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DIETETIC FACTORS INFLUENCING THE GLUCOSE TOLERANCE AND THE ACTIVITY OF INSULIN.

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It has long been recognized in the therapeutics of diabetes mellitus that the composition of the diet exerts a pronounced effect upon a diabetic's tolerance for carbohydrates, but it is only recently that attention has been drawn to the effect of different diets upon a healthy individual's tolerance for glucose. That starvation lowered the carbohydrate tolerance of a normal person was clearly recognized by Claude Bernard [1877], and it is noteworthy that he did not confuse such cases of glycosuria with cases of early diabetes mellitus or symptomless glycosuria (renal glycosuria), even though the only criterion of impaired or normal tolerance at his disposal was the presence or absence of glycosuria. Within the last ten years sporadic papers have appeared on the effect of diet upon this aspect of carbohydrate metabolism, and in 1927 Sweeney published results showing the effect of each class of foodstuff upon the glucose tolerance of healthy men. It is now established that the sugar tolerance is impaired by starvation or the taking of diets with a high content of fat, whilst it is improved by taking diets containing an excess of carbohydrate. This latter improvement would appear to be linked with a phenomenon observed by Hamman and Hirschman [1919], the so-called Staub-Traugott effect. These workers noticed that if the same dose of glucose were given at intervals of 90 min. the blood-sugar tolerance curve improved with each successive dose.

The effect of variations in diet upon insulin action has, until recently, been almost completely neglected. Abderhalden and Wertheimer [1924] and Bainbridge [1925] demonstrated that animals were more sensitive to insulin when receiving a carbohydrate diet than when they were eating a fat diet or were starving. Tiitso [1925–6] showed that, in starving animals, the blood sugar fell more slowly after insulin than in

those allowed a full diet. Recently Hynd and Rotter [1931] have demonstrated that there is a definite relation between the composition of the diet and the time after insulin injection that hypoglycæmic symptoms occur; animals on a high carbohydrate diet showed symptoms earlier than animals on a high fat diet.

It appeared to the writer that a correlation existed between the effect of diet upon the subsequent sugar tolerance and its effect upon the susceptibility of an animal to insulin, and it was shown, in healthy men [Himsworth, 1933], that those diets which caused an improvement in sugar tolerance also permitted a more rapid depression of the blood sugar by insulin, whilst those which produced a diminished sugar tolerance always retarded the rate at which the blood sugar fell after insulin injection. In the present paper it will be shown that identical results can be obtained on animals and that this inter-relationship, between sugar tolerance and insulin action, holds good in a wide variety of conditions.

Methods.

Male rabbits, approximately 2 kg. in weight, were used.

The *diets* given were of two kinds. The carbohydrate diet was composed of 250 g. of fresh cabbage and approximately 150 g. of a mixture of oats and bran in the proportion of 1 to 3. The fat diet was made up of 50 g. of fresh cabbage and 120 g. of soya-bean meal moistened with water. This amount of soya bean was about the maximum that a 2 kg. rabbit would eat in 24 hours. On analysis the soya-bean meal yielded 17 p.c. of ether soluble substances. A considerable proportion of this fraction was made up of lecithine, and it is of interest, in view of the recent work of Best and Hershey [1932], that its presence did not prevent the usual adverse effect of fat upon the sugar tolerance. Judged by human standards the fat diet contained a comparatively small proportion of fat, but judged by the standard of the diet habitually eaten by rabbits it will be seen that the fat bulks relatively largely.

All the animals receiving soya bean were of our own breeding, and were placed on this diet when they were 12 weeks old, but were not used for experimental purposes until they had reached the age of 6 months. The animals ate the soya bean with avidity and, although they appeared somewhat thinner than rabbits on a carbohydrate diet, their condition continued to be excellent. It should be noted that no experiment was performed on an animal until it had been taking the required diet for at least 2 months. All experiments were commenced at 10 a.m., 18 hours after withdrawal of food.

The *blood sugar* was estimated in 0·1 c.c. of whole blood by the Hagedorn-Jensen method.

Blood samples were taken from the marginal vein of the ear. As it was thought that vasomotor changes in the ear might affect the sugar content of the blood, by altering the rate of blood flow through the tissues, precautions were taken against this source of error. The animals remained at rest in a special box and were kept warm throughout the whole experiment, after previously having been accustomed to this lengthy procedure and also to the necessary handling. In the earlier experiments, 2 months before being used, the sensory nerves to both ears were cut and the superior cervical sympathetic ganglion on each side was removed. Later it was found that, provided the animals were well trained, and that they were kept comfortably warm, this precaution was unnecessary.

No attempt to obtain samples was made until the ear veins were fully distended with blood. A cut with a sharp Hagedorn needle was then made in the long axis of the vein, the specimen taken, and a small square of filter paper pressed lightly on to the cut for 30 sec., and then left in position. If the cut had been made cleanly, blood quickly ceased to escape and yet the flow continued undisturbed through the vein underneath. Removal of the filter paper with a squeezing sliding motion restarted the bleeding.

The times of sampling were decided before the experiment commenced. The operator was warned just previous to the scheduled time, and the exact second that the blood filled the pipette was noted by an assistant. In experiments after injection of insulin (insulin depression curves) samples were taken in the early part of the curve every $1\frac{1}{2}$ min. and later every 3 min. In the different types of glucose tolerance curves the time and frequency of sampling varied, but in the same type of experiment the corresponding samples for each curve were taken at exactly the same minute.

The accuracy with which blood-sugar samples can be obtained by the above technique and the constancy of the results under the same conditions can be judged from Figs. 1 and 2.

Insulin injections were all given intravenously into the marginal vein of the ear not being used for blood sampling. For investigation of the effect of insulin upon the blood sugar the subcutaneous route is unsatisfactory owing to the slow and irregular absorption from the site of injection. A solution of *crystalline* insulin, 10 units per c.c., for a sterilized solution of which I am greatly indebted to Dr J. W. Trevan of the Wellcome Physiological Research Laboratories, was used in all experi-

ments, as commercial insulin had been found unsuitable, owing to its containing impurities which cause a transient initial hyperglycæmia on intravenous injection. In the insulin depression curves one-half unit of insulin was given by means of a syringe of fine bore accurately calibrated.

Glucose injections were given into the marginal vein of the ear from which blood was not being withdrawn. The glucose was made up as a 20 p.c. solution in normal saline and sterilized by boiling. At each injection 5 c.c. of this solution (1 g.) was given at a uniform rate which was

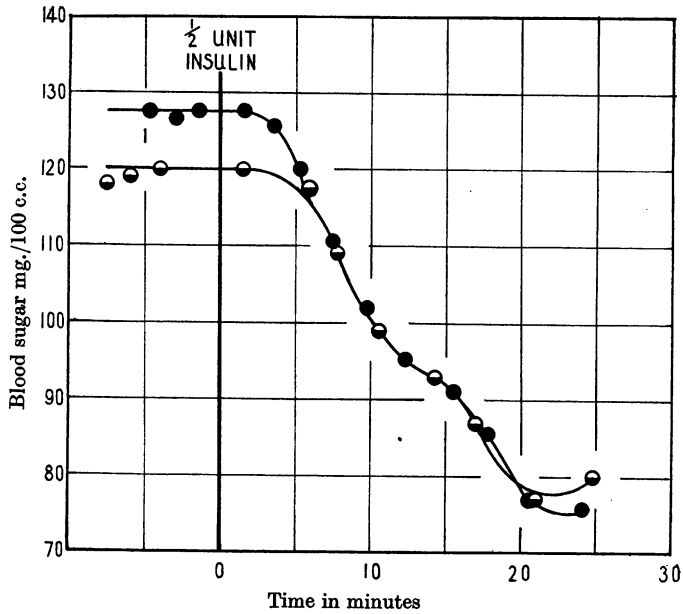

Fig. 1. Insulin depression curves after one-half unit of insulin obtained from the same animal, on two different occasions, whilst taking the carbohydrate diet.

timed so that the whole injection took one minute. As the animals were all of approximately the same weight no attempt was made to regulate dosage by surface area. In experiments in which consecutive doses of glucose were given (consecutive tolerance curves) all injections were carried out at the same time intervals. When insulin was injected in the course of a consecutive tolerance curve the dose chosen was 5 units, and it was given immediately before the last blood sample of one curve and 2½ min. before the next glucose injection.

Adventitious factors affecting blood-sugar curves after injection either of glucose or of insulin may either impair or improve the animal's capacity

to remove sugar from the blood. Excitement and hæmorrhage both interfere with the removal. The effect of excitement can be seen from Fig. 7. Any animal which, after two or three periods of training, did not

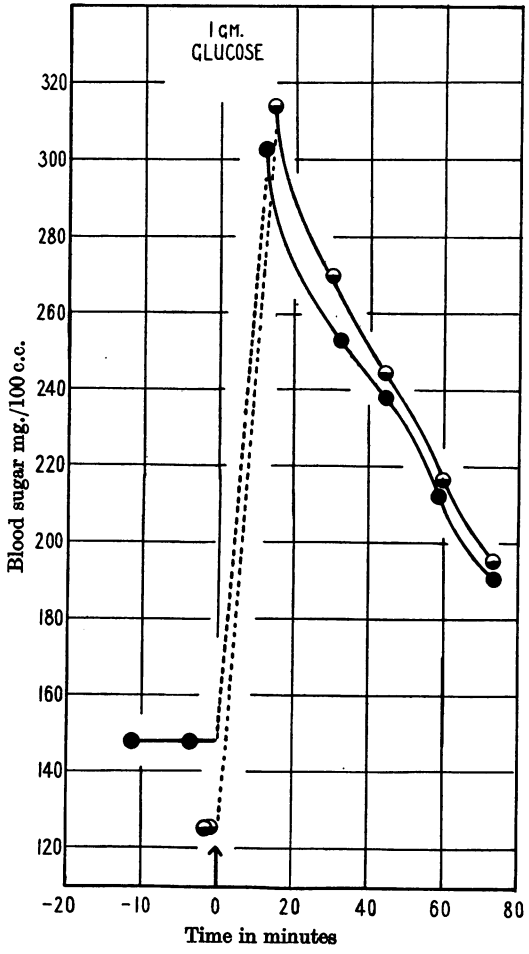

Fig. 2. Two glucose tolerance curves, after the injection of 1 g. of glucose intravenously, obtained on two different occasions from the same animal, whilst taking the fat diet.

sit quietly in the box but showed hurried respirations, retracted eyelids or intermittent struggling, was discarded as unsuitable for this work. Considerable hæmorrhage is required to produce an adverse effect upon the sugar-removing mechanism. If, the moment a blood sample is taken, an assistant stops the bleeding, there will be no error from this source, but

as a precaution it is advisable to reduce the number of samples to a minimum in experiments of considerable duration. Improvement in an animal's capacity to remove blood sugar may be found if a second experiment is performed too soon after one in which much glucose was given to the animal. After a glucose tolerance curve 6 weeks should elapse before any further experiments are performed on that animal.

Comparison of experiments. In this paper the term "glucose tolerance" is used to signify the ability of an animal to remove injected glucose from the blood. Comparison of this ability may be made in two ways, firstly by comparing the slopes of different blood-sugar curves, and secondly by comparing the absolute height of the blood-sugar values at standard times after the injection of glucose. As the fall of the blood sugar is hyperbolic the first method is of little value unless numerous samples are taken. By the second method, if we find that under the dietetic conditions being investigated, the blood-sugar level at the chosen time is lower than when the animal was under standard conditions, then the animal has been able to remove more sugar from the blood under the new conditions in the same length of time and the sugar tolerance is increased, and *vice versa*. As the differences in sugar tolerance under the influence of different diets is so definite we have not scrupled to compare curves on different animals of the same weight (Fig. 4).

In order to compare two insulin depression curves it is necessary that in each experiment the blood-sugar value in the resting state should be at the same level. As this level in one rabbit may vary over a range of 30 mg./100 c.c. it is often necessary to take several curves on the same animal before obtaining two that can be compared. The necessity for this tedious precaution was anticipated from a consideration of the mass action theory as applied to the blood sugar [Himsworth, 1931], and was often verified by experience. If, under the same conditions, on one occasion the resting blood-sugar level is 140 mg./100 c.c., and on a second occasion 115 mg./100 c.c., then three-quarters of an hour after one-half unit of insulin it will be found that the blood-sugar values will be at approximately the same level. It will appear that in the first experiment one-half unit of insulin had in the same period of time depressed the blood sugar 25 mg./100 c.c. more than in the second experiment. If, now, these two curves are charted so as to start from the same resting level the first experiment will appear to indicate a greater susceptibility of the animal to insulin (cf. Fig. 1). That this is not so will be revealed when, under the same dietetic conditions, two insulin depression curves are obtained which actually start from the same resting level. The curves are

then identical. It may be taken as a general rule that a standard dose of insulin will, in an animal on a fixed diet, cause a greater and more rapid fall of blood sugar when the resting blood sugar is high than when it is low. It will be seen that, ideally, the resting blood sugar values in curves to be compared should be at the same level (Fig. 5), but that if we are expecting the conditions under investigation to increase the effect of

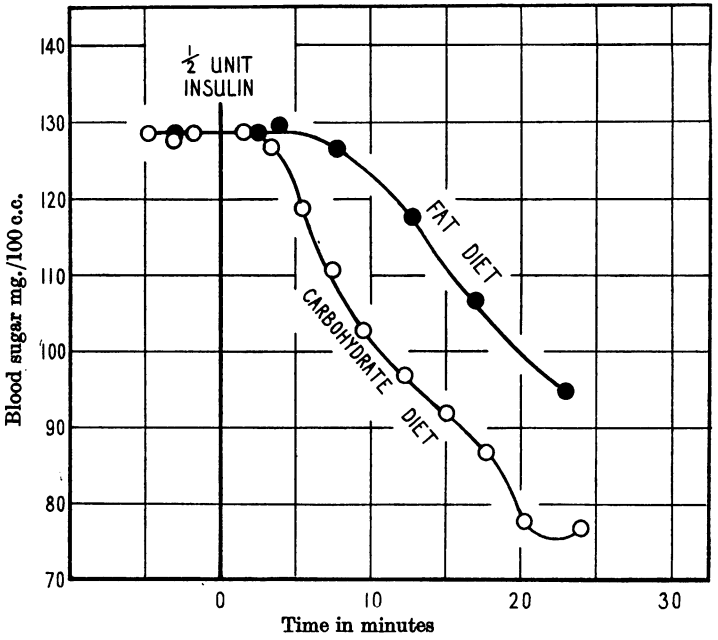

Fig. 3. Two insulin depression curves from the same animal, one (discs) obtained on the fat diet, the other (circles) obtained on the carbohydrate diet. The resting blood-sugar level on the fat diet was 134 mg./100 c.c. whilst on the carbohydrate diet it was 128 mg./100 c.c. Under the same dietetic conditions this difference in the resting levels would have tended to produce a more rapid and deeper fall in the curve commencing at 134 mg./100 c.c. As this tendency acts against the result expected, and obtained above, it has been considered allowable to compare these two curves despite their commencing at different resting levels.

insulin then if the resting blood sugar under these conditions is lower than under the standard conditions we may compare the curves, but if it is higher then we may not. Similarly if the new conditions are expected to decrease insulin effect we may only compare the curves if the resting blood sugar under these conditions is higher (Fig. 3). It is hardly necessary to add that only curves obtained from the same animal may be compared.

Results.

The figures show typical results from each group of experiments.

In Fig. 3 are shown two insulin depression curves obtained on the same animal, one whilst receiving a carbohydrate diet and the other whilst taking a fat diet. It will be observed that, after the injection of insulin, there is a short latent period during which the blood sugar is un-

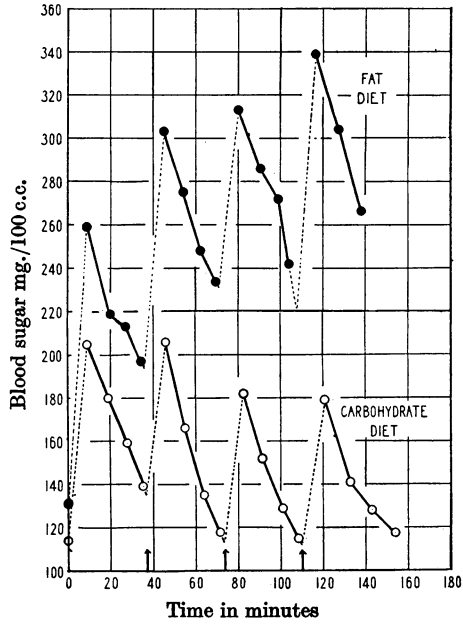

Fig. 4. Two consecutive glucose tolerance curves after injection of 1 g. of glucose every 35 min., the one (discs) obtained on the fat diet, the other (circles) obtained on the carbohydrate diet. The times of injection are indicated by the arrows.

affected, and that this period lasts for only 2 min. in the curve on the carbohydrate diet whilst it lasts for 6 min. in the curve on the fat diet. The significance of the latent period has been discussed elsewhere [Himsworth, 1933]. After this delay, depression of the blood sugar commences, and the experiments show that on the carbohydrate diet one-half unit of insulin exerts a more rapid and profound effect upon the blood sugar than when the animal is receiving a fat diet.

Fig. 4 demonstrates that the sugar tolerance is decreased by giving a fat diet. The experiments with four injections of glucose belong to the consecutive glucose tolerance group, but from comparison of the curves

after the first glucose injection in each experiment the diminished tolerance, on the fat diet, is obvious.

Fig. 5 shows that intravenous injection of glucose sensitizes the animal to the influence of insulin so that the same dose exerts a greater and more rapid effect upon the blood sugar after glucose than before. In

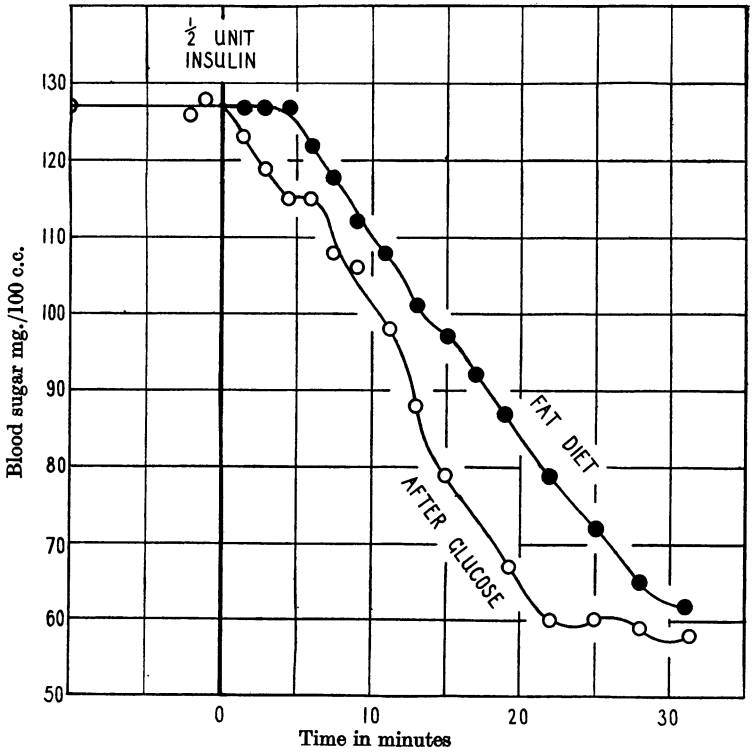

Fig. 5. Insulin depression curves, both obtained whilst the animal was on the fat diet, the one (discs) taken under the routine basal conditions, the other (circles) 40 min. after the blood sugar had fallen to rest after four injections of 1 g. of glucose at intervals of 35 min. The resting blood-sugar level in each case was 127 mg./100 c.c.

this experiment four injections of 1 g. glucose were given intravenously at intervals of 35 min. and then the blood sugar allowed to return to normal levels. Insulin was not injected until 40 min. after the blood sugar had returned and remained steady at the resting level. It was necessary to allow this period to elapse, otherwise it might, quite justifiably, have been maintained that the more rapid and profound effect of insulin after the glucose injections was due to a continuation of the fall of

the final sugar tolerance curve and not due to a true increased susceptibility of the animal to insulin. This experiment was carried out thirteen times with the same result on different animals. Six of the animals had been on a fat diet for over 3 months; the rest were on a carbohydrate diet. The difference between the two groups was not striking, but indicated that animals living on a carbohydrate diet showed a greater increase in sensitivity to insulin after four glucose injections than did those taking a fat diet.

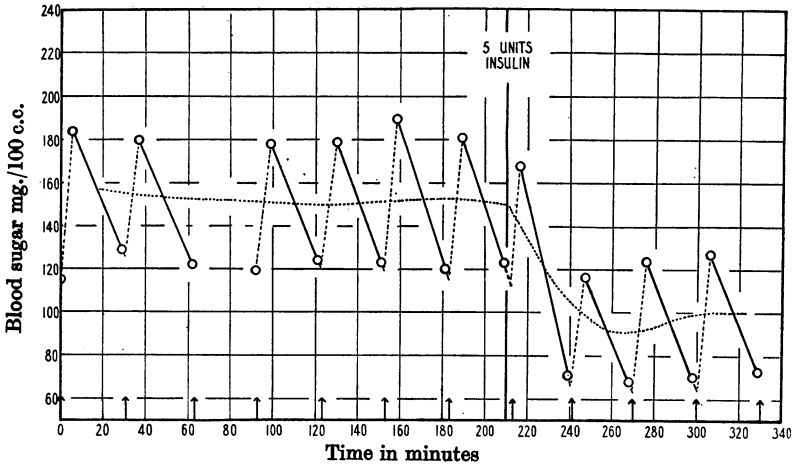

Fig. 6. Experiment showing the consecutive glucose tolerance curves obtained after injection of 1 g. of glucose every 30 min. when the animal was receiving a carbohydrate diet. Five units of insulin were given intravenously before the eighth injection of glucose. The times of injection of glucose are indicated by the arrows, and the time of insulin injection by the heavy vertical line.

The next group of experiments was concerned with the improving tolerance to carbohydrate that occurs after each successive dose of glucose, the so-called Staub-Traugott phenomenon. Figs. 4, 6, 7 and 8 show the effects of successive doses of glucose intravenously. The lower curve in Fig. 4, the first seven curves in Fig. 6, the first four curves in Fig. 7, and the first five curves of the lower experiment in Fig. 8 demonstrate this phenomenon. For convenience of visualization a curve has been drawn (dotted line) through the average figure of the two blood-sugar values for each curve at the time midway between the two samples. It will be seen in the curves mentioned that each subsequent blood-sugar curve begins and ends slightly lower than did the preceding one and that the "average curve" falls. Thus the phenomenon is present in rabbits

after intravenous injection and does not require that glucose be absorbed from the intestine. The writer has come to regard this effect as one of the most delicate phenomena in carbohydrate metabolism. In Fig. 7 the first four curves show an improving ability of the animal to deal with glucose. During that time the animal was perfectly quiet. As the fifth injection of glucose was about to be given the rabbit struggled violently in the box, and, as we discovered afterwards, injured its back. The disturbance did not last above 30 sec., but its effect upon the subsequent tolerance curve is evident.

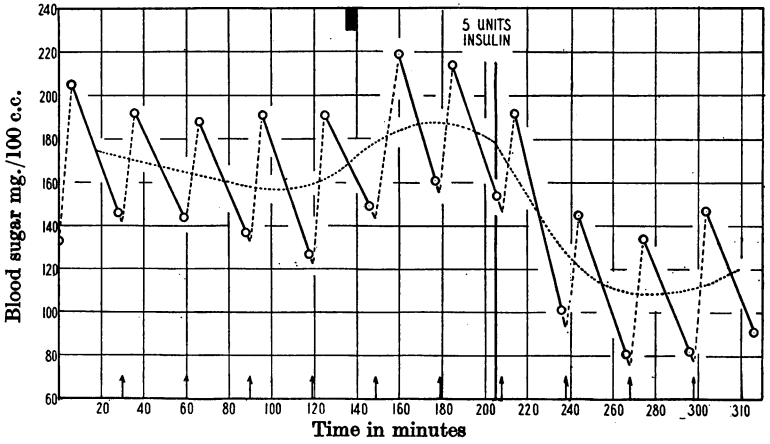

Fig. 7. Consecutive glucose tolerance curves obtained after injection of 1 g. of glucose every 30 min. when the animal was receiving a carbohydrate diet. Times of glucose injection indicated by arrows. During the fifth curve the animal struggled violently and the time of this occurrence is shown by the rectangle. Five units of insulin were given intravenously before the eighth injection and this time is marked on the chart by the heavy vertical line.

Now all animals in which this phenomenon of improving tolerance commenced after the first injection of glucose were receiving a carbohydrate diet. In animals on a fat diet the effect was either not observed or did not appear until several glucose injections had been given. Thus in the upper curves of Figs. 4 and 8 the blood sugar rises progressively higher after each injection of glucose. In the upper experiment shown in Fig. 8 there is no sign of improvement until after the sixth injection. This influence of diet is invariably the same. On a high fat diet not only does the tolerance not improve, but it is at first unequivocally worse after each injection, until finally after several injections a tardy improvement appears.

In the final group of experiments a comparison of the ability of a standard dose of insulin to suppress the hyperglycæmia after the intravenous injection of glucose was made between animals taking the two types of diet. These experiments were the logical sequel of those previously described, in which it was shown that the ability of insulin to

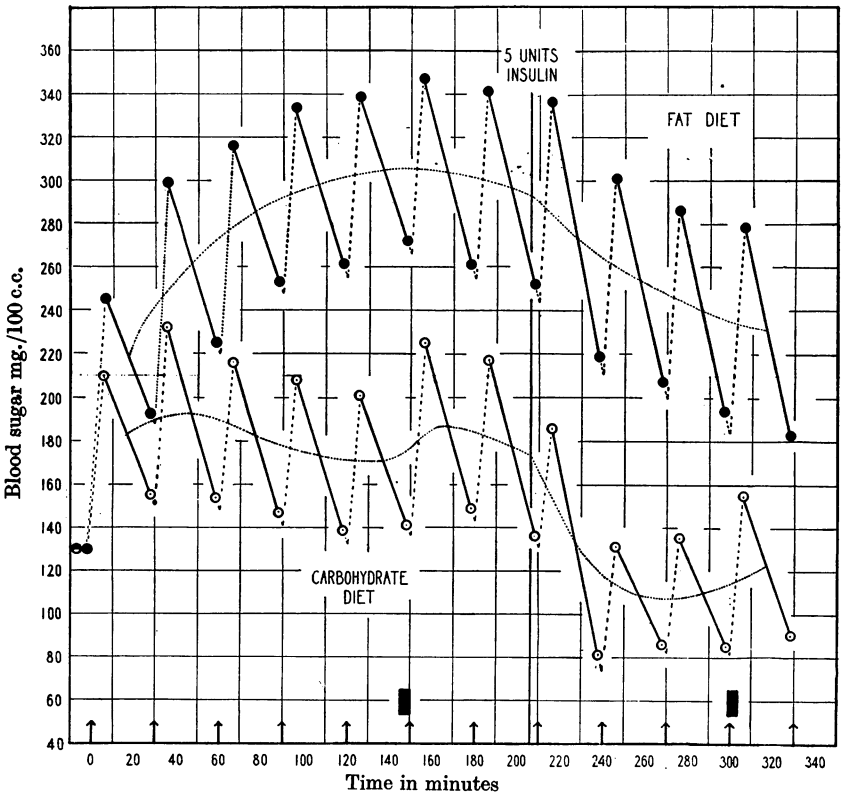

Fig. 8. Two series of consecutive glucose tolerance curves performed on the same animal after injection of 1 g. of glucose every 30 min. The upper curves (discs) were obtained when the animal was receiving a fat diet, the lower (circles) when taking a carbohydrate diet. For each experiment the arrows indicate the times of glucose injection and the heavy vertical lines the times when five units of insulin were given intravenously. The black rectangles indicate periods of struggling during the performance of the lower experiment.

depress the blood sugar was increased after intravenous injections of glucose. It was argued that if insulin were injected during the course of a consecutive glucose tolerance curve, then the increasing susceptibility of the animal to insulin consequent upon the preceding injection of glucose

would be revealed by an increasing ability to suppress hyperglycæmia. The dose of insulin injected intravenously was 5 units and it was preceded either by two, four or seven injections of 1 g. of glucose at intervals of 30 min. Four further glucose injections were given after the insulin and the tolerance curves obtained from these were compared with the curves obtained before insulin. In Figs. 6, 7 and 8 are shown the results of these experiments in which seven preceding injections were given. In Figs. 6 and 7 the animal was receiving a carbohydrate diet, and the marked effect of insulin in suppressing the rise of blood sugar is obvious. In Fig. 8 are shown two experiments of this type obtained on the same animal, the upper when it was taking a fat, the lower when taking a carbohydrate diet. It will be seen that when the experiment was performed during the period of fat feeding, insulin exerted little effect in suppressing hyperglycæmia, but that when the same experiment was carried out in the period of carbohydrate feeding, insulin exerted its effect in a pronounced degree.

Discussion.

The results presented in the previous section can at this point conveniently be summarized as follows.

The fat diet decreases sugar tolerance; retards and diminishes the action of insulin upon the blood sugar; prevents or delays the progressive improvement of sugar tolerance which occurs on injection of consecutive doses of glucose; and impairs the ability of insulin to diminish the hyperglycæmia following intravenous injection of glucose.

The carbohydrate diet improves sugar tolerance; accelerates and increases the depression of the blood sugar by insulin; favours the progressive improvement of sugar tolerance after consecutive injections of glucose; and does not impair the action of insulin in reducing the hyperglycæmia after intravenous administration of glucose.

Reference has been made to observations by previous authors on the effect of starvation upon the sugar tolerance curve and the depression of the blood sugar by insulin. It has been found that sugar tolerance is reduced, insulin action is diminished, and [Du Vigneaud and Karr, 1925] the ability of one dose of glucose to reduce the hyperglycæmia produced by a second is impaired. Thus it appears that starvation produces the same deleterious effect upon the ability of the body to deal with sugar as the giving of a diet with a high fat content.

Various theories have been put forward in explanation of the effects of starvation, administration of glucose, fat diets and carbohydrate diets upon the organism's sugar tolerance, but little attention has been paid

to the effects of the same factors upon insulin action. So far no attempt at correlating the two sets of observations appears to have been made. In the light of the correlation demonstrated by the above experiments the previous theories on these conditioned variations of sugar tolerance become untenable.

The previous theories are of two types, those which attribute the change in tolerance to variations in the reaction of the tissues, and those which ascribe the change to alteration in sensitivity of the insulin-secreting mechanism. Prior to 1925 the "tissue reaction theory" was put forward by workers whose observations had been concentrated on the effect of diet on sugar tolerance, whilst the "theory of varying insulin secretion" was held by those who had more particularly investigated the effect of consecutive doses of glucose upon the sugar tolerance. After this date the latter phenomenon was correlated with the effect of variations in diet, and at the present time the "theory of varying secretion" is the most generally quoted. It is evident, however, that the two hypotheses were not mutally exclusive.

The "tissue reaction theory" will be considered first.

Haldane, Wigglesworth and Woodrow [1924] showed that rapid and temporary changes in tissue reaction, either to the alkaline or to the acid side, influenced sugar tolerance adversely. Du Vigneaud and Karr [1925] give glucose tolerance curves from rabbits after starvation and 18 hours after administration of either a fat or carbohydrate meal. Starvation and a previous fat meal had the usual adverse and a carbohydrate meal the usual beneficial effect on the sugar tolerance. These authors claimed that the administration of sodium bicarbonate before the test neutralized the effect of starvation or ingestion of fat. Their further results, however, did not indicate that this effect was dependent upon change in the *p*H or alkali reserve of the blood. Since this time various authors have investigated this problem in man but the results have been conflicting. My own results on this point [Himsworth, 1933, complete results not yet published] showed that long-continued ingestion of large doses of ammonium chloride or sodium bicarbonate had no effect upon the sugar tolerance and resulted only in a reduction or augmentation of the alkali reserve, which was fully compensated, within the extremes of the normal range of *p*H by changes in the CO_2 tension of the blood. It was possible to show that, in man, the *p*H of the arterial blood remains at the normal level whether a high fat or a high carbohydrate diet is given, that if, on the high fat diet, sufficient alkali were taken daily for ten days to produce a persistent compensated alkalosis, the decreased sugar tolerance

still persisted unchanged, and that if, on the carbohydrate diet, sufficient ammonium chloride were given to produce a persistent compensated acidosis the high tolerance for sugar remained unchanged. It must, however, be understood that it is not suggested that these results exclude the possibility of some change in tolerance being produced by sudden and more extreme variations in *p*H consequent upon single massive doses of acid or alkali, nor that the taking of single large amounts of fat or carbohydrate by an organism adjusted to an ordinary diet may not produce a significant change of the *p*H. It is, however, believed that these results prove beyond doubt that the changes in sugar tolerance consequent on a high fat or a high carbohydrate diet in man are not due to changes in reaction of the tissues.

Hamman and Hirschman [1919], who first described the improvement in tolerance consequent upon ingestion of repeated doses of glucose, merely observed that the phenomenon was due to stimulation of the mechanism which removed glucose from the blood. Traugott [1922] ascribed the improvement to stimulation of liver function. In this year data on the effects of administration of insulin became available, and Foster [1922] attributed the greater tolerance seen after a second dose of glucose to the effect of insulin secreted in response to the first dose. Thalheimer *et al.* [1926] showed that continuous injection of glucose, at a uniform rate, resulted in a rise of blood sugar which was followed by a steady decline to normal or subnormal levels, and they also ascribed this result to the secretion of insulin consequent upon the continuous stimulation of the islet cells by the inflow of glucose. In 1927 Sweeney published his paper on the effects of starvation and of fat, protein and carbohydrate diets upon the sugar tolerance. He definitely regarded the beneficial effect of carbohydrate as being due to an increasing sensitization of the insulin-secreting mechanism to changes in the blood sugar, as a result of the repeated stimulus of excessive carbohydrate consumption, whilst the adverse effect of fat and of starvation he attributed to the desensitization of the same mechanism consequent on the absence of this stimulus. Macleod [1930] endorsed this explanation and said "...we may imagine that when there is abundance of pre-formed carbohydrate (free sugar and glycogen) entering the circulation, the islets of Langerhans are stimulated to produce larger amounts of insulin than usual, and to secrete it more abundantly, in response to temporary increases in blood sugar."

We shall now discuss this theory in the light of our results. In any of the consecutive glucose tolerance curves on the carbohydrate diet the

improvement in tolerance can be seen after each dose of glucose (Figs. 4, 6, 7). This improvement is slight, but when we compare these curves with those obtained on the fat diet (Figs. 4, 8), it is evident that the impressive thing is not that the improvement after glucose is small in degree, but that, after each glucose injection, a great decrease in tolerance does not occur. It is evident that to prevent such a decrease a mechanism of considerable power is brought into play more strongly on the carbohydrate than on the fat diet. In contrast with the results on a fat diet the improvement seen on the carbohydrate diet though slight is significant.

It has been shown that insulin exerts a greater effect on the blood sugar when it has been preceded by an injection of glucose. Glucose injection, therefore, results in the production of a state of increased susceptibility to insulin, *i.e.* each unit of insulin on injection has, actually or apparently, become more active. Zunz and la Barre [1927] have shown that hyperglycæmia is an adequate stimulus for the secretion of pancreatic insulin. If we now consider the phenomenon of improving tolerance after consecutive doses of glucose in animals on a carbohydrate diet (Figs. 4, 6, 7), we see that after the first dose of glucose at least two processes must occur. Insulin is secreted in response to the hyperglycæmia but, in addition, there is produced some degree of the state of increased susceptibility to insulin. These two factors combine to reduce the blood sugar. Let us suppose that the curve is allowed to return to rest at the original level. The experiments with insulin demonstrate that the susceptible state persists and, therefore, the pancreas must now be secreting less insulin than it did before glucose was given, otherwise, the animal being more susceptible, the blood sugar would sink and remain at levels considerably lower than the original. Neither in rabbits nor man does this occur. The second dose of glucose is now given. Insulin is again secreted in response to hyperglycæmia but, owing to the animal being more susceptible, each unit of insulin secreted now exerts a greater effect than in the preceding curve. Thus less insulin will be required to bring the blood sugar back to normal. But it has been found that the degree of susceptibility increases with each subsequent injection of glucose up to at least eight injections. If, after four injections, the blood sugar is allowed to return to rest, it will be found that it remains steady within a few mg. per 100 c.c. of the original level, and yet, by direct experiments, it has been demonstrated (Fig. 5) how much more susceptible the animal now is to insulin. The secretion of pancreatic insulin now required to maintain the blood sugar at this level must be very much less than was required in the pre-experimental state. These deductions indicate that, not only is the current

explanation of increasing insulin secretion after each consecutive injection of glucose untenable, but that any explanation which does not recognize that insulin is secreted in decreasing amounts after each dose of glucose is equally incompatible with experimental results. It would appear then that insulin plays a decreasingly important part in reducing the blood sugar in the consecutive glucose tolerance curves, and that in such experiments the improving tolerance, or absence of decreasing tolerance, is mainly due to an increase in the organism's susceptibility to insulin.

This conclusion is accentuated by the results of experiments in which consecutive tolerance curves were performed on animals taking a high fat diet (Figs. 4, 8). In the upper experiment of Fig. 8 it will be seen that first the tolerance decreases and then that an improvement sets in after the sixth injection of glucose. Just before the eighth injection five units of insulin were given intravenously. A very slight effect on the tolerance resulted. This result is strikingly different from the same experiment carried out on the same animal when receiving a carbohydrate diet (lower experiment, Fig. 8) (cf. also Figs. 6, 7). Whatever was lacking in the animal on the fat diet which the same animal on the carbohydrate diet possessed, it is evident that this was not insulin. The animal on the fat diet was practically insusceptible to insulin. It is worthy of note that almost certainly this result was not due to the presence of adrenaline. Fig. 7 shows the effect of an injury on the sugar tolerance. The injury was probably followed by secretion of adrenaline which caused the subsequent diminution in tolerance, yet in this experiment five units of insulin exerted its usual marked effect (cf. Fig. 7). We can only conclude that the impairment of sugar tolerance shown on the fat diet is due to the animal's diminished susceptibility to insulin and its impaired ability to develop this state in response to administration of glucose. This conclusion is fully endorsed by those experiments in which the action of insulin on the blood sugar was directly compared in the same animal under conditions of fat and of carbohydrate feeding (Fig. 3).

The idea derives further support from the results obtained by various authors when investigating the effects of starvation. Dann and Chambers [1930] have shown, in dogs, that after a 3 weeks' fast the administration of 50 g. of glucose results in hyperglycæmia and glycosuria; that in normal animals sugar causes a definite rise of R.Q., but that in the starving ones the same amount of glucose produces practically no change from the resting value. In an attempt to compensate for this suppression of the ability to oxidize carbohydrate, large doses of insulin

were given, to the starved animals, along with the first dose of glucose. A rise of R.Q. of normal magnitude was not obtained; even enormous doses of insulin could not restore the normal specific dynamic action of glucose. The missing factor was evidently not insulin. It was found, however, that administration of 50 g. of glucose on consecutive days produced a progressive improvement of the resulting sugar tolerance curve so that, on the fifth day, this had returned to normal. This improvement was paralleled by a gradually increasing respiratory response until, on the fifth day, 50 g. of glucose again produced the normal rise of R.Q. Thus, by exactly the same means that had been shown to increase the sugar tolerance and susceptibility to insulin, the normal metabolic response to glucose had been restored. It is of interest in this connection that Baur [1929] showed in men that, after 85 g. of glucose, the oxygen intake was higher after a carbohydrate rich diet than after a carbohydrate poor diet or fast. It appears justifiable to conclude that, in starvation, the decreased sugar tolerance, the impaired effect of insulin on the blood sugar, the small improvement in tolerance after successive doses of glucose, and the impairment of the specific dynamic action of glucose, are all dependent upon the inefficiency of one common mechanism, the mechanism which is responsible for the development of the state of susceptibility to insulin.

Our final conclusion, therefore, after a consideration of the effects of starvation, fat diets, glucose administration and carbohydrate diets is, that the varying ability of an animal to deal with carbohydrate characteristic of each of these conditions is not due to a variation in response of the insulin secreting mechanism, but is due to a change in the animal's susceptibility to insulin. The mechanism behind the development of this susceptible state remains unknown, but it would appear justifiable to suggest that it is probably intimately connected with insulin action. Two simple possibilities present themselves. The diminished susceptibility following starvation or the taking of a fat diet may be due to the production in excess under these conditions of an inhibitor of insulin. The increased susceptibility consequent upon the administration of glucose or carbohydrate diets may result from increased production of an activator of insulin, it being postulated that insulin is prepared and as secreted by the pancreas is an inactive precursor of that substance which, in the body, produces the effects we recognize as following injection of insulin. It will be seen that both possibilities may also co-exist, each exerting its influence on the phenomenon under observation in proportion to its concentration in the body at that time. In previous papers [Himsworth,

1932, 1933] other evidence has been brought forward in favour of the activation hypothesis, and it will be seen that all the experimental results recorded in this communication can be explained on the basis of an activation of insulin, an activation in which the speed is dependent upon the quantity of activator which has been transferred to the tissues in proportion to the stimulus of carbohydrate administration.

Summary.

1. Experiments are recorded showing:

(*a*) That the administration to a rabbit of a diet rich in fat decreases the sugar tolerance; retards and diminishes the action of insulin upon the blood sugar; prevents or delays the progressive improvement of sugar tolerance which occurs on injection of consecutive doses of glucose; and impairs the ability of insulin to diminish the hyperglycaemia following intravenous injection of glucose.

(*b*) That the administration of a diet rich in carbohydrate improves the sugar tolerance; accelerates and increases the depression of the blood sugar by insulin; favours the progressive improvement of sugar tolerance after consecutive injections of glucose; and does not impair the action of insulin in reducing the hyperglycæmia after intravenous administration of glucose.

2. From the results of other workers it is shown that starvation produces the same effect on glucose tolerance and insulin action that we have demonstrated as characterizing the high fat diet. The diminished specific dynamic action of glucose found after starvation and after a high fat régime is brought forward as another point of similarity.

3. The current theory that the diminished tolerance after starvation and the taking of a fatty diet is due to a diminished sensitivity of the insulin secreting mechanism, and that the improved tolerance after glucose injection and administration of a carbohydrate diet is due to increased sensitivity of the same mechanism, is shown to be incompatible with the observed results.

4. It is concluded that these results can be completely explained on the basis of the experimental observation that fat diets and starvation diminish the susceptibility of an animal to insulin, whilst carbohydrate diets and administration of glucose increase the susceptibility to insulin.

5. It is suggested that the susceptibility of an organism to insulin can be explained by the hypothesis that insulin as prepared and as secreted by the pancreas is an inactive substance, that it is activated in the body by an

unknown factor and that the production of this unknown activator is increased when carbohydrates are given, and diminished when they are withdrawn.

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