

THE EFFECTS OF INSULIN ON EXPERIMENTAL HYPERGLYCEMIA IN RABBITS

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In a previous paper it was shown that a marked fall occurs in the percentage of blood sugar in normal rabbits when they are injected subcutaneously with insulin. Taken in conjunction with the fact that insulin also reduces often to the normal level, or even below it, the high percentages of sugar found in the blood of depancreated dogs and of diabetic patients, it would appear that its action must be a fundamental one in the control of the blood sugar level (1). The present investigation was undertaken to obtain further evidence of the scope of the action of insulin by studying its effect on the various experimental conditions that are known to cause marked hyperglycemia in rabbits.

It is unnecessary to review here the extensive literature which bears on the methods used to cause these various forms of hyperglycemia. We will only refer to those investigations which have a direct bearing on our own results, in connection with each of the varieties which we have investigated.

METHODS. The rabbits used were as uniform in size and breed as possible and they were fed for some days preceding the experiments an abundance of oats and hay, sometimes with sugar added. The blood was collected at frequent intervals in 1 cc. quantities from the ear veins and the percentage of sugar determined in the samples by the Schaffer-Hartman method. At the termination of the experiments, whenever possible, the percentage of glycogen was determined in the liver by Pfüger's method, using the Schaffer-Hartman method for measurement of the reducing power of the hydrolyzed solutions. The insulin used was not always of uniform potency since during the progress of this research we were also engaged in working out the most suitable method for its preparation. As a preliminary to each experi-

ment it was therefore the practice to inject the preparation of insulin into normal rabbits. Provided the preparation was found to be active, either the same rabbit or another (normal) rabbit was subjected to one or other of the procedures for the production of hyperglycemia. These were piqûre, injection of epinephrin, mechanical asphyxia, carbon monoxide poisoning and ether.

RESULTS: *Piqûre*. In order to be certain that hyperglycemia will result in this experiment it is necessary to make sure that the liver contains adequate amounts of glycogen and that the puncture is not too far above the calamus of the medulla and is near the midline.

With regard to the latter condition it is our opinion that certainty of correct puncture can best be assured by reflecting the skin from the occipital bone under local (ethyl chloride) anesthesia and then, with the head bent as far forward as possible, puncturing at the occipital tubercle in the direction of the outer canthi of the eyes until the point of the instrument is felt to come against the basilar process. We have found that an ordinary trochar is a suitable instrument to use. This operation necessarily wounds the cerebellum with the result that after it the animal shows forced movements. Usually also there is a certain amount of hemorrhage into the 4th ventricle. Stewart and Rogoff (2) recommend actual exposure of the 4th ventricle by Eckhard's method, which involves separating the muscles lying over the occipito-atlanto-ligament, which is then incised. We believe however that this is unnecessary and that it is an advantage to avoid it because of the danger of hemorrhage. At the termination of each experiment the exact position of the puncture was determined by post-mortem examination.

The results of piqûre on three normal well-fed rabbits were as follows:

1. *Rabbit II, 16; weight, 2.3 kilos*

No normal blood sample taken.

Piqûre at 11:25 a.m.

Blood at 11:35—0.151 per cent sugar.

Blood at 12:05—0.282 per cent sugar.

Blood at 12:35—0.350 per cent sugar.

Blood at 2:50—0.387 per cent sugar.

Blood at 3:35—0.324 per cent sugar.

Blood at 4:00—0.280 per cent sugar.

Blood at 5:00—0.170 per cent sugar.

Blood at 5:45—0.161 per cent sugar.

Glycogen determination not made. Post mortem of the medulla showed two punctures (see fig. 2), one 2 mm. to the right of the midline, 3 mm. above the calamus scriptorius, and the other 2 mm. to the left, 8 mm. above the calamus.

2. Rabbit III, 3

Normal blood at 10:15 a.m.—0.137 per cent sugar.

Piqure at 11:00.

Blood at 11:30—0.305 per cent sugar.

Blood at 12:05—0.420 per cent sugar.

Blood at 12:50—0.457 per cent sugar.

Blood at 2:20—0.386 per cent sugar.

Blood at 3:20—0.244 per cent sugar.

Blood at 4:25—0.187 per cent sugar.

Blood at 12:30—0.170 per cent sugar (next day).

Animal killed at 12:30 next day.

The liver at this time showed a glycogen content of 2.0 per cent. Post mortem showed a puncture through the vermis of the cerebellum in the midline, and through the medulla 1 mm. to the left of the midline, 11 mm. above the calamus.

3. Rabbit IV, 1; weight, 2.15 kilos.

Normal blood at 9:15 a.m.—0.174 per cent sugar.

Piqure at 10:45 a.m.

Blood at 11:30—0.370 per cent sugar.

Blood at 12:00—0.390 per cent sugar.

Blood at 1:00—0.380 per cent sugar.

Blood at 2:00—0.400 per cent sugar (boiled too long, therefore too high).

Blood at 4:00—0.330 per cent sugar.

Blood at 4:30—0.317 per cent sugar.

Blood at 8:30—0.203 per cent sugar.

Animal killed at 3:30 p.m. next day. Liver showed a glycogen content of 0.59 per cent. The puncture of the medulla was oblique, 1 mm. to the right of the midline and extending from 8 to 15 mm. above the calamus scriptorius.

These results are given in curve form (fig. 1) along with two of Stewart and Rogoff's and the position of the punctures is shown in figure 2. It will be seen that the rise in blood sugar follows the piqure very rapidly indeed, the maximum being reached in about one hour, and that the return to normal is much slower occupying from 5 to 8 hours. The glycogen content of the rabbit showing the steepest curve (no. 2) was found to be 2 per cent on the day following the experiment. It was lower, 0.6 per cent, in another (no. 3) animal in which the curve was somewhat lower. The considerable variability in the position of the punctures in these three definitely positive cases of hyperglycemia shows that some latitude is permissible.

Turning now to the experiments in which the piqure was performed on animals under the influence of insulin very different results are evident.

4. Rabbit IV, 2

Normal blood at 12:50—0.123 per cent sugar.

At 3:30, 5 cc. of insulin.

Blood at 4:30—0.083 per cent sugar.

Piqûre at 4:45.

Blood at 5:15—0.081 per cent sugar.

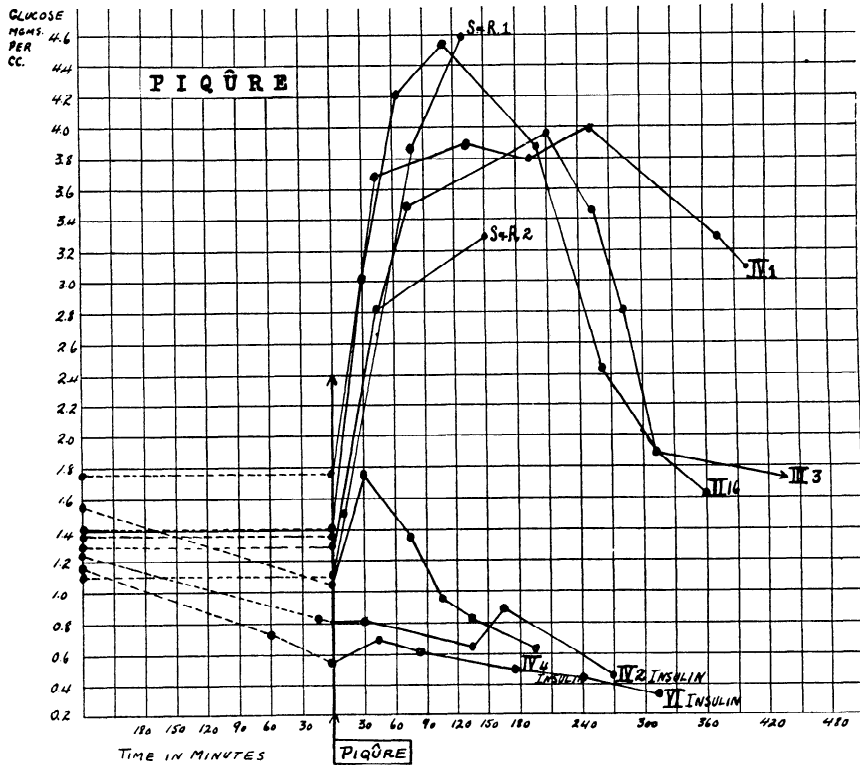


Fig. 1

At 5:20 4 cc. of insulin.

Blood at 6:00—0.064 per cent sugar.

Blood at 6:30—0.093 per cent sugar.

Blood at 8:13—0.045 per cent sugar.

Blood at 3:00—0.124 per cent sugar (next day).

At 8:15 the rabbit was in convulsions as described in the previous paper and dextrose solution was injected subcutaneously, resulting in rapid recovery. The rabbit was killed at 3:30 the next day, and the liver showed only 0.27 per cent

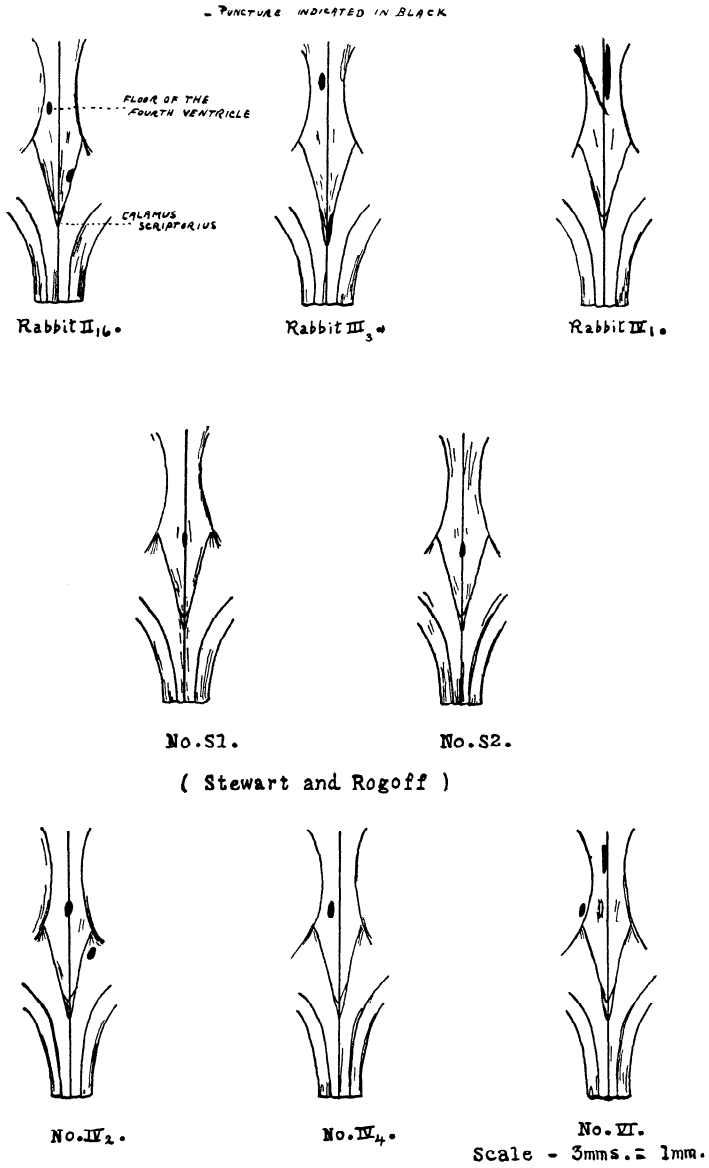


Fig. 2. Diagram indicating the seat of puncture in the floor of IV ventricle.

glycogen. The medulla showed two punctures, one on the right cerebral peduncle, 6 mm. above the calamus, and the other 10 mm. above in the midline (no. 4 in fig. 2).

5. Rabbit IV, 4; weight 1.8 kilos

Normal blood at 9:25—0.158 per cent sugar.

At 10:35, 4 cc. of insulin.

Blood at 11:15—0.106 per cent sugar.

Piqûre at 11:30.

Blood at 12:00—0.177 per cent sugar

At 12:05, 3 cc. of insulin.

Blood at 12:45—0.138 per cent sugar

Blood at 1:15—0.096 per cent sugar.

Blood at 1:45—0.085 per cent sugar.

Blood at 2:45—0.063 per cent sugar.

Animal killed at 3:15 p.m. Liver contained 4.4 per cent glycogen. Post mortem showed a puncture of the medulla 2 mm. to the left of the midline, 10 mm. above the calamus (fig. 2, no. 5).

6. Rabbit VI; weight, 2.4 kilos

Normal blood at 9:50—0.117 per cent sugar.

At 10:00—7 cc. of insulin.

Blood at 11:00—0.075 per cent sugar.

Blood at 11:45—0.056 per cent sugar.

Piqûre 11:50 (2 punctures) about 5 cc. of blood lost.

Blood at 12:35—0.068 per cent sugar.

Blood at 1:10—0.062 per cent sugar.

Blood at 2:40—0.052 per cent sugar.

At 3:00—3 cc. of insulin.

Blood at 3:45—0.042 per cent sugar, mild convulsions.

Blood at 4:55—0.035 per cent sugar, mild convulsions.

Animal killed at 5:00. Liver contained 2.64 per cent glycogen. Puncture through vermis in midline, two in floor of 4th ventricle (fig. 2, no. 6).

The punctures of the medulla in these three cases were such as to insure hyperglycemia. The amounts of glycogen found present in the livers were entirely adequate in two cases (5 and 6) but rather small in one (4). In this case the animal was not killed until twenty-two hours after the operation, during which time a considerable amount of glycogen may have been hydrolyzed. In experiment 4 the blood sugar during the first four hours following piqûre did not rise above 0.093 per cent, a figure considerably under the normal level. In no. 5, it will be noted that the sugar was not reduced to the same extent as in no. 6, before piqûre was performed; in thirty minutes it had risen to 0.177 per cent slightly above normal, and then, following another dose of extract, fell off gradually to 0.063 per cent. (These results are shown in graphic form in fig. 1.)

From these experiments, we may conclude that the severe hyperglycemia which occurs in rabbits whose livers are rich in glycogen, following piqûre, may be markedly reduced, if not entirely inhibited, if the operation is performed subsequent to the injection of suitable doses of insulin. The experiment, no. IV, 2, in which 0.27 per cent of glycogen was found in the liver, is the only one in which there can be any doubt as to the inhibiting action of insulin. It is somewhat similar to one described by Stewart and Rogoff (*loc. cit.*) in which no hyperglycemia occurred in a piqûred rabbit with 0.34 per cent of glycogen in the liver. In this case the blood sugar rose only from 0.124 per cent to 0.143 per cent following piqûre and to 0.151 per cent following asphyxia. In our case, as a result of insulin, the blood sugar fell to 0.045 per cent and then recovered to 0.124 per cent by next day. This recovery probably indicates that the glycogen had been drawn on to restore the blood sugar to its normal level. In light of the corroborative nature of the results of other experiments on piqûre we do not consider it necessary at the present to add further observations.

Epinephrin hyperglycemia. Bang (3) found that the subcutaneous injection of 1 mgm. epinephrin in rabbits caused the blood sugar to rise to a maximum in 2 to 3 hours returning to the normal in 7 to 9 hours. The curve was similar in starved and well-fed animals except that it did not begin to rise quite so quickly in the former. Reference to other investigations are given by Bang, the most significant point being that injection intravenously of the above amount of epinephrin causes only a transient and slight increase in blood sugar.

In order to satisfy ourselves that marked hyperglycemia invariably follows subcutaneous injection of epinephrin in rabbits, the following experiments were done using solutions of adrenalin chloride.

1. *Rabbit IV, 3; weight 1.45 kilos; well-fed*

Normal blood at 9:15—0.154 per cent sugar.

At 10:20, 2 cc. adrenalin chloride solution 1—1000 injected subcutaneously.

Blood at 10:55—0.364 per cent sugar.

Blood at 11:30—0.397 per cent sugar.

Blood at 12:00—0.440 per cent sugar.

Blood at 1:00—0.440 per cent sugar.

Blood at 2:05—0.410 per cent sugar.

2. *Rabbit II, 6; well-fed*

Normal blood at 10:50—0.141 per cent sugar.

At 2:35, 2 cc. adrenalin chloride solution 1—1000 injected subcutaneously.

Blood at 3:10—0.34 per cent sugar.

Blood at 4:55—0.36 per cent sugar.

This is the same adrenalin solution as used in the insulin exper. (III, 6).

3. Rabbit III, 2

Normal blood at 9:55—0.120 per cent sugar.

At 9:58, 2.0 cc. adrenalin chloride 1—1000, subcutaneously.

Blood at 10:35—0.291 per cent sugar.

Blood at 11:05—0.340 per cent sugar.

Blood at 11:35—0.390 per cent sugar.

Blood at 12:20—0.416 per cent sugar.

Blood at 1:15—0.391 per cent sugar.

Blood at 2:15—0.349 per cent sugar.

Blood at 3:15—0.317 per cent sugar.

4. Rabbit III, 3

Normal blood at 9:35—0.124 per cent sugar.

At 9:47, 1.0 cc. adrenalin chloride.

Blood at 10:17—0.195 per cent sugar.

Blood at 10:47—0.296 per cent sugar.

Blood at 11:25—0.340 per cent sugar.

Blood at 12:30—0.369 per cent sugar.

Blood at 1:30—0.364 per cent sugar.

Blood at 2:30—0.354 per cent sugar.

5. Rabbit IV, 6; weight 1.65 kilos; sugar-fed

Normal blood at 9:50—0.117 per cent sugar.

At 9:55, 1.0 cc. of adrenalin chloride 1—1000¹ injected subcutaneously.

Blood at 10:25—0.223 per cent sugar.

Blood at 10:55—0.270 per cent sugar.

Blood at 11:55—0.273 per cent sugar.

Blood at 1:10—0.158 per cent sugar.

To study the influence of insulin on this form of hyperglycemia, two methods were used. In the following two experiments 2 cc. adrenalin and 5 cc. of insulin were injected at the same time.

1. Rabbit III, 6; well-fed

Normal blood at 12:00—0.122 per cent sugar.

At 2:45, 5 cc. of insulin and 2.0 cc. adrenalin chloride 1—1000 were injected subcutaneously.

Blood at 3:30—0.090 per cent sugar.

Blood at 4:15—0.132 per cent sugar.

Animal died at 5:40 p.m. No glycogen determination made.

¹ This is the same adrenalin solution as used in the insulin experiments IV, 5 and IV, 6.

2. Rabbit X

Normal blood at 9:50—0.138 per cent sugar.
At 9:56, 4.0 cc. insulin + 2 cc. adrenalin chloride 1—1000.
Blood at 10:30—0.232 per cent sugar.
Blood at 11:00—0.303 per cent sugar.
Blood at 11:30—0.347 per cent sugar.
Blood at 12:10—0.365 per cent sugar.
Blood at 1:10—0.388 per cent sugar.
Blood at 2:10—0.325 per cent sugar.
Blood at 3:10—0.233 per cent sugar.
Blood at 4:10—0.165 per cent sugar.
This insulin caused convulsions in a control animal.

In the following three experiments 2 cc. adrenalin were injected after the hypoglycemic effect of insulin had become evident.

1. Rabbit VII, weight, 4.25 kilos

Normal blood at 10:15—0.127 per cent sugar.
At 10:17, 4 cc. insulin.
Blood at 11:15—0.106 per cent sugar.
2 cc. adrenalin chloride 1—1000, subcutaneously.
Blood at 11:45—0.183 per cent sugar.
Blood at 12:15—0.227 per cent sugar.
Blood at 12:45—0.240 per cent sugar.
Blood at 1:15—0.244 per cent sugar.
Blood at 1:45—0.307 per cent sugar.
Blood at 2:15—0.324 per cent sugar.
Blood at 2:45—0.304 per cent sugar.
Blood at 3:45—0.235 per cent sugar.
Blood at 4:45—0.147 per cent sugar.
Blood at 6:30—0.115 per cent sugar.

2. Rabbit VIII

Normal blood at 11:00—0.122 per cent sugar.
At 12:15, 2.0 cc. insulin.
Blood at 2:15—0.095 per cent sugar.
Blood at 4:00—0.067 per cent sugar.
Blood at 4:30—0.065 per cent sugar.
At 4:35, 2.0 cc. adrenalin chloride.
Blood at 5:05—0.136 per cent sugar.
Blood at 6:00—0.182 per cent sugar.
Blood at 6:55—0.269 per cent sugar.
Blood at 7:25—0.321 per cent sugar.

3. Rabbit IX

Normal blood at 11:00—0.125 per cent sugar; 2.0 cc. insulin.
Blood at 2:15—0.085 per cent sugar.

Blood at 4:00—0.069 per cent sugar.
 Blood at 4:30—0.068 per cent sugar.
 At 4:35, 2.0 cc. adrenalin chloride.
 Blood at 5:10—0.110.
 Could not obtain blood.

In the following five experiments 1 cc. adrenalin was injected after the hypoglycemic effect of insulin had become evident.

1. Rabbit XII

Normal blood at 9:05—0.105 per cent sugar.
 At 9:10, 2.0 cc. insulin.
 Blood at 11:15—0.083 per cent sugar.
 Blood at 12:15—0.050 per cent sugar.
 1.0 cc. adrenalin chloride 1-1000.
 Blood at 1:15—0.054 per cent sugar.
 Blood at 2:15—0.090 per cent sugar.
 Blood at 3:20—0.090 per cent sugar.
 Blood at 4:15—0.090 per cent sugar.
 Glycogen W_3 = 2.26 per cent.

2. Rabbit XIII

Normal blood at 9:25—0.100 per cent sugar.
 At 9:30, 2.0 cc. insulin.
 Blood at 11:25—0.066 per cent sugar.
 Blood at 1:10—0.074 per cent sugar.
 1.0 cc. adrenalin chloride 1-1000.
 Blood at 2:10—0.120 per cent sugar.
 Blood at 3:15—0.131 per cent sugar.
 Blood at 4:10—0.127 per cent sugar.
 Glycogen B_3 = 1.80 per cent.

3. Rabbit XIV

Normal blood at 10:10—0.130 per cent sugar.
 At 10:12, 3 cc. insulin.
 Blood at 12:10—0.099 per cent sugar.
 Blood at 1:25—0.092 per cent sugar.
 1 cc. adrenalin subcutaneously.
 Blood at 2:25—0.250 per cent sugar.
 Blood at 3:25—0.265 per cent sugar.
 Blood at 4:25—0.248 per cent sugar.

4. Rabbit IV, 7; well-fed

No normal blood sample obtained.
 At 5:00, 10 cc. of insulin injected subcutaneously.
 Blood at 6:00—0.042 per cent sugar (rabbit in convulsions).
 At 6:15, 1.0 cc. adrenalin chloride 1-1000 subcutaneously.
 Blood at 7:20—0.030 per cent sugar.

Blood at 8:05—0.035 per cent sugar.

Blood at 8:55—0.060 per cent sugar.

Blood at 10:45—0.056 per cent sugar (taken from heart).

Animal killed at 10:45. Liver contained 0.77 per cent of glycogen. Throughout this experiment the animal was subject to convulsions of from one to two minutes duration at about fifteen-minute intervals, between which its condition seemed to be considerably improved.

5. Rabbit XI

Normal blood at 9:00—0.111 per cent sugar.

At 9:30, 4.0 cc. insulin subcutaneously.

Blood at 11:35—0.090 per cent sugar.

At 11:40—1 cc. adrenalin chloride.

Blood at 12:30—0.166 per cent sugar.

Blood at 11:40—0.180 per cent sugar

Blood at 1:30—0.188 per cent sugar } Obtained from heart.

Blood at 2:30—0.176 per cent sugar }

It is clear that insulin is capable of greatly reducing the hyperglycemia caused by epinephrin, provided the latter be not given in massive doses. Out of a total of five experiments in which 1 cc. adrenalin chloride was injected after the hypoglycemic effect of insulin had become evident, the blood sugar did not rise above 0.130 per cent in three, in one it rose to 0.190 and in another to 0.265. In the two last mentioned cases however the insulin used was evidently extremely weak, the blood sugar being only reduced to about 0.09 per cent prior to the injection of adrenalin, thus contrasting with the marked reduction in the other cases. In the three experiments in which 2 cc. adrenalin were injected following insulin a decided hypoglycemic effect remained in one, but distinct increase in blood sugar occurred in the other two. In two experiments in which 2 cc. adrenalin were injected at the same time as insulin, a marked hyperglycemia developed in the one but not in the other. Taking these results as a whole it is plain that much more work must be done before it can be told to what extent insulin can antidote the adrenalin effect. We are hopeful that it may be possible to determine the dosage of insulin in terms of the amount capable of antidoting the effect of a standard dose of adrenalin. These results are given in curve form in figures 3 and 3a.

Asphyxial hyperglycemia. There is no more certain means for causing a marked degree of hyperglycemia than asphyxia brought about by constriction of the upper air passages. In rabbits the most practical way for doing this is by placing a piece of water-proof material over the

snout and holding it there until the heart rate becomes definitely slowed. The animal is then allowed to breathe freely for a few breaths when it is again asphyxiated. This procedure is kept up for twenty minutes. With so many observations of this type on record (cf. Stewart and

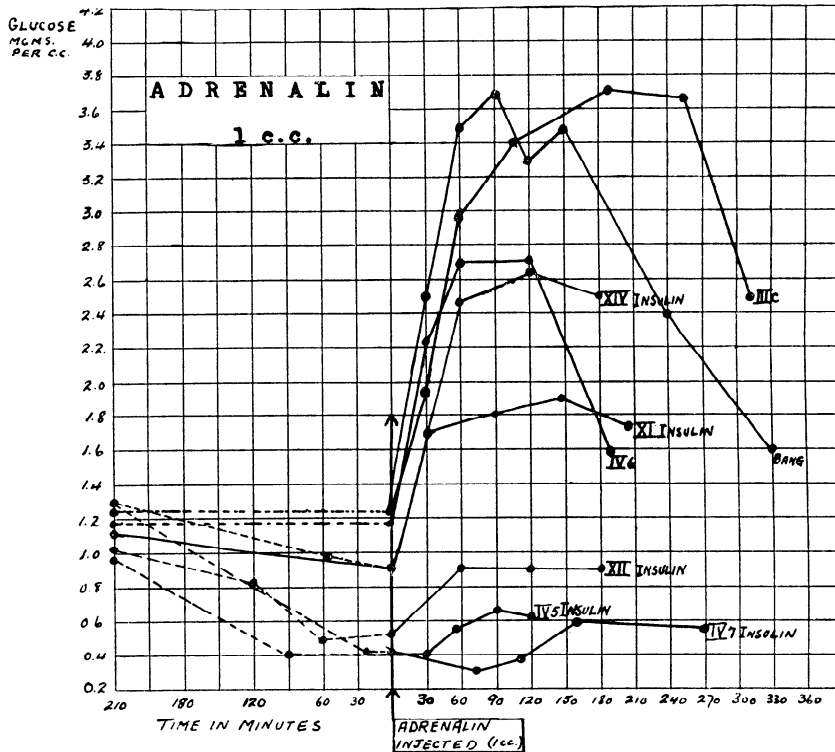


Fig. 3a

Rogoff, and Macleod) it was not considered necessary to perform more than one control experiment of which the following is the result.

1. Rabbit IV, ♂; weight 2.3 kilos; well-fed

Normal blood at 9:50—0.140 per cent sugar.

Mechanical asphyxia from 9:55–10:20.

Blood at 10:23—0.383 per cent sugar.

Blood at 10:55—0.376 per cent sugar.

Blood at 11:25—0.370 per cent sugar.

Blood at 11:55—0.222 per cent sugar.

Blood at 12:25—0.200 per cent sugar.

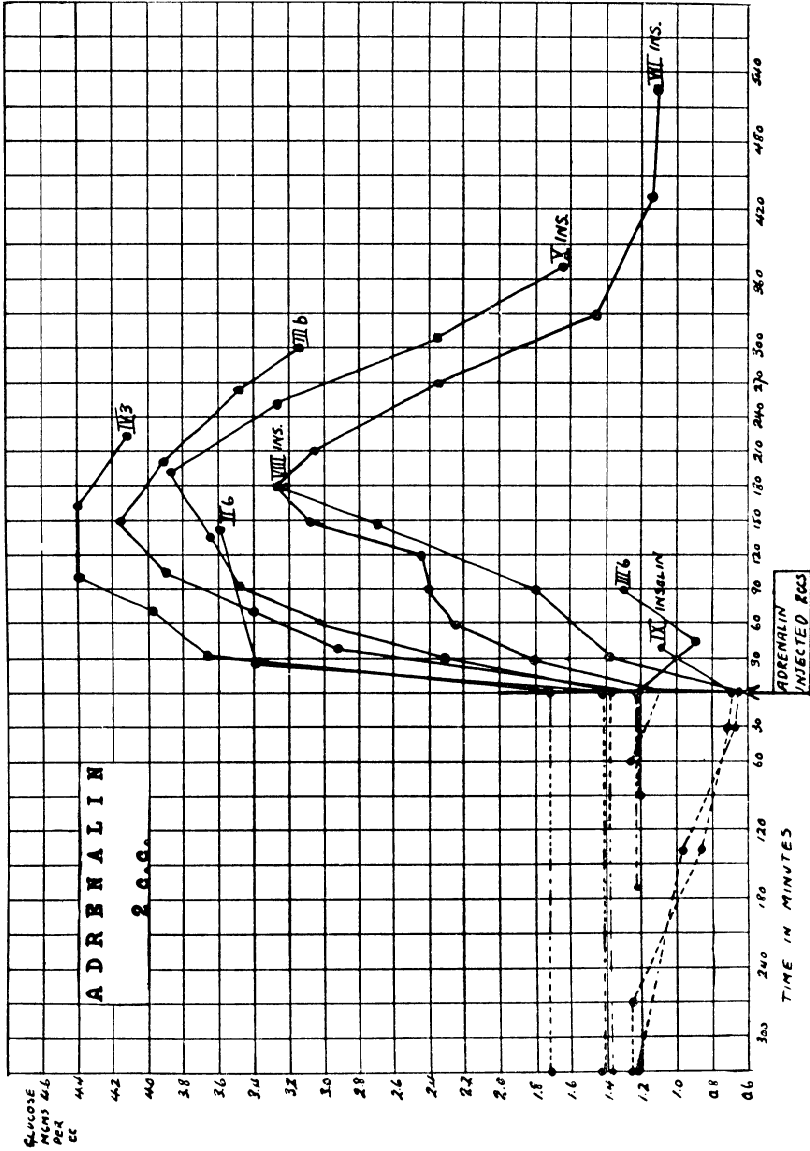


Fig. 3b

Three experiments were then performed on well-fed rabbits which were asphyxiated for twenty minutes following the injection of insulin.

2. Rabbit IV, 4; weight 2.05 kilos

Normal blood at 9:50—0.124 per cent sugar.
 At 10:00, 3 cc. insulin injected subcutaneously.
 Blood at 11:00—0.077 per cent sugar.
 At 2:00, 3.0 cc. insulin (same) subcutaneously.
 Blood at 2:20—0.045 per cent sugar (animal hyperexcitable).
 Mechanical asphyxia from 2:22—2:40.
 Blood at 2:45—0.159 per cent sugar.
 Blood at 3:15—0.075 per cent sugar.
 Blood at 3:45—0.046 per cent sugar.
 Animal was asphyxiated again, and died during the operation.
 Blood at 4:15—0.144 per cent sugar obtained from the heart after death.
 Liver contained 2.04 per cent glycogen.

3. Rabbit IV, 9; weight, 2.3 kilos; sugar-fed

Normal blood at 10:00—0.117 per cent sugar.
 At 10:05, 4.5 cc. insulin injected subcutaneously.
 Blood at 11:45—0.083 per cent sugar.
 At 12:40, 4.0 cc. insulin subcutaneously.
 Blood at 2:00—0.079 per cent sugar.
 At 2:55, 12.0 cc. insulin (same) subcutaneously.
 At 3:10 the animal was in convulsions; it was asphyxiated from 3:15 to 3:35.
 Blood at 3:40—0.075 per cent sugar; animal hopping about.
 Blood at 4:20—0.090 per cent sugar.
 Blood at 4:50—0.035 per cent sugar; animal in convulsions.
 Mechanical asphyxia again from 4:55—5:15, animal seemed to be considerably recovered.
 Blood at 5:20—0.077 per cent sugar.
 Blood at 5:55—0.039 per cent sugar.
 At 6:10 animal was in violent convulsions. Killed at 6:15. Blood from the heart at 6:15—0.062 per cent sugar. Glycogen in the liver, 2.69 per cent.

4. Rabbit IV, 10; weight 2.65 kilos; sugar-fed

Normal blood at 9:40—0.139 per cent sugar.
 At 9:41, 5.0 cc. of insulin subcutaneously.
 Blood at 10:25—0.075 per cent sugar.
 Blood at 11:15—0.062 per cent sugar.
 At 11:25, 6.0 cc. of insulin subcutaneously.
 Mechanical asphyxia from 11:30—11:50.
 Blood at 11:50—0.075 per cent sugar.
 Blood at 12:20—0.064 per cent sugar.
 Blood at 12:50—0.056 per cent sugar.
 Animal killed at 1:00. Liver contained 5.3 per cent glycogen.

These results are shown in curve form in figure 4 and it will be seen that insulin had the effect either of preventing entirely any asphyxial rise in blood sugar or of greatly reducing the rise which usually occurs. In Experiment IV, 4, the sugar rose from 0.045 to 0.159 per cent during a twenty-minute asphyxial period, an increase of 0.114 per cent as com-

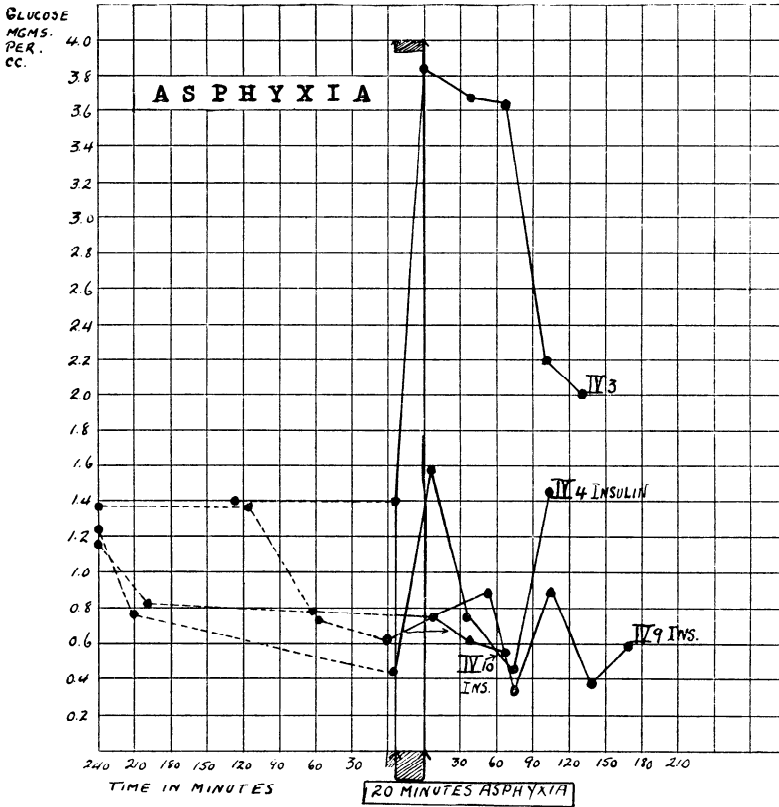


Fig. 4

pared with 0.243 per cent in the control experiment (no. IV, 3) but it will be observed this rise was of a very temporary nature, the percentage returning to its pre-asphyxial level in one hour. A similar sharp return to the pre-asphyxial level is also to be observed in the second period of asphyxia in experiment IV, 9.

Carbon monoxide poisoning. In order to study the effect of a less acute form of asphyxia than the foregoing we have also investigated

carbon monoxide poisoning. The method was to place the animal in an air-tight box just large enough to contain it comfortably, and provided with an observation window. A mixture of air and illuminating gas was then allowed to circulate slowly through the box at the rate of 0.6 liter per minute. This mixture contained 0.8 per cent carbon monoxide by calculation. It was probably considerably less than this since Haggard and Henderson found that 0.4 per cent was sufficient. In the 0.8 per cent atmosphere hyperpnea developed early and in forty-five minutes the animal was in a semi-conscious condition and was removed from the box. It was then unable for several minutes to stand on its feet and some time elapsed before the hyperpnea disappeared. Four of the five experiments performed were carried out on two well-fed rabbits, each of which was first subjected to forty-five minutes' gassing, and then in a week's time, underwent a second period of gassing, after being given insulin. The fifth experiment is an additional control performed on a normal rabbit. The results of the five experiments are as follows:

1. (a) *Rabbit III, 10; weight, 2.2 kilos; well-fed*

Normal blood at 2:30—0.149 per cent sugar.
Gassed from 2:30—3:18, 0.8 per cent CO (by calculation).
Blood at 3:19—0.245 per cent sugar.
Blood at 3:49—0.334 per cent sugar.
Blood at 4:19—0.347 per cent sugar.
Blood at 4:49—0.320 per cent sugar.
Respirations at 3:20—240 per minute
Respirations at 3:45—250 per minute.
Respirations at 4:30—190 per minute.

(b) *Same animal seven days later*

Normal blood at 10:00—0.125 per cent sugar.
At 10:05, 3.0 cc. of insulin, subcutaneously.
Blood at 10:40—0.089 per cent sugar.
At 10:45, 5.0 cc. insulin, subcutaneously.
Gassed from 10:52—11:37, 0.8 per cent CO.
Blood at 11:40—0.081 per cent sugar.
Blood at 12:10—0.083 per cent sugar.
At 12:15, 2.5 cc. insulin subcutaneously.
Blood at 12:40—0.070 per cent sugar.
Blood at 1:10—0.060 per cent sugar.
Blood at 2:30—0.070 per cent sugar.
Blood at 3:30—0.062 per cent sugar.
Animal was in very weak condition when removed from the chamber.

2. (a) *Rabbit III, 11; weight 2.4 kilos; well-fed*

Normal blood at 1:30—0.137 per cent sugar.

Gassed from 1:37-2:02 (only twenty-five minutes).

Blood at 2:03—0.211 per cent sugar.

Blood at 2:33—0.219 per cent sugar.

Blood at 3:03—0.203 per cent sugar.

Blood at 3:33—0.171 per cent sugar.

Blood at 9:30—0.136 per cent sugar (next day)..

Animal removed in twenty-five minutes because it seemed to be in an unconscious condition.

(b) *Same rabbit three days later*

Normal blood at 2:45—0.135 per cent sugar.

At 2:50, 5.0 cc. insulin subcutaneously.

Gassed 3:02-3:46, 0.8 per cent CO (by calculation).

Blood at 3:46—0.143 per cent sugar.

Blood at 4:16—0.150 per cent sugar.

Blood at 4:46—0.127 per cent sugar.

Blood at 5:16—0.095 per cent sugar.

Animal was in a very weak condition when removed from the chamber.

3. *Rabbit III, 12; weight, 2.3 kilos*

Normal blood at 9:40—0.119 per cent sugar.

Gassed from 9:45-10:29, 0.8 per cent CO.

Blood at 10:30—0.286 per cent sugar.

Blood at 11:00—0.257 per cent sugar.

Blood at 11:30—0.145 per cent sugar.

Blood at 12:00—0.129 per cent sugar.

Animal seemed to recover rapidly after removal from the chamber.

In experiment 1 (b) the results are very striking; the blood sugar immediately after the animal was removed from the chamber, was slightly lower than when the animal was inserted forty-four minutes before. The same rabbit seven days previously had responded to the same period of gassing, without the injection of insulin, by a rise in blood sugar to as high as 0.347 per cent. In experiment 2 (b) the sugar rose very slightly (from 0.135 to 0.150 per cent) when subjected to carbon monoxide after administration of insulin, but in this case, the insulin was given only twelve minutes before the animal was placed in the chamber, so that the full force of its reducing powers might not have come into effect. In the control experiment, performed previously on the same rabbit, the blood sugar rose to 0.22 per cent after only twenty-five minutes of carbon monoxide.

No glycogen determinations were made on the livers of these animals, but it is evident, from the initial hyperglycemia following carbon monoxide, that an ample amount of this material was present, and the

animals were fed until the second experiment, on the same carbohydrate-rich diet.

These results are also shown in curve form in figure 5 and they indicate that insulin prevents, or at least greatly depresses, the hyperglycemia which follows the administration of carbon monoxide to well-fed rabbits.

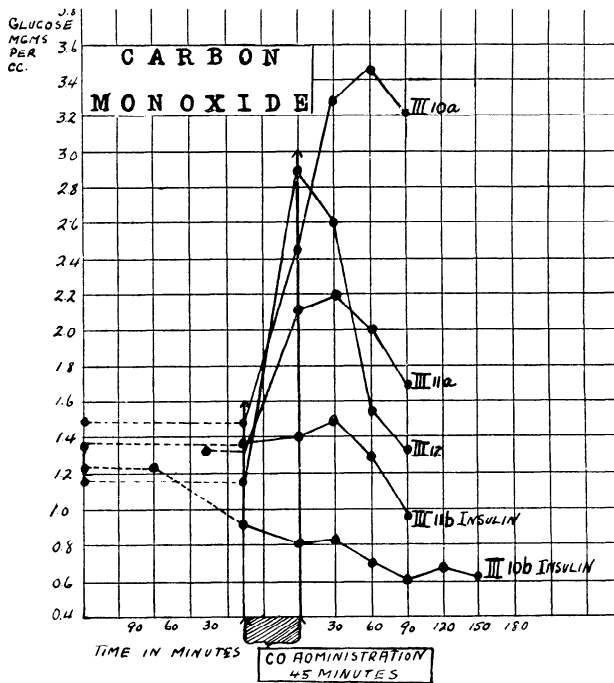


Fig. 5

Ether hyperglycemia. It is well known that the percentage of blood sugar rises during ether anesthesia. In rabbits the degree of this hyperglycemia, according to Fujii, is proportional to the intensity of the etherization. Of course it is impossible to be certain that the animals used in a series of experiments such as this, are all etherized to the same degree, but nevertheless, even under very light anesthesia, a certain degree of hyperglycemia always develops, provided of course the liver contains an ample supply of glycogen. The following experiments illustrate the changes in blood sugar following ether administrations to normal well-fed rabbits.

1. Rabbit IV, 7; weight, 1.75 kilos

Normal blood at 12:00—0.147 per cent sugar.
 Ether commenced at 12:15.
 Blood at 12:50—0.45 per cent sugar.
 Blood at 1:25—0.50 per cent sugar.

2. Rabbit II, 14a

No normal sample of blood was examined.
 Ether commenced at 10:15, continuous to 1:15.
 Blood at 10:30—0.220 per cent sugar.
 Blood at 11:30—0.355 per cent sugar.
 Blood at 12:30—0.381 per cent sugar.
 Blood at 1:15—0.459 per cent sugar, obtained from the heart.
 Animal died 1.15.

3. Rabbit II, 14b

No normal sample of blood was examined.
 Ether commenced at 10:35, continuous to 5:00.
 Blood at 10:50—0.201 per cent sugar.
 Blood at 11:50—0.365 per cent sugar.
 Blood at 12:50—0.415 per cent sugar.
 Blood at 1:50—0.447 per cent sugar.
 Blood at 2:50—0.435 per cent sugar.
 Blood at 3:50—0.440 per cent sugar.
 Blood at 4:50—0.461 per cent sugar.
 Animal died at 5:00.

From these results, which are also plotted in curves, figure 5, we see that the rise in blood sugar is rapid, and that it persists as long as the etherization: in each case until the death of the animal. Turning now to the experiments, in which ether was administered after injecting the animal with insulin, the following results were obtained:

Rabbit IV, 10; weight, 2.7 kilos; sugar-fed

Normal blood at 9:50—0.095 per cent sugar.
 At 10:00, 7.0 cc. of insulin, subcutaneously.
 Blood at 10:45—0.073 per cent sugar.
 At 11:45, 5.0 cc. of insulin, subcutaneously.
 Blood at 1:25—0.057 per cent sugar.
 Blood at 2:30—0.052 per cent sugar.
 At 2:35, 5.0 cc. of insulin, subcutaneously.
 At 2:40 ether commenced, continuous to 4:25.
 Blood at 3:10—0.065 per cent sugar.
 Blood at 3:40—0.060 per cent sugar.
 Blood at 4:10—0.071 per cent sugar.
 Animal died at 4:25. Liver contained 3.91 per cent glycogen.

Rabbit IV, 8; weight 2.5 kilos

Normal blood at 11:00—0.127 per cent sugar.
At 11:15, 10.0 cc. of insulin, subcutaneously.
Blood at 2:00—0.037 per cent sugar.
Ether commenced at 3:00, continuous to 6:00.
At 3:01, 10.0 cc. of insulin, subcutaneously.
Blood at 3:45—0.095 per cent sugar.
Blood at 4:30—0.081 per cent sugar.
Blood at 5:30—0.045 per cent sugar.
Blood at 6:05—0.035 per cent sugar obtained from heart.
Animal killed 6:00. Liver contained 2.8 per cent glycogen.

These results are also given in curve form, figure 6, and they show that even with considerable amounts of glycogen in the liver there was only a slight and transient increase in the percentage of blood sugar while the animal was under ether.

In another case, insulin was not given until some time after the animal had been lightly under ether, with the following results:

Rabbit IV, 6; weight, 1.85 kilos; sugar-fed

Normal blood at 1:30—0.114 per cent sugar.
Ether commenced at 1:45 p.m., continuous to 5:15 p.m.
Blood at 2:15—0.142 per cent sugar.
Blood at 2:45—0.150 per cent sugar.
At 2:50, 10.0 cc. of insulin, subcutaneously.
Blood at 3:20—0.120 per cent sugar.
Blood at 3:50—0.082 per cent sugar.
Blood at 4:40—0.060 per cent sugar.
Blood at 5:10—0.047 per cent sugar.
Animal killed at 5:15. Liver contained 0.77 per cent glycogen.

The degree of hyperglycemia in this experiment is somewhat less striking possibly because of the relatively low glycogen content of the liver. The effect of insulin is however quite definite.

The effect of insulin in inhibiting the hyperglycemia of ether anesthesia is of importance from both experimental and clinical standpoints. From the former because it offers greater opportunities for the experimental investigation of the exact mechanism of the physiological action of insulin and from the latter, because it offers a means by which hyperglycemia may be controlled when surgical anesthesia is necessary in diabetic patients.

DISCUSSION. Considering these results as a whole there can be no doubt that insulin in suitable dosage more or less inhibits the development of hyperglycemia in rabbits subjected to various conditions which

otherwise cause it. The degree of this inhibition is usually sufficient entirely to mask any rise in blood sugar but sometimes a rise occurs to a certain extent. This rise may be quite marked when the insulin is

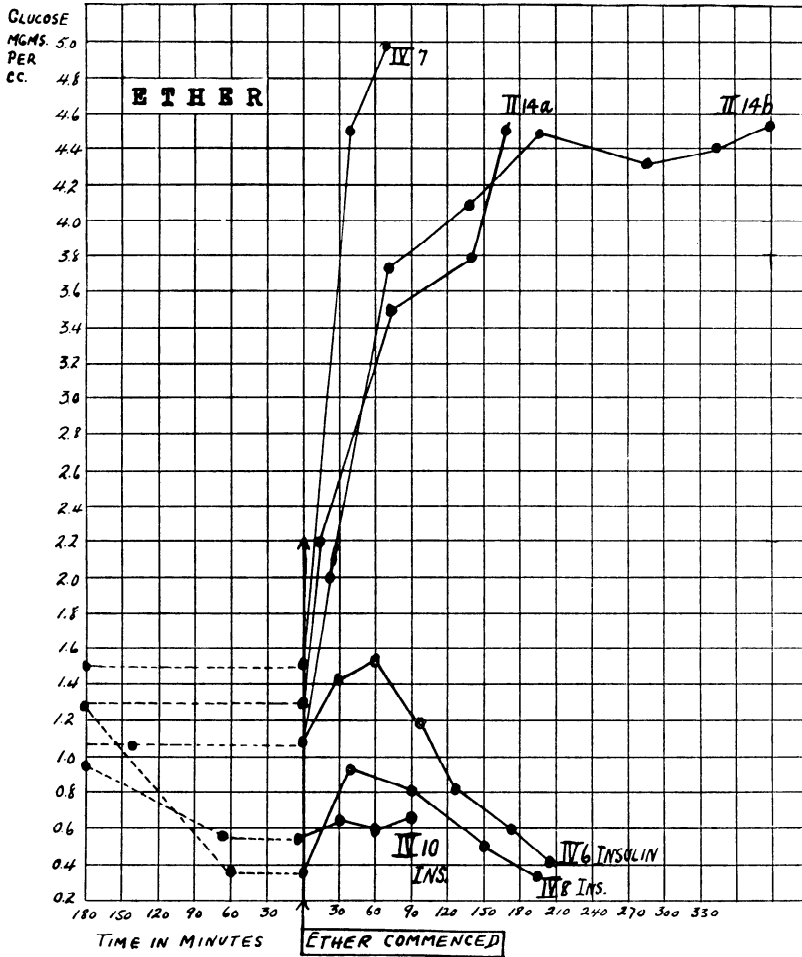


Fig. 6

injected at the same time as the application of the stimulus used to cause hyperglycemia, as is especially evident when this is epinephrin. In the latter case, indeed, the degree of hyperglycemia may be as great as the usual when the injections of epinephrin and insulin are made simultaneously. This would seem to indicate that there is a considerable

latent period before insulin unfolds its full action, a conclusion which is borne out by the preliminary observations which we have made on the behavior of the respiratory quotient following insulin. During this preliminary stage a powerful hyperglycemia-producing stimulus like epinephrin can apparently almost annul the effect of insulin. Further work on this aspect of the problem is in progress.

That insulin acts not only in the experimental forms of hyperglycemia discussed in this paper but also in that following pancreatectomy and in diabetes in man indicates that its action on carbohydrate metabolism is fundamental. Its effect on blood sugar is just as striking in a pancreatic diabetic animal with only a trace of glycogen in the liver as on a normal one whose liver is loaded with this material. In the former case insulin also influences the excretion of ketone bodies, the mobilization of fat and the respiratory quotient, which must be interpreted as meaning that it is essential in the regulation of the series of intermediary metabolic changes that culminate in the complete utilization of both fat and carbohydrate. By more intensive investigations of the metabolism of pancreatic diabetic animals treated with insulin it is therefore to be hoped that some light may be thrown on the problem of ketogenesis.

Concerning the modus operandi of insulin in preventing the purely experimental form of hyperglycemia, we have no hypothesis to offer. Before any such is attempted it will be necessary to obtain precise data on the amounts of glycogen in the liver and muscles before and during and after insulin action. Only then can it be known whether insulin actually stimulates glycogenesis to the extent that it takes glucose away from the systemic blood.

CONCLUSIONS

1. When the fall in blood sugar due to subcutaneous injection of insulin is thoroughly established, piqûre, epinephrin, mechanical and carbon monoxide asphyxia and ether do not cause the usual degree of hyperglycemia. There may be a distinct increase in the percentage of blood sugar but very seldom is this sufficient to raise it to the normal level existing before insulin was given.

2. Even when the insulin is given at the same time as the animal is subjected to the experimental condition used to cause hyperglycemia, the latter may be either entirely absent or greatly diminished.

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