Not warmed.	No convulsions. Blood sugar at death - .036%
Dog 18	5 hrs. 19 mins.	Not warmed.	No convulsions.
Cat 11	2 hrs. 46 mins.	Not warmed.	No convulsions.

that convulsions might supervene if an animal were kept for a sufficient length of time at this temperature. Two insulated dogs were kept at 25° C. for 8 hours and for 10½ hours respectively without showing any convulsive symptoms, although the blood sugar was below the convulsive level during the entire time (Dogs 17 and 10).

Before concluding that insulin convulsions are inhibited by mere lowering of body temperature, it was necessary to show that amytal anesthesia is not the determining factor. Experiments on non-anesthetized animals were, accordingly, attempted. A cat (No. 5) was cooled without anesthesia to 25° C., and insulin was injected. Although the blood sugar concentration remained below 0.045% for 2 hours and 25 minutes at this temperature, no convulsions occurred. Warming was then commenced, and 2 hours and 50 minutes later, when the body temperature had reached 38.5° C., strong tonic convulsions were exhibited. These were preceded by premonitory signs commencing at 32° C. and by weak convulsions at 37° C. (Cat 5). Since the behavior of this cat was the same as that of the amytalized animals, the conclusion can be drawn that insulin convulsions were prevented in these animals by mere lowering of body temperature.

Cats were used because it was impractical to cool dogs to 25° C. without anesthesia. Even then, considerable difficulty was experienced. Three animals died in convulsions, either during immersion in the cold bath, or soon after removal, without having received insulin. They also showed a hyperglycemia, varying from 0.224%to 0.440%, and a decreased carbon dioxide combing power of 38.7 to 19.7 volumes per cent. This reaction on the part of cats is brought forward as an explanation of one of our experimental results - the only case in which convulsions were observed following insulin administration at low body temperature. This particular non-anesthetized cat remained in a hypoglycemic condition for 1 hour, when it suddenly exhibited a weak, though definite, tonic convulsion followed by several strong clonic convulsions. A

Van Slyke determination showed a decreased alkali reserve of 27.1 volumes per cent. The animal was given glucose intravenously, and five minutes later, when the blood sugar was 0.500%, again passed into convulsions. Hence, these convulsions can be ascribed to causes other than insulin hypoglycemia.

Our results in connection with inhibition of convulsions at lowered body temperature are in line with those of Krogh. This observer found that administration of large doses of insulin to mice rarely causes convulsive symptoms, but that the animals show great muscular weakness and pass into a comatose condition with a fall of body temperature to a very low level. He also noted that placing the animals in an incubator at 28° C. causes convulsions of the ordinary type to supervene.

In view of the facts mentioned above that frogs develop insulin convulsions only 4 to 5 days after the injection of even very large doses of insulin at room temperatures, and only after 24 hours when kept at 25° C., it is possible that convulsions would have occurred in our animals had they been left for a sufficiently long time. In other words, lowered body temperature may not inhibit, but simply delay, insulin convulsions. This is still an unsettled question. If the blood sugar determination made by Dubois for the dormant European marmot can be accepted, it would seem that decreased body temperature in these animals, at least, prevents entirely the occurrence of convulsions.

V. HYPOGLYCEMIA ABOLISHES SHIVERING.

In the course of the above experiments it was noted that, when the blood sugar fell to 0.045% or slightly above this level, the animals ceased to shiver. During the whole of the cooling process they shivered strongly, even when their temperature was 25° C. The shivering was continuous at first, but, as the temperature fell, it occurred only with inspiration. One and a half to three hours after the injection of insulin, when the blood sugar had reached a level of 0.063% to 0.053%, shivering was markedly diminished. Soon it ceased altogether; at this point the blood sugar varied from 0.052% to 0.034%, the most common figure being 0.045%. (Table IV.) Once abolished, the shivering reflex never returna spontaneously. In the animals that were subsequently warmed, no shivering whatsoever was observed, even as the normal temperature level was approached. The first signs of movement were muscular tremors, not related to any particular phase of the respiratory cycle, and these were soon followed by convulsions. No shivering was observed during a period of 10 hours and 45 minutes in an animal left at 25° C. with a low blood sugar (Dog 10).

This behavior is in marked contrast to the reaction shown by the non-insulinized animal. A dog or cat with a normal blood sugar shivers as soon as it is exposed to cold, and continues to shiver during the entire time that its temperature is below the normal level. Thus, one cat (No. 3), used as a control, was anesthetized like the rest, and cooled to 25° C., but was not given insulin. It continued to shiver vigorously after removal from the bath, and in a short time had

TABLE IV.

	Temp.°C. when shivering ceased.	Blood sugar when shivering ceased.	Glucose injection.	Remarks.
Cat 1	25° C.	.040%	No glucose	Warmed to 38.6° C. No shivering during warming.
Cat 2	24.5°	.040	No glucose	Warmed to 38.5° C. No shivering during warming.
Cat 3	Control	-	-	Cooled to 25° C. then warmed to 39° C. Shivering throughout.
Cat 5	26.0°	.034	No glucose	Warmed to 38° C. No shivering during warming.
Dog 12	35.0o	.044	No glucose	Warmed to 38o C. No shivering during warming.
Dog 10	26.2°	.053	No glucose	No shivering during 10 hrs. 45 mins. following cessation.
Cat 9	25.0o	.036	Glucose intraven.	Shivering began immediately.
Cat 11	26.3°	.043	Glucose subcutan.	Shivering began 5 mins later.
Dog 18	38.0°	.045	Glucose intraven.	Shivering began immediately.
Dog 18	25.2°	.045	Glucose subcutan.	Shivering began 9 mins 5 secs. later.
Dog 19	35.4°	.052	Glucose intraven.	Shivering began 2 mins 15 secs. later.
Dog 20	25.5°	.050	Glucose subcutan.	Shivering began 6 mins later.
Dog 21	37.5°	.043	Glucose subcutan.	Shivering began 1 min. later. No amytal.

had raised its own temperature to 30° C. and was able to move about freely.

Whether hypoglycemia is the essential factor in the abolition of the shivering reflex could be determined only by the injection of a blucose solution. Accordingly six animals were given glucose after this reflex had disappeared. In two of these, intravenous injections induced shivering immediately - indeed before the injection was completed. In other cases, where subcutaneous injections were given, shivering came on gradually. It was observed first in the flank, then in the gluteal region and shoulder muscles. The lower and upper limbs were then involved. Soon the shivering became general and increasingly vigorous as the temperature rose. One animal, having recovered its ability to shiver, was again given insulin (10 units). The shivering soon became less violent, and 1 hour and 45 minutes after insulin had been given had once more disappeared. The possibility that the restoration of shivering was due to a difference in temperature between the injected solution and the animal's body was disposed of by injecting Ringer's solution intravenously at a temperature of 11° C. into an hypoglycemic dog at 25° C. This had no effect, whereas glucose given 8 minutes later under similar conditions caused the animal to shiver at once.

Later we were able to demonstrate that hypoglycemia prevents shivering in non-anesthetized dogs at normal temperature. A wet dog placed out-of-doors for a few minutes (external temperature 7° C.) began to shiver immediately. The animal was returned to the room and given insulin. Two hours and fifteen minutes later there was

TABLE V.

Dog 10, Wt. 9 K., amytal anesthesia.

Time	Temp. of dog	Temp. of room	Heart rate	Resp. rate	Blood sugar	Remarks
7.08 p.m.	26.0° C.	23.0° C.	79	8	.113%	45 units insulin injected.
8.30	26.8	20.0	--	-	.081	Strong shivering.
9.00	26.5	--	--	-	.064	Shivering was diminished.
9.15	26.2	--	73	7	.053	Shivering stopped.
9.30	26.0	--	--	-	.043	
10.15	25.0	23.5	56	6	.037	
11.00	25.0	22.0	54	18	lost	
12.30 a.m.	25.0	24.0	54	18	.028	
2.00	25.0	23.5	48	16	.023	
2.26	24.7	23.5	46	18	---	
3.00	24.1	24.0	48	17	1021	
4.00	24.0	23.5	39	10	.018	
5.00	23.8	22.5	40	8	.018	
7.00	23.0	23.0	38	5	.016	
8.00	23.0	23.0	38	5	---	
8.00-9.00	--	--	--	-	---	Animal died.

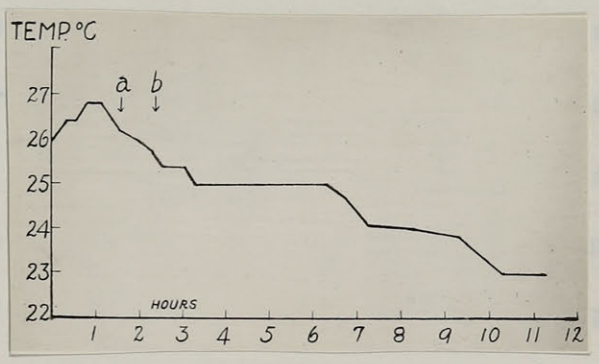

Fig. III. Body temperature curve.
a. Shivering diminished.
b. Shivering abolished.

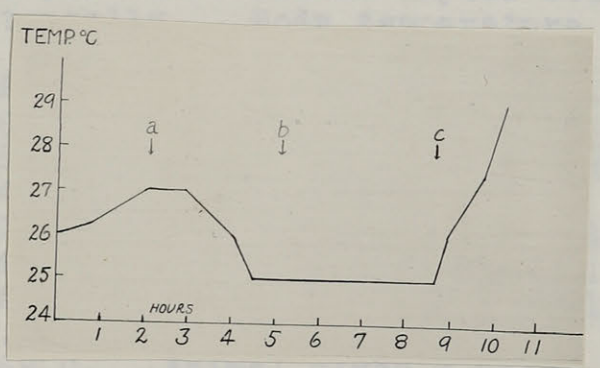

Fig. IV. Body temperature curve.
a. Shivering diminished.
b. Shivering abolished.
c. Glucose injected.

VII. EXPERIMENTS WITH A NATURALLY HIBERNATING SPECIES.

Since all of the experiments described above were carried out with dogs and cats, animals that do not hibernate naturally, one necessarily hesitates to draw conclusions from them concerning the mechanism of hibernation. Hence, as soon as a supply of woodchucks became available, the above experiments were repeated using these animals instead of cats and dogs, the method of procedure in the first few experiments being precisely. The protocol of one of these experiments follows:

Woodchuck 1. Weight 1.51 kilograms.

10 : 51	Given 0.5 cc. of 10 per cent. sodium amytal solution per kilogram intraperitoneally. Body temperature 38.7° C.
11 : 25	Immersed in a cold bath. Vigorous shivering.
11 : 40	Body temperature 22.52° C. Removed from bath.
12 : 00	Body temperature 20° C. Blood sugar 116 mgm. per cent.
12 : 05	Given 2 units insulin per kilogram. Shivering vigorously.
12 : 25	Body temperature 24° C. Animal wetted.
12 : 50	Body temperature 23.3° C. Shivering vigorously.
2 : 50	Body temperature 24° C. Shivering vigorously.
3 : 15	Body temperature 25.1° C. Shivering vigorously.
3 : 40	Blood sugar 56 mgm. per cent.
4 : 15	Body temperature 26° C. Animal given 3 units insulin per kilogram.
4 : 25	Body temperature 27° C. Immersed in cold bath.
4 : 21	Body temperature 22.6° C. Removed from bath.
4 : 45	Body temperature 19.7° C. Animal still shivering.
5 : 26	Blood sugar 67 mgm. per cent. Given 2 units insulin per kilogram.
6 : 20	Body temperature 21.7° C. Shivering vigorously.
7 : 30	Blood sugar 51 mgm. per cent. Given 5 units insulin per kilogram.
8 : 30	Body temperature 25° C. Shivering vigorously.
8 : 35	Given 5 units insulin per kilogram. Immersed in cold bath.
8 : 45	Body temperature 23.5° C. Removed from bath. Shivering vigorously.
11 : 00	Body temperature 25.5° C. Blood sugar 44 mgm. per cent. Shivering vigorously. Animal killed.

In the above experiment shivering was not abolished at any time,

and the temperature of the animal tended to rise whenever it was below normal. It was inferred that this was due to the fact that the blood sugar concentration did not at any time fall to a sufficiently low level to abolish shivering, even though large amounts of insulin had been injected. In order to produce a more profound hypoglycemia, another woodchuck was given insulin before immersion in the cold water; for, as has long been known, the emotional stimulation caused by the sudden exposure of an animal to cold produces a rise in the blood sugar concentration. The details of this experiment are given below:

Woodchuck 2. Weight 1.55 kilograms.

June 21.

10 : 45 Given 0.5 cc. of 10 per cent. sodium amytal solution per kilogram.
11 : 05 Given 0.1 cc. amytal per kilogram.
11 : 30 Given 0.1 cc. amytal per kilogram.
11 : 50 Body temperature 38° C. Blood sugar 161 mgm. per cent.
12 : 00 Given 5 units of insulin per kilogram subcutaneously.
4 : 00 Blood sugar 44 mgm. per cent.
4 : 05 Given 5 units of insulin per kilogram subcutaneously.
6 : 55 Animal completely relaxed.
7 : 10 Body temperature 37° C. Blood sugar 36 mgm. per cent.
8 : 18 Body temperature 37° C. Feeble tonic convulsion.
8 : 30 Immersed in cold bath. No shivering.
8 : 40 Slight shivering. Removed from bath. Blood sugar 97 mgm. per cent.
8 : 45 Immersed in bath.
9 : 07 Removed from bath. Slight shivering. Body temperature 22° C.
9 : 28 Body temperature 21.5° C.
10 : 55 Body temperature 18° C. Shivering vigorously.
11 : 25 Blood sugar 70 mgm. per cent. Given 5 units of insulin per kilogram subcutaneously.

June 22.

12 : 15 Body temperature 26° C. Shivering vigorously. Immersed in bath.
12 : 27 Body temperature 20.8° C. Shivering vigorously. Removed from bath.
12 : 50 Body temperature 20.2° C. Shivering vigorously.
1 : 40 Body temperature 26.5° C. Shivering vigorously. Given 10 units of insulin per kilogram.
9 : 50 Animal in a state of profound torpidity, lying partly turned on its back with limbs outstretched. Body temperature 24° C. Temperature of room 22° C.
10 : 54 Body temperature 24° C. Blood sugar 19 mgm. per cent.
2 : 12 Body temperature 25° C. Temperature of room 23.5° C.
4 : 36 Body temperature 25.5° C. Temperature of room 23.4° C.

9 : 30	Body temperature 25.7° C. Temperature of room 23.5° C.
June 23.	
9 : 00	Body temperature 30° C. Temperature of room 24° C. Blood sugar 59 mgm. per cent. Animal semi-torpid and capable of slow movement, if stimulated. Wetted with cold water. No shivering.
9 : 10	Given 5 units of insulin per kilogram.
11 : 15	Body temperature 21.5° C.
5 : 21	Body temperature 22.5° C. Blood sugar 60 mgm. per cent. Feeble movements and slight shivering. Animal died during removal sample.

The blood sugar concentration of this woodchuck fell to 36 mgm. per cent. 7 hours and 10 minutes after the first administration of 5 units of insulin per kilogram. When the animal was immersed in a cold bath, no shivering was observed until 10 minutes had elapsed. At the moment when shivering was first noticed, the blood sugar had risen to 97 mgm. per cent. This indicates that hypoglycemia abolishes shivering in the woodchuck, but that immersion of an hypoglycemic woodchuck in cold water causes a marked increase in the blood sugar concentration, and this is followed by shivering.

Once the body temperature of the woodchuck had been lowered, and profound hypoglycemia induced by means of repeated injections of insulin, the animal passed into a state of artificial hibernation. Before assuming this state of torpidity, the eoodchuck was shivering vigorously, and its temperature rose from 20.3° C. to 26.5° C. in 50 minutes. Several hours later, when the blood sugar had fallen to 19 mgm. per cent. and shivering had disappeared, the animal's temperature fell to 24° C. and remained at approximately this level for 12 hours, the surrounding temperature varying during this period from 23° C. to 23.5° C. The following morning (June 23) the temperature of the animal had risen to 30° C., and the woodchuck was in a semi-

torpid condition, i.e., stimulation evoked slow body movements. It was found that the blood sugar concentration had increased to 59 mgm. per cent. The woodchuck was wetted with cold water, and given an additional 5 units of insulin per kilogram. Its temperature fell rapidly to 21.5° C., and the animal once more became completely torpid. However, the tendency of the blood sugar level to rise was still marked; for, in spite of the insulin administration, the blood contained 60 mgm. per cent. of sugar at 5:21 p.m. At this time the animal began to shiver and move about, although its temperature had risen only 1° C.

From the above it became apparent that it would be necessary to administer insulin at intervals in order to keep these animals in a state of torpidity for a considerable period of time. In order to simulate more closely natural conditions, it was decided to give insulin to woodchucks and then to place them in a cool chamber. This procedure made the use of amytal unnecessary, and, in addition, eliminated the excessive stimulation caused by immersion in cold water. It was also possible to keep the animals completely dry, so that evaporation of water would not be a factor in lowering their body temperature. The cooling chamber was the same as that used by Tait and Britton (1923) in a series of experiments on artificially cooled woodchuck. It was a large, felt-covered, double-walled chamber with a glass window thru which the animal could be observed. The space between the walls was filled with ice. The animals were placed in a wire cage within the chamber, to prevent them from coming into contact with the cold walls.

As a control experiment, a woodchuck without insulin was placed in the cooling chamber for a period of 8 hours. It was able to maintain a body temperature of 37.3° C. without shivering or moving about to any considerable extent. The temperature of the chamber varied from 6° C. to 13° C. The animal was then given 30 units of insulin per kilogram. The following morning this woodchuck was partially torpid and a few hours later was completely dormant. U Its temperature at this time was 28° C.

Another woodchuck was given insulin (25 units per kilogram). at 3 p.m. on June 24th. At 6 p.m. the animal was inactive, and at 10:15 p.m. semi-torpid. On the morning of June 25th the animal was in a state of profound torpor, its temperature having fallen to 12.1° C. The temperature of the chamber was 11° C. At 8:30 that evening the temperature of the chamber had risen to 16.5° C. and the temperature of the animal was 17.8° C. The woodchuck was given a second injection of insulin. The next morning (June 26th) it was still completely torpid in spite of the fact that its temperature had risen to 28° C. At this time the temperature within the chamber had risen to 22.5° C. The chamber was repacked with ice. At 10:05 p.m. the temperature within the chamber had fallen to 11° C. and that of the animal to 10° C. The woodchuck was completely dormant and even the corneal reflex was absent. The next evening (June 27th) the animal's temperature was 11.3° C., that of the chamber being 15° C. The animal received a third injection of insulin (10 units per kilogram). On the afternoon of June 28th this woodchuck was still asleep. The temperature of the animal was the same as that of the chamber - 19° C. The relation of the temperature of this animal to that of its environment

is shown in Fig. V. At 2:18 p.m. this animal received 2 cc. of adrenaline (1:1000 solution) subcutaneously, in order to increase the blood sugar concentration and thus cause the animal to shiver (Cassidy, Dworkin and Finney, 1926). Fifteen minutes after the injection there was a marked increase in the rate and depth of respiration. Six minutes later the woodchuck began to shiver and to move about. Unfortunately the animal died during an attempt to obtain a sample of blood from the heart. A similar experiment was carried out on an amytalized woodchuck. This animal remained dormant for $2\frac{1}{2}$ days at the end of which time it died.

As previously mentioned, Tait and Britton (1923) found that woodchucks recovered their normal temperature spontaneously and promptly after having been cooled artificially to temperatures varying between 12° C. and 3° C. As has been described above, a woodchuck with hypoglycemia becomes poikilothermic; its temperature closely parallels that of its surroundings. The change from the homoiothermic to the poikilothermic condition is undoubtedly a consequence of the loss of the capacity for shivering and the ability to carry out spontaneous movements. It has been shown by Morgulis (1924) that the increase in metabolic rats produced by the exposure of an animal to cold is due entirely to increased muscular activity.

The degree of torpidity observed in these animals varies from time to time, and seems to be influenced by outside stimulation. Often when subjected to continued manipulation a torpid woodchuck begins to execute slow feeble movements; as soon as it is left undisturbed, it becomes completely torpid again. The animals lie

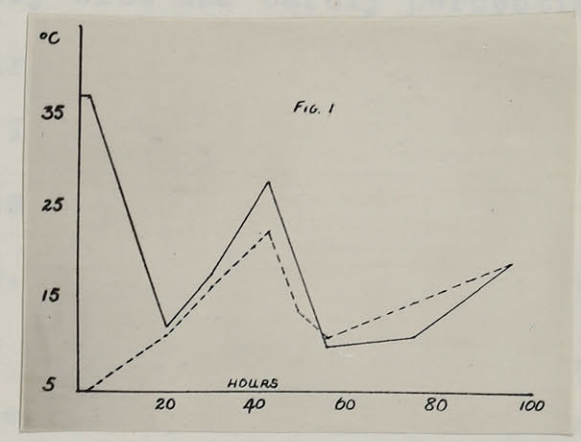

Fig. V. Curve showing the relation between the body temperature of a woodchuck and its surroundings.
Full line: animal's temperature.
Dotted line: temperature of chamber.

inert and are usually partly turned on their back. During some periods there is little or no muscular tone, the knee-jerk and corneal reflexes are absent, and the animals do not respond to painful stimulation. At other times there is considerable muscular tone, and the knee-jerk and corneal reflexes are present. During profound torpor the respirations are very slow and barely perceptible. Occasionally a long deep respiration occurs. Biot breathing was observed in some instances. Immediately before becoming dormant the animals passed uring and faeces. During torpidity small amounts of urine were occasionally voided. Whereas insulin convulsions may be produced readily in woodchucks at normal body temperature, no convulsions were observed in these animals when their body temperature had been lowered. Hence lowered body temperature inhibits insulin convulsions in woodchucks as it does in dogs and cats.

Two grams per kilogram of glucose in 20 per cent. solution were injected into a woodchuck after artificial hibernation had been induced. Shivering began 4 minutes later and became vigorous within 8 minutes. Sixteen minutes after receiving the sugar the animal was actively moving about and interested in its surroundings. Within 4 hours its temperature had risen to 26.5° C., and the woodchuck was entirely normal.

VIII. DISCUSSION.

While it would be premature to conclude that the state induced in these animals is identical with true hibernation, nevertheless several features are common to the two conditions: (1) the tendency of the animal to assume the temperature of its surroundings; (2) the absence of shivering and of spontaneous movements; (3) loss of consciousness; (4) profound reduction in the rate of metabolism; (5) a prolonged state of hypoglycemia without convulsions.

The changes brought about by injection of glucose into the blood stream resemble those observed during awakening. Both respiration and heart rate are quickened; there is violent shivering, and just as Horvath (1878) has remarked of the marmot, when once the shivering movements have commenced, nothing can prevent the animal from awakening and its temperature from rising. For example, one (non-anesthetized) cat (No. 9) from a state of dormancy recovered full consciousness in a short time, and would have regained its normal temperature if allowed to do so.

It is plain that in this transition glucose plays the important part. Here we may again adduce the conclusions of Pembrey that during the awakening of the marmot the metabolism is that of carbohydrate combustion.

When the temperature of an animal has fallen, recovery of normal temperature depends in great part upon the capacity for shivering. This in turn is determined by the concentration of the blood sugar. When the blood sugar level is below 0.045%, the heat-regulating

mechanism is markedly impaired if not completely inhibited.

Whether liver metabolism or muscular activity is the chief source of increased heat production in the awakening animal has been a matter of debate. The view of Dubois already quoted is that the liver plays the important part, but the observations of Pembrey and White (1896) and of Pembrey (1901) indicate that the chief cause of the rise in temperature is the increased metabolism of the muscles. In our opinion the latter view is correct. The findings of Dubois may have been due to the fact that he interfered with the glucose output of the liver. The observations of Claude Bernard, of Voit, and of Dubois, may once more be cited, to the effect that during hibernation the liver contains much glycogen, which it loses as the temperature rises upon awakening.

Since from these experiments it seems very probable that natural hibernation is brought about by the combined effect of lowered body temperature and hypoglycemia, one is tempted to speculate as to how these two requirements are simultaneously fulfilled in nature. The following sequence of events seems probable to the writer. It is well known that animals which hibernate naturally consume large quantities of carbohydrate food in the autumn. This ingested carbohydrate stimulates the pancreas to produce insulin which is thrown into the blood stream. It has been shown by Banting (1925) and by Jordan (1927) and others that the islets of Langerhans are directly stimulated by hyperglycemia. Now, if a woodchuck which had been feeding in this way for a considerable period were suddenly to stop eating on a cold day, it is quite a possibility that there would be sufficient insulin in the

circulation to lower the blood sugar to 0.045%. If this occurred when the environmental temperature was sufficiently low, torpidity would ensue. Due to the lowered body temperature all of the organs of the body would become less active, and lack of adrenal secretion would be sufficient to account for the maintenance of a low blood sugar concentration for a long period. In the spring, it is possible that the adrenal glands again become active. As a result, glycogen would mobilized, the blood sugar concentration would rise, shivering would commence, and the animal would return to an active state within a few hours.

IX. CONCLUSIONS.

1. Lowering of the body temperature of a dog to 25° C. under amytal anesthesia does not appreciably change the concentration of the blood sugar.

2. In dogs and cats insulin reduces blood sugar with the same rapidity whether the body temperature be 25° C. or 38° C. In some animals, however, at 25° C. there is a delay of 40 to 60 minutes before the fall of blood sugar commences.

3. When the body temperature has been lowered to 25° C. before the injection of insulin, the form of the insulin curve for dogs does not differ appreciably from the normal.

4. When the blood sugar has been lowered by insulin in dogs and cats at a body temperature of 25° C., the sugar concentration shows no tendency to return to normal.

5. Lowering of the body temperature of cats and dogs to 25° C. inhibits insulin convulsions.

6. Lowering of the blood sugar by means of insulin abolishes the shivering reflex. Administration of glucose causes its reappearance.

7. The combined action of cold and insulin in cats and dogs produces a state simulating hibernation. Subsequent injection of glucose restores the animal to normal.

8. When a woodchuck is given an amount of insulin sufficient to produce profound hypoglycemia, it loses its power of temperature control. If it then be placed in an environment even moderately cool, it passes into a state of artificial hibernation. The characteristics presented by an animal in this condition are: (1) the woodchuck becomes poikilothermic; (2) it is unable to shiver or execute spontaneous movements; (3) consciousness is lost, and the animal is insensible to painful stimulation; (4) the convulsions associated with insulin hypoglycemia do not occur; (5) the metabolic rate is greatly decreased. The state of torpor thus induced can be prolonged by the administration of insulin at intervals. The injection of glucose terminates this condition. Shivering begins almost immediately, the temperature rises at a rapid though variable rate, and the animal returns to normal

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