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On the mechanism of insulin action.

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The generally accepted explanation of the blood sugar lowering the effect of insulin when injected into the mammalian organism, either subcutaneously or intravenously, is that an interaction takes place between the injected hormone and the cells or tissue fluids of the body. The chemical structure of insulin is unknown, and the nature of its chemical reactions within the body has not been determined up to the present time.

Mueller and Corbitt[1] found that in rabbits there was a difference in the blood sugar lowering effect between the intradermal and the subcutaneous administration of insulin. By intradermal injection the blood sugar lowering effect was found to be of longer duration than by subcutaneous injection.

This observation led to further research work upon the effect of the two methods of administration of insulin in human subjects. The results form the basis of the present communication. Diabetics as well as individuals with a normal carbohydrate metabolism were studied. The experiments were done in the morning, with the subject in a fasting condition, or following a breakfast of definite composition, finished at a known time. For the injections the U 40 strength of Insulin Lilly (Iletin) was employed. Two experiments on the same individual, employing like doses of insulin from the same bottle and under the same dietary conditions, but differing in the method of administration of the insulin, constitute a series and are so referred to in the text to follow. A total of 33 such series are here reported.

The blood sugar was determined immediately before the insulin injection, and at one hour, two hours and four hours following the injection. In a sufficient number of cases the same blood sugar curve, but omitting the insulin injection, was determined.

[1] Mueller and Corbitt, *J. Lab. and Clin. Med.*, 1924, ix, 3.

In six additional series of one hour only, the blood sugars were determined at twenty minute intervals following the injections.

Twenty-nine (88 per cent) of the thirty-three series show a greater reduction in the blood sugar level at the first hour following the intradermal injection than following a subcutaneous injection of an equal dose.

At the second hour, twenty-three (70 per cent) of the intradermal injections resulted in a lower blood sugar level than did the subdermal injections.

At the fourth hour, twenty, or 67 per cent, of the series showed a greater hypoglucemia with intradermal administration.

The difference in the response to the two methods of administration, as manifested by the hypoglucemic effect, is most striking with the smallest doses, (*i. e.*, 5 units) and at the first hour after the injection.

This difference in response to the two methods of administration varies from case to case. The greatest differences in the hypoglucemic effect between the intradermal and the subdermal methods occur in the cases of moderately severe diabetes. The least differences are noticeable in non-diabetics. A consistently less great response to the intradermal injection was noted in only one case of severe diabetes. The figures show characteristic curves obtained by plotting the blood sugar values, following the insulin injection, expressed in per cent of the initial blood sugar before the injection, against time. Figure IV represents the composite curves of all the 5 and 10 unit series. The fact that the maximum difference falls at the first hour, and the gradual decrease in this difference at the subsequent time intervals, is shown in this curve.

Insulin injected subcutaneously enters the blood stream and is brought to the tissues more promptly than when the same dose is given between the layers of the skin. Kasahara[2] showed that the resorption of true solutions, colloidal solutions and cell and bacterial suspensions when introduced intracutaneously is materially delayed as compared to identical injections administered subcutaneously. Our results cannot therefore be explained by a quicker resorption of the intradermally introduced insulin. From the 20-minute series (Fig. V) it is apparent that there is a blood

[2] Kasahara, *Ztschr. f. d. ges. exp. Med.*, 1925, xliv, 294.

sugar lowering effect of intradermally injected insulin before any of the hormone can possibly be present in the blood stream, for the subdermally administered equal dose of insulin shows no such effect twenty minutes after the administration. We conclude from the observations reported above that insulin injected intracutaneously must have an initial specific action produced in some way other than the usual contact of the hormone with the tissue fluids and cells.

Our findings imply the transmission of a specific stimulus between the intracutaneous area of the skin and the organ or organs the activity of which governs the carbohydrate metabolism in the mammalian body. We postulate the pathway which carries this stimulus to be the fibres of the parasympathetic nervous system. Claude Bernard showed that stimulation of certain parts of the parasympathetic system results in increased glucose formation in the body. M. Eiger,[3] working with turtles showed that stimulation of the peripheral end of the cut vagus caused increased glycogenesis, despite exclusion of the pancreatic activity. H. Meyer showed that injection of pancreatic extract inhibits glycogenolysis in the liver. From these experiments it is to be assumed that stimuli may travel via the vagus nerve to the liver, and there cause actual increased liver cell metabolism, and also that such stimuli may travel to the pancreas whereby an equivalent internal secretory influence of the liver cell metabolism may be registered. In both cases vagus stimulation results in the inhibition of glycogenolysis.[4] Minkowski's experiments have shown that not only extirpation of the pancreas, but also disconnection of the nerves between pancreas, liver and duodenum results in diabetic symptoms.

The response of the liver cell in the form of glycogenetic activity is governed to a large extent by the amount of glycogen in the liver at the time, and this response will be decreased if the liver is rich in glycogen. The blood sugar levels following the injections of 10 and 20 unit doses of insulin intradermally as well as subcutaneously on two non-diabetics are shown in Figure II. The quick recovery to the normal blood sugar level in both, as well as the reversed response of one of the subjects to the experiment, is probably due to the degree of glycogen saturation of the

[3] Eiger, M., *Centralbl. f. Physiol.*, xxx.

[4] Quoted from L. R. Mueller, *Die Lebensnerven*, Berlin, Springer, 1924, 282.

liver cell. Experiments are now being done in the attempt to prove that the effect of an intracutaneous injection of insulin is greatest when the liver is poor in glycogen, and that with the liver rich in glycogen, in the same individual, the effect of a subdermal injection will be as great, or greater than that of a like dose injected intradermally.

The glucolysis occurring in the bloods drawn before insulin injection and at stated intervals thereafter was determined in nine

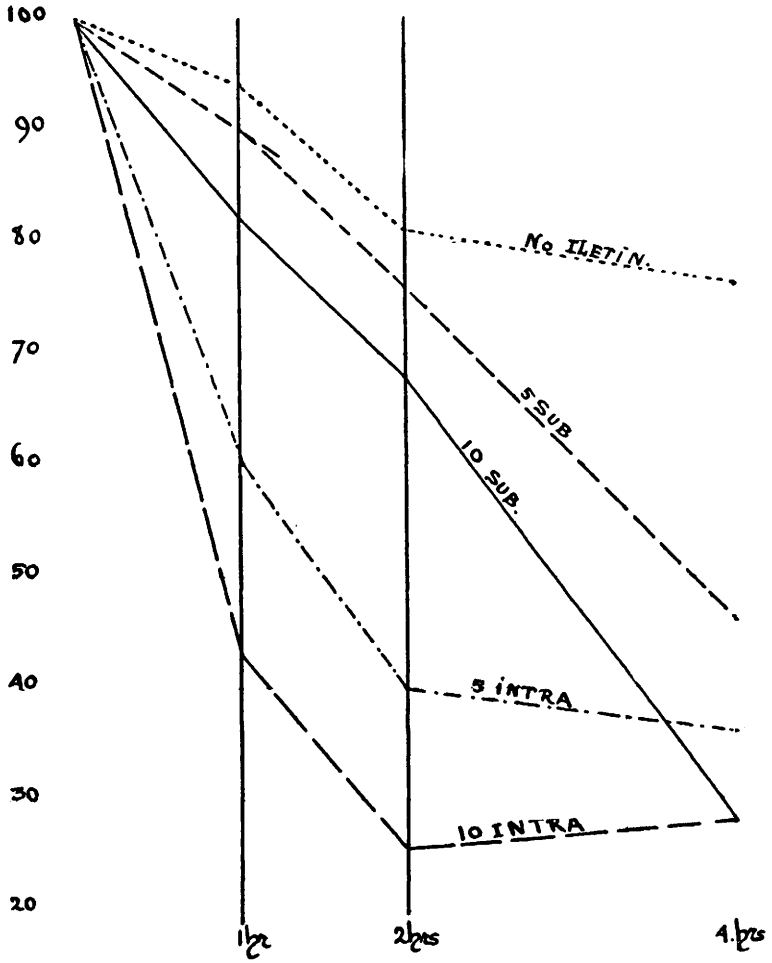

FIG. 1.

Blood sugar in per cent of initial value, following insulin injection. Diabetes Mellitus (moderately severe).

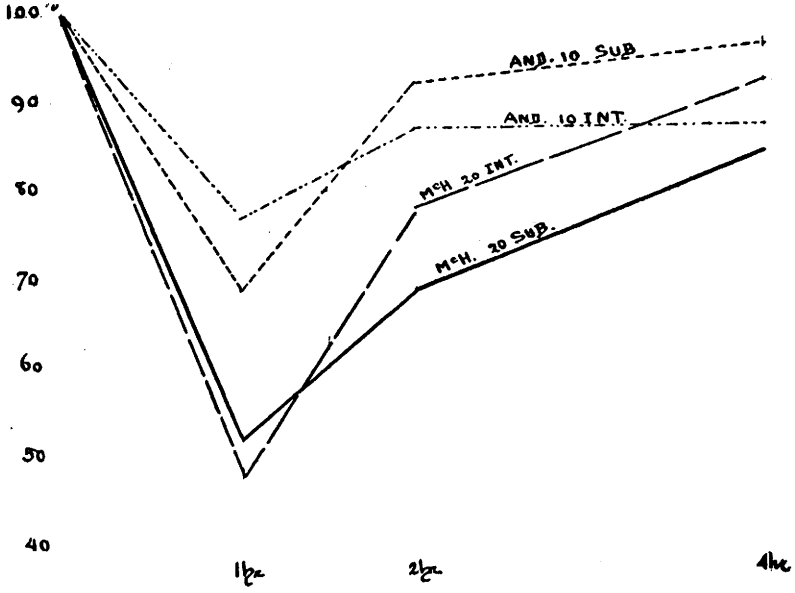

FIG. 2.
Blood sugar in per cent of initial value, following insulin injection in non-diabetics.

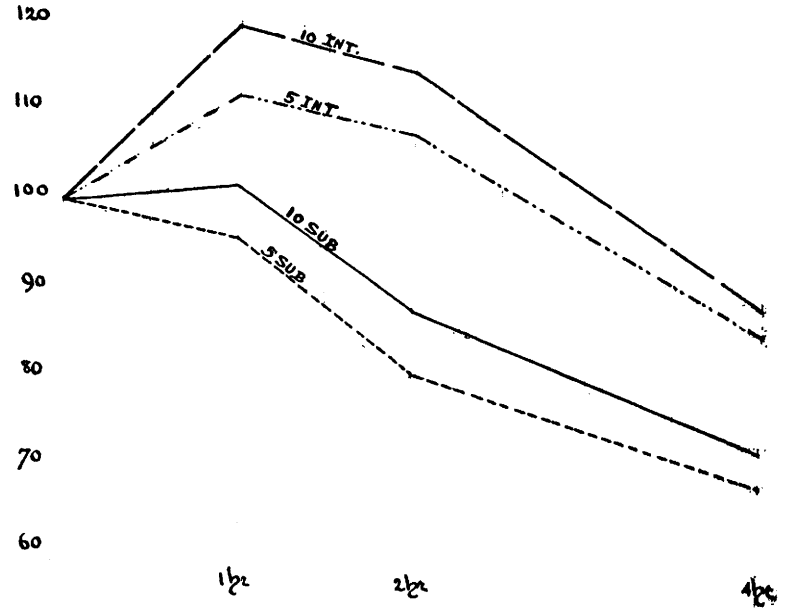

FIG. 3.
Severe diabetes mellitus.

series. The average of the absolute values in mg. per cent of the sugar destroyed when the blood was allowed to remain in the incubator, at 37° C., for two hours were as follows:

	Insulin by intradermal method	Insulin by subdermal method
Before injection	24 mg. per cent	21 mg. per cent
One hour after injection	19 mg. per cent	28 mg. per cent
Two hours after injection	22 mg. per cent	30 mg. per cent
Four hours after injection	15 mg. per cent	21 mg. per cent

As may be seen from the table the one-hour post intradermal injection blood failed to manifest greater glucolytic activity than did the blood drawn before insulin injection. On the other hand the one hour post subcutaneous injection blood manifested a greater glucolytic activity than did the blood drawn before the injection. In fact the glucolytic activity of the bloods drawn following intradermal injection remained constant or diminished slightly as the blood sugar level decreased, whereas by the subcutaneous method the glucolytic activity of these parallel bloods was increased at the two-hour post injection period as well as at the one-hour post injection period.

In severe diabetes the glycogenetic function of the liver is markedly reduced or is even absent. The severity of the diabetic condition is mirrored by the results of the various series as done in these experiments in that the concentration of glycogen in the liver cells governs the degree of difference in response to the two methods of injection.

This is well illustrated by Figure III from a case with severe diabetes mellitus.

Experiments done by one of us[5] on rabbits proved that if the parasympathetic pathways are blocked by atropin the normal blood sugar lowering effect of intradermally injected insulin does not appear. In control tests it was shown that atropin itself in the doses used does not effect the blood sugar level. If rabbits are injected with atropin every 30 minutes, and the parasympathetic pathways are thus cut off during the entire period of observation, the result upon the animal's reaction to various methods of insulin administration is as follows: The blood sugar curve following intravenous administration is the same as the curve in the same animal without atropin. After subdermal injection the hypoglucemic effect is slightly less marked than in the

[5] E. F. Mueller. To be published.

non-atropinized animal. If the insulin is administered intracutaneously in an atropinized rabbit the blood sugar level is not affected for a period of almost an hour. During the two succeeding hours a hypoglucemic effect is noted, but it is only half as great as in a non-atropinized animal.

These experiments indicate that the fibres of the parasympathetic nervous system may carry the specific stimulus initiated by the insulin injection. That area in the body which is most rich in the parasympathetic nerve endings will serve as the most effective area for the localization of the depot if the reaction to this effect is to be be given the greatest prominence. This effect is totally lacking in the intravenous administration, partly demonstrable by the subdermal administration, and very much more definitely present when the insulin is deposited intracutaneously. The degree of intensity of this stimulus does not depend upon the dosage; the effect of this stimulus, though gradually diminishing, will persist as long as the deposit of active insulin remains. With the beginning of resorption of the insulin from the depot, wherever located, into the circulation the hormone effect is initiated and

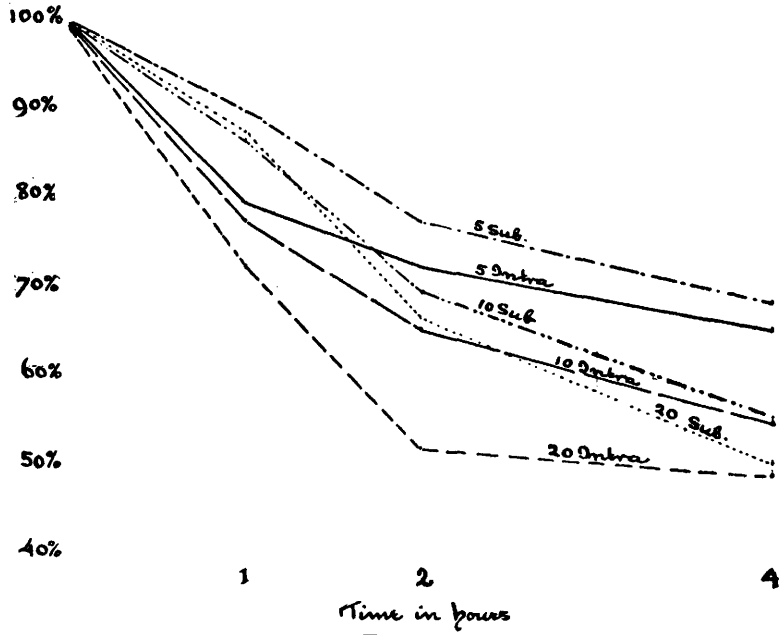

FIG. 4.

Blood sugar in per cent of initial value, following insulin injection. Composite curves.

increases with increasing concentration of the active insulin in the circulation. As resorption into the circulation proceeds, the hormone effect increases and reaches its maximum, and the specific effect of the stimulus via the parasympathetic nervous system gradually diminishes and disappears.

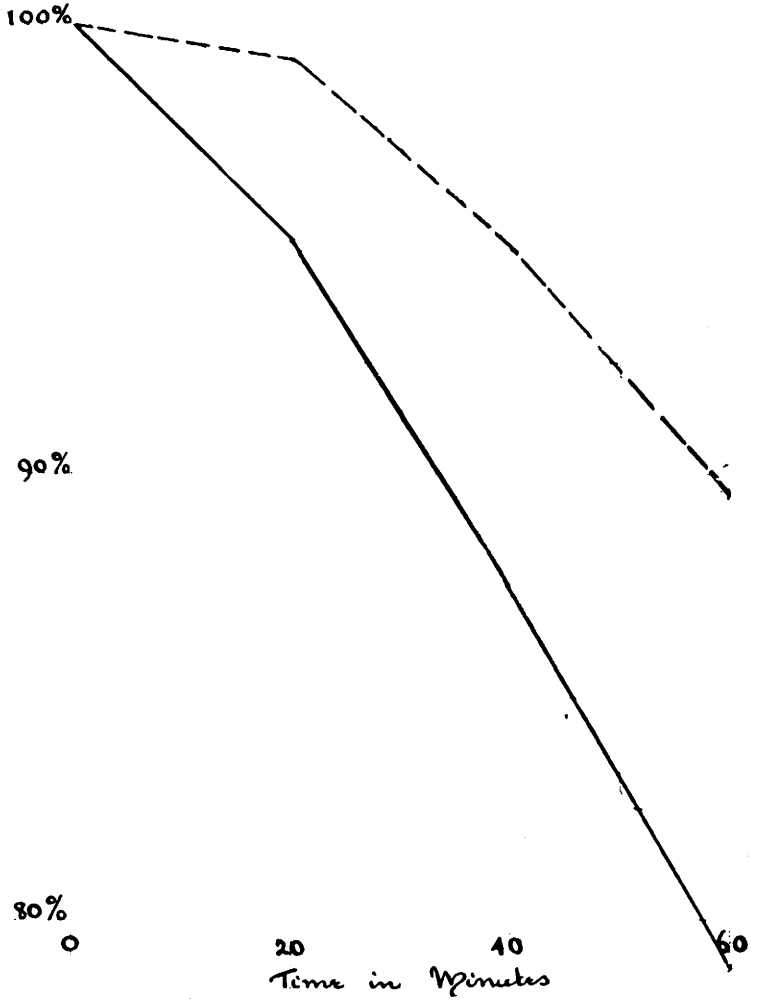

FIG. 5.

Blood sugar in per cent of initial value, following insulin injection.

———— Intradermal.

— — — — Subcutaneous.

Composite curves.