CLII. THE PERIPHERAL ACTION OF INSULIN IN NORMAL ANIMALS.

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SINCE the discovery of insulin little advance has been made in knowledge of its mode of action and of the changes which it brings about in the tissues.

Macleod [1934], summarising the work of the past 15 years, concludes that the conversion of blood sugar into muscle glycogen is not a conspicuous feature of insulin action in normal animals. In so far as oxidation of glucose in the tissues is responsible for the fall in the blood sugar, attention is called to the demonstration by Cori [1931] of a relationship between sugar disappearing from the blood and sugar metabolised after administration of insulin to normal animals. Macleod cites experiments by himself and collaborators which show that the respiratory quotient does not rise during the period when the blood sugar is falling, but only when the convulsive level of hypoglycaemia is reached, indicating that increased oxidation is not responsible for the lowering of blood sugar in the early stages of insulin action. Since it requires only the removal of 75 mg./100 ml. to reduce the blood sugar to the convulsive level in a rabbit, Macleod believes that his hypothesis, that insulin acts by converting the glucose into some intermediate form in the tissues still holds good, although the evidence is against this form being hexosephosphate.

A new approach to this problem is now possible as a result of the recent coordination of views on tissue respiration [*cf.* Keilin, 1933] and of conceptions of intermediary carbohydrate metabolism [*cf.* Cori and Cori, 1933].

This method of approach is illustrated in Fig. 1, which indicates the possible correlation of the respiratory system of the cell and the various intermediate stages in the breakdown of glucose to lactic acid. Certain agents are well known to inhibit various stages of cellular respiration in tissue slices and to interfere with intermediate stages in the conversion of glucose into lactic acid by the action of minced muscle preparations, pressed muscle juices and extracts. In Fig. 1 these inhibiting agents are indicated by frames. The arrows extend from the frames to the points where their specific retarding actions are exercised. Ethylurethane and cyanide inhibit dehydrogenase and oxidase respectively [Keilin, 1933], sodium fluoride has been used to prevent formation of pyruvic acid, and iodoacetic acid inhibits the interaction of pyruvic and glycerophosphoric acids [see Pollard and Pryde, 1933]. We have based our conclusions on the assumption that the same processes take place *in vivo* and that massive inhibition at any of the stages indicated in Fig. 1 will interfere with carbohydrate metabolism in the tissues generally, resulting in hyperglycaemia during the period when the inhibitor is active. Such behaviour is known to result from the action of cyanides [Ohmi, 1933, 1, 2] and of iodoacetic acid [Irving, 1934]. The hyperglycaemic action of anaesthetics and some narcotics is well known; as indicated later we have shown that fluorides have a similar effect. It has

indeed been shown that insulin can lower the high blood sugar values produced by iodoacetic acid, cyanides and anaesthetics, but massive doses of insulin have been used and the observations have been made by different individuals so that it is impossible to assess any relative values to the insulin-antagonising actions

Fig. 1.

of the above-mentioned compounds. We have therefore attempted to ascertain the point of maximum activity of insulin by using a standard amount of the latter and studying its relative effects on animals which have been given maximum tolerated doses of one of the inhibitors. A preliminary report has already been published [Laughton and Macallum, 1934].

During the progress of this study 73 different animals were used. The maximum tolerated dose of each of the compounds was ascertained and records of the blood sugar under its action obtained over a period of time. In other groups of animals the maximum tolerated doses of the respective agents were administered to animals which had received 1 rabbit unit per 2 kg. body weight of insulin 30 or 60 minutes previously, and also in some cases insulin and the inhibitor were administered simultaneously; in a few cases insulin was given 60 minutes after the retarding substance. In all cases the blood sugar values were observed at specified intervals for a period of 3 hours.

Effects of inhibitors on action of insulin.

Cyanide. Curve 1, Fig. 2, represents the effect of 1 rabbit unit of insulin per 2 kg. on a normal rabbit. Curve 3 shows the blood sugar curve of a normal rabbit to which 1.2 mg. of KCN per kg. were administered intravenously, while curve 2 indicates the effect on the blood sugar of a rabbit of the standard dose of insulin at the beginning of the experiment followed 30 minutes later by intravenous injection of 1.2 mg. of KCN per kg. As indicated by the immediate

rise in blood sugar, cyanide exercised a temporary inhibiting effect on the insulin which was comparable in duration to its hyperglycaemic effect in the normal rabbit.

Fig. 2.

Fig. 2. 1. Insulinised rabbit; 2. Insulinised rabbit. *Cyanide injected; 3. Cyanide hyperglycaemia.

Fig. 3.

Fig. 3. 1. Insulinised rabbit; 2. Insulinised rabbit. *Urethane administered. 3. Insulin and urethane administered simultaneously; 4. Urethane hyperglycaemia; 5. Urethane hyperglycaemia. *Insulin administered.

Ethylurethane. Curve 1, Fig. 3, shows the normal action of the standard dose of insulin. Curve 2 shows the blood sugar values following the administration of the standard dose of insulin at the beginning of the experiment, followed 30 minutes later by intraperitoneal injection of 2.5 g. urethane per kg. (15 % soln.). Curve 3 gives the effect of simultaneous injection of the standard doses of insulin and urethane at the beginning of the experiment. Curve 4 shows the characteristic hyperglycaemia following the administration of the standard dose of urethane alone. Curve 5 indicates the effect of the standard amount of urethane given at the beginning of the experiment followed 60 minutes later by the standard dose of insulin.

Fluoride. Here again Curve 1 is the effect on a normal rabbit of the standard dose of insulin. Curve 3 shows the influence of 75 mg. of NaF (5 % soln.) given intraperitoneally and curve 2 that of the standard dose of insulin followed 60 minutes later by 75 mg. of NaF per kg.

Iodoacetic acid. Curve 1 shows the effect of the standard dose of insulin. Curve 2 gives the blood sugar values in a rabbit which was given the same dose of insulin followed 60 minutes later by intraperitoneal injection of 35 mg. of iodoacetic acid (1 % soln.) per kg., and curve 3 those of an animal which

received 35 mg. iodoacetic acid per kg. and 60 minutes later the standard dose of insulin. Curve 4 represents the changes in blood sugar resulting from the administration of 75 mg. of iodoacetic acid per kg. at the beginning of the experiment.

Fig. 4.

Fig. 4. 1. Insulinised rabbit; 2. Insulinised rabbit. *Fluoride administered; 3. Fluoride hyperglycaemia.

Fig. 5.

Fig. 5. 1. Insulinised rabbit; 2. Insulinised rabbit. *Iodoacetic acid administered; 3. Iodoacetic acid hyperglycaemia. *Insulin administered; 4. Iodoacetic acid hyperglycaemia.

DISCUSSION.

It will be noted that the inhibitors by themselves all produce marked hyperglycaemia. It is possible and probable that this effect is caused by inhibition of glucose breakdown at the points indicated in Fig. 1. In the case of cyanide it is obvious that insulin does not counteract the hyperglycaemic action (curve 2, Fig. 2), but that the hypoglycaemic action of insulin is delayed and reappears after the effect of cyanide has passed off. In the presence of insulin the duration of the inhibiting action of cyanide approximates to the duration of its hyperglycaemic action in the normal animal.

In the case of urethane the animals were deeply narcotised and the effect persisted throughout the duration of the experiment (curve 4, Fig. 3). It is also shown by curves 2, 3 and 5 that the urethane effect inhibited the usual action of insulin (curve 1).

In the animals treated with sodium fluoride steadily increasing hyperglycaemia was noted upon which insulin was without influence.

The most striking effects were found after administration of iodoacetic acid. As seen in curve 2, Fig. 5, where iodoacetic acid was administered 60 minutes after the standard dose of insulin, the hypoglycaemic action of insulin still persisted although it was somewhat delayed. The effect of insulin was again demonstrated (curve 3, Fig. 5) when it was injected 60 minutes after the standard amount of

iodoacetic acid; here the lowering of blood sugar was not immediate, but in the course of an hour there was a distinct drop in the hyperglycaemia in direct contrast to the effect shown in curve 4 which followed the administration of iodoacetic acid alone.

A survey of the previously noted results indicates that insulin exercises its maximum activity at the point where pyruvic acid reacts with glycerophosphoric acid to form lactic acid and glyceraldehydephosphoric acid [see Pollard and Pryde, 1933]. It is also evident that interference with cell respiration (urethane and cyanides) lowers the efficiency of insulin and that sodium fluoride, which prevents the formation of pyruvic acid from glyceraldehydephosphoric acid is antagonistic to insulin. These results must be considered on a strictly relative basis since it is well known that the hyperglycaemia of narcosis and anaesthesia can be reduced by massive doses of insulin.

The principal criticism to be advanced against the preceding conclusions is that the hyperglycaemia is due to rapid mobilisation of liver glycogen rather than to retardation of tissue metabolism.

Fig. 6. 1. Adrenaline hyperglycaemia; 2. Adrenaline hyperglycaemia. *Insulin administered.

This criticism can be refuted by the experiment shown in Fig. 6 which illustrates the effect of the standard dose of insulin on adrenaline hyperglycaemia. In this series of experiments the peripheral metabolism was intact and here we see in curve 1 the characteristic response to adrenaline alone (1 ml. of 1:10,000 per kg.). In the experiment illustrated by curve 2, 90 minutes after the adrenaline was given, the rabbit received the standard dose of insulin used throughout the course of this work as a result of which there was a characteristic hypoglycaemic reaction terminating in a convulsion 90 minutes after the injection of insulin. Similar results were obtained in other animals in this series. We may then conclude that the amounts of insulin which have been used in the course of this work will effectively control hyperglycaemia originating from mobilisation of liver glycogen and consequently it is both possible and probable that the hyperglycaemias following the actions of the inhibitors are due to the specific actions of the latter in the respiratory cycle of the tissues and in the intermediate stages of glucose breakdown; this view has already been expressed by Irving [1934] in regard to iodoacetic acid. These experiments tend to support Macleod's view that insulin converts the sugar into an intermediate form in the tissues.

SUMMARY.

1. The action of maximum tolerated doses of cyanide, urethane, iodoacetic acid and sodium fluoride produces a hyperglycaemia which, except in the case of iodoacetic acid, neutralises, at least temporarily, the action of 1 rabbit unit of insulin per 2 kg. body weight.

2. Iodoacetic acid in maximum tolerated doses delays but does not neutralise the effect of 1 rabbit unit of insulin per 2 kg. body weight.

3. Insulin exercises its maximum effect upon the reaction between pyruvic and glycerophosphoric acids.

4. Mobilisation of liver glycogen is not the sole factor in the hyperglycaemias resulting from the substances used in the preceding experiments.

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