PROTOCOLS.

Dog 7. Exposure 3-20-24. On 5-28-24, a definite chronic ulcer, 14 mm. long, 6 mm. wide and 2 mm. deep, present. A scar on the posterior serous coat. On 2-26-25, ulcer still present, practically same size, edges more indurated. Animal still alive.

Dog 8. Exposure 5-11-24. On 3-12-25, a definite chronic ulcer present. An area about 2½ cm. in diameter, indurated and roughened. In the center of this area, a fissure 8 mm. in length, 3 mm. in width, and about 3 mm. in depth. No scar on the posterior serous coat. Animal still alive.

Dog 9. Exposure 6-13-24. On 3-5-25, a chronic pear-shaped ulcer, 1 cm. in diameter and 2 mm. deep, present. Floor of the ulcer granulating. Dog still alive.

Dog 15. Exposure 2-24-25. On 5-15-25, an ulcer 4 mm. long, 2 mm. wide, oval in shape, punched out appearance. Dirty grey floor, edges indurated, surrounded by a hyperæmic area. Dog still alive.

Dog 12. Exposure 3-17-25. On 5-19-25, died of a generalized peritonitis due to a gastric perforation. Perforation about 6 mm. in diameter, edges thickened. Microscopic examination of the margin of the perforation shows but little sign of inflammatory reaction. Most of the necrotic tissue digested away.

Proper anesthesia was used in all cases.

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Analogous action of insulin and epinephrin on the liver.

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Insulin and epinephrin are frequently considered as antagonistic because hypoglykemia and hyperglykemia follow after the respective administration of these substances.

Since we know the mechanism of insulin action,[1] the alleged

[1] E. F. Müller, H. E. Wiener, and R. vE. Wiener, PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 375.

antagonism of insulin and epinephrin gained interest for us. We have previously demonstrated that insulin has a two-fold action depending upon whether or not insulin is absorbed at the site of injection.[2]

From a tissue depôt (an intradermal injection) insulin acts purely by nerve stimulation, increasing the glycogenetic function of the liver. This glycogenesis finds its expression in a consequent lowering of the blood sugar. Insulin absorbed from its tissue depôt, or injected intravenously does not bring about this glycogenetic action.

Insulin injected intravenously or absorbed from a subcutaneous injection becomes effective after making contact with the tissues of the body. This process is one that takes a certain period of time, and the effect will not be noted for 30 to 40 minutes after injection, while the nerve action is immediate. Insulin injected subcutaneously or intravenously, for comparison with epinephrin action, will enable us to compare only the hormone action of insulin, *i. e.,* its glycolytic action, with the effect of epinephrin. Such an antagonism is quite improbable. The antagonistic process would consist of building up sugar.

Pertinent experiments of MacLeod and his associates[3] have been made as follows: Epinephrin was given to animals one hour after insulin injection, with a resulting equalization of both insulin and adrenalin effect on the blood sugar. We do not believe that this observation justifies the assumption of an antagonistic effect of epinephrin and insulin.

In a series of animals we have first determined the relative dosage of epinephrin and insulin which would produce a corresponding percentage augmentation or reduction, respectively, of blood sugar. The result showed that an average .05 mg. of epinephrin and 0.5 units of insulin per kilogram body weight increased or diminished the blood sugar approximately 50 per cent in two hours time. Simultaneously injected (subcutaneously, either separately or mixed) insulin and epinephrin do not show an antagonistic action in the majority of cases. In more than 60 per cent the result is an increased insulin action, a typical lowering of the blood sugar, in spite of the presence of enough epinephrin to theoretically balance the insulin effect.

[2] E. F. Müller and W. F. Petersen, same, in print.

[3] J. J. MacLeod, *Physiol. Reviews,* 1924, iv, 21.

If we give epinephrin 20 minutes before the subcutaneous injection of insulin, a decrease of the blood sugar follows. This decrease exceeds the effect of a single corresponding insulin injection in the same animal in the majority of cases; in some instances a decrease of 60 per cent is reached in two hours.

Since we know the two-fold action of insulin, the explanation of these observations is simple. Epinephrin acts by increasing vasocontraction and diminishing endothelial permeability; it postpones and diminishes the absorption of insulin injected simultaneously or later. Consequently the insulin, excluded from the circulation, does not come into contact with the tissues, and glycolysis does not take place; but from the tissue depôt the nerve action of the insulin becomes manifest. Glycogenesis is stimulated, and continues active as long as insulin is not absorbed. So epinephrin, through its vaso-constricting effect, postpones insulin absorption, makes possible its glycogenetic nerve effect, and eliminates for this period of time its hormone action. The glycogenetic effect of insulin is presumably much stronger than the glucose mobilization initiated by an epinephrin deposit in the tissues because an increase in blood sugar is usually absent.

Examining our results in connection with those of MacLeod, who injected epinephrin during the period of most intensive insulin action, the following might be mentioned before discussing the underlying explanation of these findings. In a previous communication[4] we have shown that subcutaneous injection of insulin is followed without exception by an increase of blood sugar, while intradermal injections result in an immediate blood sugar decrease. We have also demonstrated that intradermal injections act glycogenetically, while insulin present in the circulation (as after subcutaneous and intravenous injections) mobilizes glucose. Thus glycogen is depleted in the liver.

It may be of interest to mention that probably insulin, *per se,* when present in the blood stream, acts in mobilizing sugar. Were this glucose mobilization to depend only on a replacement basis (sugar vacuum of the tissues) it would not be demonstrable in the short interval following injection, nor would it reach the high levels which we have obtained during the period immediately after injection (50 per cent or more). During this time the *in vitro* effect of insulin is not apparent.

[4]E. F. Müller and W. F. Petersen, *J. Am. Med. Assn.,* in print.

Insulin present in the blood stream almost instantly mobilizes glucose, and in this way increases the blood sugar level. The mechanism of this action is still unknown. Insulin may act by direct contact or by a sympathetic effect on the liver.

The result of the mobilization of glucose from the liver, cannot be determined longer than about 20 to 30 minutes after the injection, because at that time the hormone effect becomes apparent and conceals the further mobilization of glucose, which is instantly acted upon by the combined tissue-insulin effect. With these considerations in mind we believe that MacLeod's, as well as our own experiments, can be interpreted as follows:

(1) Epinephrin given before or simultaneously with insulin delays the absorption of insulin; the nerve effect of the insulin depôt initiates glycogenesis in the liver, and decrease in blood sugar follows.

(2) Epinephrin given an hour after insulin injection (when insulin has been completely absorbed and has reached its maximum effectiveness) mobilizes glucose from the liver, and thereby increases the already existing glucose mobilization. The glycogen store of the liver may thereby be practically exhausted, but the blood sugar level is maintained by the sufficiently rapid mobilization of glucose from the liver.

This interpretation, that insulin and epinephrin have an analogous influence upon the liver, may be confirmed in various ways. We have already succeeded in demonstrating that the effect of the injection of insulin (subcutaneous and intravenous) acts in mobilizing glucose.

It is also known that animals respond to drugs with a varying degree of intensity. For instance, rabbits which responded only slightly to insulin administration, react very markedly to epinephrin. One rabbit, for example, reacted with a decrease of only 22 per cent of its previous blood sugar content (uniform dosage of 0.5 units per kilogram body weight); while another showed a decrease of 65 per cent in the same interval. It was of interest to find that the former showed an increase of 125 per cent of its sugar level after the administration of epinephrin, while the latter responded with a 40 per cent increase (uniform dosage of 0.5 mg. per kilogram body weight). More examples of such individual differences were observed, not all of the same striking degree.

The individual difference is explicable on the basis already suggested of an analogous action of the epinephrin and insulin. Both epinephrin and insulin act by depleting the liver of glycogen. If the liver response is individually intensive, much glucose will be mobilized and will be found in the circulation after epinephrin administration. This same animal will also release considerable amounts of glucose during insulin action, thus almost completely replacing the metabolized sugar, and the sum total of the insulin effect as observed in the blood sugar curve will be relatively small.

If, on the other hand, the liver of another individual releases but small amounts of glucose to an irritation, the demonstrable epinephrin reaction will be small, and only a part of the metabolized sugar (after insulin injection) will be replaced, so that a distinct sugar deficiency becomes apparent in the blood.

The observations indicate that, while the hormone action of insulin is always in direct proportion to the amount of insulin present in the tissues, the liver response depends very largely on the individual reactivity of the particular animal, seemingly a fixed characteristic of that organism.

The individuality of the response is of importance for the study of the nerve action of both epinephrin and insulin, while it is, of course, of lesser interest for the hormone effect of insulin which must be considered an independent process.

The individuality may also be of importance for the understanding of the described analogy of insulin and epinephrin.

An analogous glycogenolytic effect of insulin and epinephrin is demonstrable if sufficient amounts of insulin and epinephrin are present in the circulation.