THE DETERMINATION OF GLUCOSE TOLERANCE

BY

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Historical introduction

One of the earliest examinations of the response of the human organism to the administration of sugar was made by Worm-Müller (1884), who published observations on the alimentary glycosuria of two healthy men. He found that these subjects, after being for some time on a diet very low in carbohydrate, showed a detectable glycosuria when given 50 gm. of glucose or sucrose or 100 gm. of lactose. This work was followed by that of Hofmeister (1889), who used dogs. His paper has the greater interest because it introduces the terms 'assimilation limit' and 'tolerance.' The former was used to imply the highest dose of sugar that the individual could take without showing glycosuria, while the latter was used, in speaking of certain diabetics of the milder type, to denote that dose a small increase upon which would produce glycosuria. Hofmeister did not, however, preserve any rigid distinction between the terms, and in fact, as Sansum and Wilder (1917) pointed out, what are really assimilation-limit determinations were for many years freely interpreted as showing limits of tolerance. He found that the assimilation limit for a given animal was constant, but that from one animal to another there was great variation. His work is also of importance at the present day for his clear recognition of 'hunger diabetes,' a phenomenon which Claude Bernard had encountered in dogs long before and which was, of course, implicit in Müller's work.

Seeking a measure of the assimilability of sugars, Linossier and Roque (1895) proposed the ratio $\frac{\text{sugar excreted}}{\text{sugar given}}$; but they obtained figures varying very widely even for the same sugar. Gilbert and Carnot (1898) showed a fairly constant ratio, however, lying between 40 per cent. and 100 per cent. when large doses (2.5–10 gm./kgm.) were given intravenously. Doyon and Dufourt (1901) found that the proportion of sugar excreted was largely dependent upon the time taken for administration, and following closely upon

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this work came that of Blumenthal (1905), who emphasized that the true conception of tolerance was not the static one, of a quantity which could be dealt with by each kgm. of tissue, but rather the dynamic one, of a quantity which could be so dealt with in each unit of time.

This fundamental idea made it necessary to have control of the rate of entry into the circulation ; and such control obviously demanded intravenous administration. It is a matter of some surprise that this point, made so very clear more than thirty years ago, has not yet been accorded its due weight in clinical practice.

Working on rabbits, Blumenthal found that a rapid injection of glucose amounting to about 0.85 gm./kgm. was tolerated without glycosuria ; but that if the dosage were either increased above this figure or repeated after too brief an interval, glycosuria occurred. He then approached an estimation of sugar tolerance as a velocity by establishing that a dose could be selected which, if given rapidly, could be repeated indefinitely at fifteen-minute intervals without the production of glycosuria. For rabbits, this dose lay between 0.6 and 1.2 gm./kgm.

Any method depending on the production of glycosuria must, as Maclean (1921) pointed out, be subject to a great source of error in the widely varying response of the kidneys to hyperglycaemia in different individuals and in different states of health and disease. For this reason, no great advance in clinical work on this subject was possible until Bang's publication (1913) of the first satisfactory method for the chemical estimation of blood sugar. This led at once to the great activity of the past twenty-five years, during which many workers have proposed tests of sugar tolerance based upon the estimation of the blood sugar after giving a test dose of sugar. The routes of administration have included the oral, the intravenous, and even the subcutaneous. The last is open to such obvious theoretical and practical criticism, however, particularly on the grounds of pain and sepsis, that it seems hardly necessary to consider it further.

General considerations

Before proceeding to discuss the oral and intravenous glucose tolerance tests, it will be convenient to consider certain factors of importance in the conduct of any test of tolerance, all concerned with the preparation and management of the patient and for the most part well recognized.

(a) **Rest.**—It was shown by Comessatti (1906), using Blumenthal's methods, that the tolerance of rabbits was improved by exercise in a treadmill, while Loeb and Stadler (1914) showed the converse to be true of resting rabbits. This result, subsequently fully confirmed in the human subject, indicates a difficulty in standardizing conditions. It is in practice satisfactory, however, that the patient remain in bed for the test after the preceding night's rest.

(b) **Preceding diet.**—Difficulties in this connexion were foreshadowed by Bang's recognition in 1913 that glucose tolerance was lowered by fasting and by Hamman and Hirschman's discovery (1919) that a second glucose tolerance curve was always lower than a shortly preceding one. These two observations

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have been the inspiration of a vast amount of work, well summed up in Hims-worth's comprehensive papers (1933-4-5). The conclusion emerges that the glucose tolerance of a healthy individual is directly proportional to, and is solely dependent upon, the amount of carbohydrate the diet has contained during the few days preceding the test. In practice, a patient whose tolerance is to be tested should, as far as possible, have taken a normal diet for three to five days. If that is not possible, it is important that the diet actually taken should be constant and well known, so that the results obtained may be adequately discounted. The importance of this matter may be gauged from the three oral glucose tolerance curves shown in fig. 1, which were derived from the same healthy child on normal, high-carbohydrate, and low-carbo-

FIG. 1.—Three oral glucose tolerance curves (30 gm.) obtained from a normal boy on normal (solid line), low carbohydrate (dashed line) and high carbohydrate (dotted line) diets.

FIG. 2.—Dietary effects upon oral glucose tolerance curves. Upper curve (solid line) from a case of clinically occurring hunger diabetes, on admission with glycosuria. Dashed curve, from same case after a period on hospital diet with 50 gm. glucose added daily. The lower solid line shows the result of giving a normal boy 60 gm. of glucose daily in addition to ordinary diet.

hydrate diets. Two instances of extreme type are shown in fig. 2. The flat curve is the result of giving a normal boy of thirteen years 60 gm. of glucose daily, in addition to his normal diet, for several months. The high curve was obtained from an otherwise healthy boy of twelve years, who, by reason of unfortunate home conditions, was inadequately fed. It was rendered normal in form (dotted curve) by a period on hospital diet with 50 gm. of glucose added each day. This case was, in fact, one of 'hunger diabetes,' for the boy was sent to hospital for the investigation of an occasional glycosuria, which was never seen after the diet was corrected.

(c) **The period of fasting.**—The blood sugar normally shows fluctuations due to the intermittent intake of food (fig. 3), and obviously it would be confusing to conduct a test during the absorptive or immediate post-absorptive period. Hence, it is desirable to allow about five hours to elapse after a meal

before conducting a test. Further, as the distribution of carbohydrate among the three or more meals may be quite uneven, it is better still to carry out the test after the longer night fast, which serves excellently as a period of equilibration as well as standardizing the conditions with regard to exercise. Thus has arisen the usual and satisfactory practice of performing tolerance tests fairly early in the morning, the patient having fasted from the previous evening meal.

(d) **The emotional state** of the patient is of great importance, particularly in children. Pain or apprehension can easily lead to a rise of 50 mgm. per cent. or so in the blood-sugar level, and any procedure must be planned to exclude this factor as far as possible. The curve in fig. 4 shows the fasting blood-sugar level in a child of two-and-a-half years, who had a clean, granulating wound in need of dressing. The specimens up to 20 minutes were taken under normal conditions, after which the cot was wheeled into a dressing ward, causing the child to whimper apprehensively. The dressing was performed (it must have been practically painless) and the cot returned to its accustomed place. By

FIG. 3.—Diurnal variation of blood-sugar in a normal subject taking three meals daily, at 6 a.m., 12 noon, and 6 p.m.

FIG. 4.—Fasting blood-sugar level of a child before, during and after emotional disturbance (see text).

45 minutes the child was asleep and the 50-minute specimen shows a return of the blood sugar to its previous level.

(e) **Infection.**—When it is not itself the subject of investigation, sepsis may produce disturbing effects upon tolerance curves. These effects have been so widely discussed as to call for no comment, beyond pointing out that even a heavy cold is sufficient to give a misleading result, especially in the case of the intravenous test.

Oral glucose tolerance tests (blood sampling)

The first observations of the blood sugar at intervals after the ingestion of a test dose were those of Bang (1913) and his co-workers. In the next few years a number of papers appeared dealing with such tests; but the widespread use of the procedure, at least in the English-speaking countries, seems to date from the appearance of Maclean's method of estimating blood sugar (1919) and his well-known paper (1921) on the estimation of glucose tolerance. Since that time many technical variants have been employed, some of little importance, while others will be more conveniently discussed in dealing with special aspects of the test.

Dosage.—Great variation is found in the bases adopted by various workers.

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Many use a fixed dose, while others vary it in proportion to the patient's weight. This variation according to weight (except in the widest sense) does not possess its apparent virtue ; for there can be little reason to think that equal weights of thin and obese, well and ill, normal and endocrinely disordered, active and inactive patients will be metabolically equivalent. Further, as Maclean (1921) pointed out, the hyperglycaemic effect of doses varying over a wide range is to

FIG. 5.—Oral glucose tolerance curves from three normal children using different doses of glucose, namely, 1 gm./kgm. (dotted line), 1.5 gm./kgm. (solid line) and 3 gm./kgm. (dashed line).

FIG. 6.—Two oral glucose tolerance curves derived from the same individual with doses of 1.4 gm./kgm. (solid line) and 2.8 gm./kgm. (dashed line).

all intents the same, except perhaps for some slight delay in the return to normal levels after a large dose. The curves in fig. 5 illustrate this in three different cases, while those in fig. 6 were obtained from the same case. It is interesting that excessive dosage tends apparently to delay the fall of the curve rather than to raise or advance its peak—a fact which is less surprising in view of the more

FIG. 7.—Four oral glucose tolerance curves from normal children, showing failure to return to fasting level in 2 1/2 hrs.

modern knowledge of absorption and its complex nature. In many hands it has proved satisfactory to give 50 gm. for adults, while for children under 12 years a dose of 30 gm. has been used, reduced to 15 gm. if the weight is less than 10 kgm. This dosage tends to be high for small patients, and the fasting level is often not quite reached by two-and-a-half hours (fig. 7).

Strength of solutions.—This is an important point, for concentrations exceeding 25 per cent. are sometimes productive of nausea and erratic curves, while a large bulk of a rather unpalatable solution may even cause vomiting. The use of a 20 per cent. solution has usually provided a satisfactory compromise. The medium is, of course, flavoured water. Normal saline should not be used, for it markedly reduces the height of the curves (fig. 8), presumably by interference with absorption.

Frequency of sampling.—From the fact that elevation of the blood sugar usually begins within some five minutes of ingestion of the dose and reaches a peak after only twenty to sixty minutes, frequent sampling is desirable if an accurate conception of the early phases is to be obtained. In practice, there is some difficulty in collecting such large specimens as are ordinarily used, and half-hourly specimens are made to suffice. Intervals of twenty minutes during the first hour, and of half an hour thereafter, have been used in the present investigations. More frequent and yet accurately timed specimens are easily

FIG. 8.—Two curves from the same normal patient, water (solid line) and normal (0.9 per cent.) saline (dashed line) being used as the solvent.

obtained, however, for estimation by the ultramicro method of Rappaport and Pistiner (1934) which will be described in some detail when the intravenous test is discussed.

Site of sampling.—The blood is usually obtained from a finger or ear-lobe puncture, though some workers, especially in America, use venous blood. Foster's work (1923) showed that the blood of the warmed pad of the dog's foot has practically the same sugar content as arterial blood; and Goldschmidt and Light (1925) showed that blood taken from the veins of the warmed hand and from the arteries had an identical oxygen content. These two observations seem to justify the use of capillary blood, taken under good conditions, to represent arterial blood. Further, Himsworth (1933) checked the glucose content of blood from finger-pad and ear-lobe and found substantial agreement. Particularly in children the ear-lobe is the site of election, for small specimens at least. A satisfactory puncture made in this situation with a good Franck's lancet is practically painless and can be used repeatedly to obtain samples of 0.02 c.c.

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The normal curve.—This is so well known as to require little or no description. It represents the changing resultant of two opposing influences, first, the tendency of the sugar entering by the portal vessels to raise the blood-sugar level, and secondly, the capacity of the body to remove sugar from the blood, thus tending to lower the level. The curve rises from a normal fasting value to a maximum, usually just below the renal threshold—say 150–170 mgm. per cent.—in half to one hour. Thence it falls in one-and-a-half to two-and-a-half hours to a few mgm. below the fasting level (unless the dose has been very large) to which it gradually returns (fig. 9, double line).

Grotesque curves.—Thus there are two aspects to the production of a normal curve—absorption into the blood and removal from it. It has been usual to

FIG. 9.—Some types of oral glucose tolerance curve : (a) normal (double line) ; (b) uncontrolled diabetic (-----) ; (c) controlled diabetic (solid line) ; (d) 'lag' (x-x-x-x) ; (e) flat (dashed).

fix attention upon the latter rather to the exclusion of the former ; that this position is far from correct will be suggested by the latter part of this paper. Since there is no, or practically no, absorption of sugar from the stomach, the first requisite for efficient absorption is normal gastric emptying ; while the second is normal function of the small intestine as regards both motility and the actual process of absorption. From time to time a number of grotesque curves have appeared in the literature, either quite flat, or flat for a time and then rising to a great height, or bifid. Elsewhere (Ross, 1938) I have published a series of three such curves derived from one patient within a short period in which no clinical change occurred, with the suggestion that they are of gastric origin. Certainly such curves, unsupported by other methods of investigation, do not offer a safe basis for any conclusion regarding tolerance. They should rather be the indication for further investigation by other methods.

Abnormal curves.—These conform to three main types, two higher and one

lower than the normal. The former are the well-known 'diabetic' and 'lag' curves, the latter the 'flat' type (fig. 9). The diabetic curve rises from an abnormally high fasting level, if the case is uncontrolled, or from a relatively normal one if insulin is being used, to a high level, whence it falls slowly and slightly. The lag curve rises from a normal or slightly high level to an unusual, often glycosuric, peak, and thence falls quite rapidly back to, or nearly to, the fasting level.

Both of these types have been accepted as indicating that sugar has entered the blood normally, but has been removed thence at an abnormally slow rate. In the lag curve this slow rate of removal has later been accelerated by stimulation of the body mechanism, by which pancreatic activity is usually implied. That pancreatic adjustment is a fact was classically demonstrated by Zuntz and la Barre (1927). But there is reason for doubting whether this is the only factor involved. Great interest has been taken in these curves for many years yet the number of reported cases in which the subjects have gone on to frank pancreatic diabetes is small. Much more usual is the experience of Hunt

FIG. 10.—Three types of low oral glucose tolerance curve : (a) rounded type (solid line) ; (b) flat and non-falling type (dashed line) ; (c) low curve of normal form (dotted line).

(1937), no one of whose nineteen cases had returned in a 'diabetic' condition. Further, dietary restriction can produce curves of this type (fig. 2), as also can sepsis and hepatic disease (unpublished cases). It is worth while to give every patient who shows a lag curve a high carbohydrate diet (say 300 gm. per diem for an adult), and repeat the test after a week. If then the curve is not normal or improved, the presence of liver disease or of some unobtrusive infection must be carefully considered.

Abnormally low curves have received, on the whole, much less attention. They fall, so far as they have been observed in this series, into three distinct types :

(a) A curve generally similar to the normal, but showing a smaller rise, often with a delayed peak or with no peak at all (fig. 10, solid line) and returning slowly to fasting level.

(b) Curves are occasionally found which approximate to a straight line, the blood-sugar level rising very slowly and very slightly and falling little, if at all, during two-and-a-half hours (fig. 10, dashed line). In the example shown, the maximal rise is 15 mgm. per cent. and the fall from this only 5 mgm. per cent.

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Both of these types have been seen in conditions of impaired absorption (Ross, 1935, *a*, *b*).

(*c*) The last type of 'flat' curve is seen, in contrast to the two already mentioned, in patients whose tolerance is excessive. In this type a small rise is followed by a greater fall, with later restitution (fig. 10, dotted line; fig. 2, lower solid line).

Obviously, accurate interpretation of these low curves is not possible without further information than the curves themselves provide; for they could result from failure of glucose to reach the intestine (gastric stasis), failure of absorption (disease states or acute intestinal hurry), or unduly rapid removal of sugar from the blood (hypertolerance). The first of these fallacies has been freely discounted, especially since Maclean's opinion was given (1921) that absorptive

FIG. 11.—Two curves from a case of coeliac disease: (*a*) early in the period of onset (solid line)—a high curve: (*b*) at a more advanced stage (six weeks later), with definite symptoms (dashed line)—a flat curve.

irregularities were not likely to influence results significantly. Later work, however, does not support that view.

A further difficulty exists, to which attention has previously been drawn (Ross, 1935, *b*). This is the ambiguous character of the 'normal curve.' As has been seen, this curve results from the absorption of sugar followed by its removal at a rate indicative of the individual's 'tolerance.' Now, when absorption is impaired, less sugar will enter the blood (tending to give a lower curve); but on the other hand, the glucose-tolerance will be impaired (for bad absorption must have the same effect as carbohydrate deprivation, and it is known (Himsworth, 1935) that this leads to impaired tolerance). Hence there is one factor making for a low curve, another for a high one. The actual resultant depends merely on the degree and duration of the absorptive disability. It is for this reason that cases of absorptive disease (e.g. coeliac disease and abdominal tuberculosis) are found which show almost normal oral curves, although the intravenous test shows their tolerance to be grossly impaired. An opportunity was afforded of watching one child during the development of coeliac disease. At an early stage, when there was no symptom of note beyond

listlessness and failure to gain weight, a fairly high curve (solid line, fig. 11) was obtained. Some six weeks later, when the condition was definitely established, a flat curve was found (dashed line). The reverse sequence of events has been noted by Fairley (1936) during the cure of sprue. Patients who have shown flat curves during the acute stage, on partial recovery show high ones, which then subside to normal when health is fully restored.

It will be seen, therefore, that when a 'lag' or a 'diabetic' curve is obtained, the oral test may be taken as sufficiently accurate for practical purposes. But when in the presence of symptoms low or normal curves are found, it is necessary to turn to other methods, and particularly the intravenous glucose tolerance test, for elucidation.

Two-dose tests and other such variants will not be discussed here, for although they are by no means without significance, they are in theory more closely germane to another paper which is in preparation.

The intravenous glucose tolerance test

The first study of the injection of sugar into the veins which has been discovered is that by F. J. von Becker (1854), who observed the glycosuria produced in rabbits by this means. Clinical use was not made of this route, apparently, until 1913, when Thannhauser and Pfitzer published some curves obtained by giving injections of 7 per cent. glucose solution, and observing the course of the blood sugar. They had to give a large volume of solution, usually some 500 c.c., and hence took fifteen minutes to make the injection. In 1915 Woodyatt, Sansum and Wilder felt the necessity of eliminating the unknown absorptive factor by using the intravenous route; but they also sought to avoid the 'wave' effects of Blumenthal's technique—to apply a perfectly even stress to the sugar-removing mechanism. They therefore devised a finely-regulable motor-pump capable of delivering a continuous and positively governed flow.

As normal subjects they used only healthy persons from twenty to forty-five years of age, in an average state of nutrition, who had been on a mixed general diet. On the day of the test no food was given, and injection commenced at 2–4 p.m. The initial rate of 0.7 gm./kgm./hr. was increased slightly each twenty to thirty minutes until glycosuria appeared. The normal tolerance rate thus determined was found to lie between 0.8 and 0.9 gm./kgm./hr.

This continuous method, however, is quite unsuited to clinical practice, and, like earlier and far cruder tests, it still embodied the unknown factor of renal function. Further, the test itself must have had the same effect as a high carbohydrate diet in influencing the tolerance as finally determined, i.e. it could not easily reveal impairment of tolerance due to acute dietary privation. Conversely, of course, it had the virtue of eliminating to some extent errors due to diet when other influences were being investigated.

It is not surprising, then, that later methods have nearly always been modifications of that of Thannhauser and Pfitzer (1913), an injection of glucose solution being given fairly rapidly and the blood sugar being estimated immediately before and at stated intervals after the injection.

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Some twenty-three of the early tests having been reviewed by McKean, Myers and von der Heide (1935), it is not necessary to recapitulate them in detail. The points in which variation has occurred, however, are dosage, strength of solution, time of injection, frequency of sampling, duration of observation and the actual method of estimation of blood sugar ; and each of these calls for some discussion.

Dosage.—The same arguments have been advanced for graded dosage in this test as in the oral one ; and much the same objections lie. It is obviously necessary to select a dose which will be safe and yet adequate to stress the sugar-removing machinery. Sansum and Wilder's figure gives an indication

FIG. 12.—Showing the areas of intravenous glucose tolerance curves obtained in health and disease using varying dosages of glucose. All points to the left of the vertical line (under 3,500 mgm. minutes) were derived from normal cases ; all those to the right are from cases of disease taken at random from various groups—coeliac disease, abdominal tuberculosis, liver disease (acute and chronic).

of what such a dose might be for a test designed to last about an hour—namely, something less than 1 gm./kgm. Jorgensen and Plum (1922) used a 20-gm. dose with considerable success. Hence in beginning work on children, I tried doses of 5 gm. up to 10 kgm., 10 gm. up to 30 kgm., and 20 gm. for bigger children or adults. This grading of dose was not intended in any sense to give a strict approximation to a weight basis, but merely to render the injections safe and convenient. That such an arbitrary method was justified is shown by fig. 12. This is designed to show the influence of dosage upon curves obtained from normal and abnormal subjects. The intravenous glucose tolerance curves are measured as shown in fig. 13 by means of a planimeter. The area measured is bounded by a vertical line at two minutes, the horizontal through

the fasting level, and the curve itself, with if necessary a vertical at sixty minutes to join the curve to the horizontal when they have not met. The areas so measured are expressed in mgm. mins.—i.e. the product of the mean elevation of blood sugar above fasting-level in mgm. per cent., and the time in minutes for which it persisted (up to one hour). This method of measurement is not intended to confer upon tolerance curves the suggestion of an accuracy which they inherently lack, but merely to render easier a comparison, in terms of tolerance, of curves differing in form. It will be seen from a study of fig. 12 that in normal subjects there is little dispersion of the tolerance areas with variation in dose, though in the abnormal cases there is some tendency to this. In fact, there is here a suggestion of Allen's famous 'paradoxical law'—the limits of tolerance in the normal cases (and within the limits of dosage shown)

FIG. 13.—Showing the mode of measuring intravenous glucose tolerance curves (see text).

seem to be virtual only ; while in the abnormal they tend to be actual. The possibility of producing such a diagram seems to dispose of any need for close standardization of dosage. Fig. 14 shows two actual normal curves obtained from different subjects with widely differing dosage (0.38 and 0.95 gm./kgm. respectively), while the two curves in fig. 15 were obtained from the same normal child using 0.5 gm. and 1 gm. per kgm. It will be seen that the curves are a little irregular, and this may be attributed to the child's extreme nervousness.

Strength of solution.—Since there is a definite limit to the speed with which fluid can safely be put into the veins, it is necessary to keep the volume of the test-dose low if rapid injection is to be made. On the other hand, concentrated solutions are not without danger, and, even short of serious mishap, may occasionally cause considerable, if temporary, vasomotor disturbance, as seen in alternate flushing and blanching of the face and neck. The compromise of

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using 20 per cent. solutions in normal saline has been successful, in that the appropriate dose can usually be given in one minute, always in two minutes, and vasomotor disturbance is rarely seen. Normal saline is the only solvent

FIG. 14.—Two normal intravenous glucose tolerance curves, obtained with widely varying dosage : (a) 0.38 gm./kgm. (solid line) ; (b) 0.75 gm./kgm. (dashed line). These curves are from two different subjects.

FIG. 15.—Two intravenous glucose tolerance curves obtained by giving 5 gm. (0.5 gm./kgm., dashed line) and 10 gm. (1 gm./kgm., solid line) of glucose to a normal child.

employed. It is well to point out that Crawford's statement (1938*a*) that water was used has since been corrected (1938*b*). In many hundreds of such injections there has been no mishap.

Purity and sterility are essential in the preparation of solutions. Use has been made of glucose fulfilling the requirements of the U.S.P. X, analytical reagent standard sodium chloride and water twice distilled over glass. The solutions were finally autoclaved in their plugged flasks. Under these conditions, there have been no reactions whatsoever.

The injection.—As has already been seen, this must be accomplished painlessly and without emotional disturbance of any kind. In dealing with children it is therefore necessary to gain the patient's confidence and to have skilled nursing assistance. A fasting specimen having been taken, the skin overlying a suitable vein is carefully anaesthetized and the vein exposed through a small oblique incision. A graduated funnel, rubber tubing and cannula are employed a little normal saline being run in to prove that the flow is free. The appropriate dose of glucose solution is evenly run in, due allowance being made for the dead space between the zero mark on the funnel and the vein. It may at first be thought impossible to achieve all this without disturbing the patient ; but with care and use the procedure is quite practicable. Control injections of normal saline alone have been made, and it was found that the incidental variation in blood sugar is not more than about ± 10 mgm. per cent. and over a period of a few minutes only. In children and nervous adults a needle method should not be used unless the vein is so large that a local anaesthetic can be employed without prejudicing the success of the injection. By the use of this simple gravitational method, with full visibility, it is not difficult to make the injection at a fairly steady rate and in a suitable time.

Time of injections.—Since the object of the test is to observe the ability of the body to remove sugar from the blood, it is theoretically desirable to create instantly a state of maximal hyperglycaemia. This, of course, is not practicable, but with the dosages and solutions mentioned it is usually possible to observe a standard injection time of one minute, which gives a fair approximation to this. It is probably unimportant if perchance the injection takes as long as two or even four minutes ; but longer periods—of ten or fifteen minutes—may lead to difficulty in interpreting certain ' humped ' types of curve (to be described later), even if these are ever seen in such circumstances. It is, perhaps, desirable to make it very clear that feats of rapid injection, which are highly dangerous and never necessary, are not advocated.

Frequency of sampling.—The hyperglycaemia produced by the injection is usually maximal at once, and there follows a more or less rapid fall, the fasting level being attained in about one hour. It is therefore necessary to collect specimens at short intervals during the first part of the curve, in order to follow the rapid changes adequately. For this reason, specimens have been taken at 2, 4, 6, 8, 10, 15, 20, 30, 40, 50 and 60 minutes after the injection. In point of fact, as will emerge from what follows, such frequent sampling is unnecessary for diagnostic work of the ordinary kind when familiarity with the test has been attained.

Duration of observation.—With the test described, this should be about one hour—more perhaps as a matter of interest—since in the normal case the fasting level is re-attained at about one hour. A further half hour will be

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necessary if it is desired to place emphasis on the time taken to return to fasting level ; but that point is probably less significant than the area and form of the curve.

Blood-sugar estimation.—It is not proposed here to discuss the relative merits of the various well-known methods of estimating the blood sugar, but to draw attention to the excellent ultramicro method—using only 0.02 c.c.—described by Rappaport and Pistiner (1934). Some such method is desirable in view of the necessity for small and accurately timed specimens in the intravenous test. As the original publication was made in German and in a journal which is not very generally available except to specialists, it will be useful briefly to recapitulate it, giving only such detail as is practically necessary.

The method is an adaptation of the ferricyanide reduction method of Hagedorn and Jensen. The specimen (0.02 c.c.) is conveniently collected in a Haldane haemoglobinometer pipette, and is immediately expelled (the pipette being washed out three times) into 1 c.c. of N/50 sodium hydroxide, freshly prepared by diluting 0.8 c.c. of 10 per cent. solution to 100 c.c. After thorough mixing, 1 c.c. of 0.45 per cent. zinc sulphate (made by recent dilution of a 22.5 per cent. solution) is added, and the tube is again agitated. As soon as possible after conclusion of the test, the tubes are boiled for three minutes in a water-bath, together with blanks of the two reagents alone. The specimens and blanks are now carefully filtered through a small wad of cotton-wool of a fine grade, washed thoroughly before and after with hot distilled water, the wool being finally pressed out with a glass rod. The filtrate should be crystal clear. To each tube is added exactly 2 c.c. of buffered ferricyanide solution. This solution consists of two parts, mixed in equal volume immediately before use. These are: (i) 0.9 gm. potassium ferricyanide made up to 1 litre with distilled water ; (ii) 21.0 gm. anhydrous dipotassium-hydrogen-phosphate and 63.75 gm. anhydrous tripotassium-phosphate dissolved in distilled water and made up to 1 litre. After addition of the ferricyanide solution, the tubes are placed in a boiling water-bath for exactly twenty minutes, and then thoroughly cooled. To each is added 1 c.c. of zinc-sulphate-potassium-iodide reagent (recently prepared by adding to a 20 per cent. sulphate solution sufficient solid potassium-iodide to give a concentration of 2.5 per cent. in the mixture) and 1 c.c. of 20 per cent. phosphoric acid (20 c.c. of syrupy acid, s.g. 1.75, made up to 100 c.c. with distilled water). A drop of starch indicator (0.5 per cent.) is added, and specimens and blanks are titrated with N/1000 thiosulphate solution, preferably from a 3 c.c. microburette calibrated in 0.01 c.c. and having a fine tip. The thio-sulphate solution is made by diluting 10 c.c. N/10 sodium thiosulphate and 12 c.c. N/1 sodium hydroxide to 1 litre. This solution should be frequently standardized by titration against a N/1000 potassium dichromate or potassium iodate solution. As the end-point is reached vigorous shaking is advisable to be sure of a sharp reading.

The reactions involved are as follows. The filtrate is heated in alkaline solution with a measured amount of ferricyanide, some of which is reduced by the glucose to ferrocyanide. With the zinc-sulphate-potassium-iodide reagent in an acid medium, the excess ferricyanide liberates iodine :

while the ferrocyanide is precipitated as the double potassium-zinc salt :

The difference between the thiosulphate required to titrate the iodine liberated

in the blank and in the unknown is a measure of the glucose present. By experiment a factor is found which will convert this difference into terms of mgm. per cent. This was found to average 174. The glucose value of the unknown is therefore : $174 \times (\text{c.c. of thiosulphate for blank} - \text{c.c. for specimen})$. The specimens are stable after the addition of the zinc sulphate until the ferricyanide is added, and in practice if there must be some delay in completing the estimation it is best to allow this to occur after the zinc-sulphate has been added. In duplicate estimations the results obtained coincide with those obtained by the Hagedorn-Jensen method within 1 mgm. per cent.

The normal intravenous curve

Two typical normal curves are shown in fig. 14 (solid line). It will be seen that, from a peak which is far above the normal renal threshold, the curve of descent (when plotted so that 50 mgm. on the ordinate corresponds to 10 minutes on the abscissa) approximates to a parabola, the blood sugar falling much more rapidly at first than in the later stages. The normal level is attained in fifty to sixty minutes, and there may be a slight following hypoglycaemia. The height of the peak is of little significance, for it must depend upon dosage per litre of blood, rate of injection in relation to both blood volume and dose, and perhaps other factors less obvious. The essential feature is the steady fall, of initially high rate, in an unbroken curve. Occasionally bifid peaks have been encountered. The meaning of these is by no means clear, unless they reflect vasomotor or emotional disturbance. Minor irregularities are found in the curve whenever the patient is emotionally disturbed; and not unnaturally these are seen most often in cases of coeliac disease and allied disorders, in young children and in nervous adults.

It is not considered desirable to publish a suggested standard of normality for this test, as investigation of a large enough number of truly normal subjects for the purpose has not been possible. Certainly, it is not safe to assume the normality of convalescents. Sheldon (1938) and Crawford (1938*a*) have both recently published curves of this kind which cannot be accepted as normal. This test is a much more delicate instrument than the oral one, and any trace of infection (even a coryza), a recent period of light diet, or a major anæsthetic given even two weeks previously may lead to definite shortcomings in the curve of an otherwise normal subject. However, it can be stated with confidence that three definite criteria are met by the normal curve :

- (a) In form it is a smooth, hollow curve, resembling a parabola, when plotted as described above.
- (b) It attains the fasting-level, or comes within about 20 mgm. per cent. of it, within one hour.
- (c) Within the limits of present experience, its area does not exceed 3,500 mgm. minutes when measured as described above.

Abnormal curves

These may be divided into a large group of curves in which the area is greater than normal; and a small one in which it is less. The former are,

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of course, all indicative of impaired tolerance, but they conform to three main types :

(a) The 'diabetic.' This is the homologue of the diabetic oral curve. It rises from a normal or high (if uncontrolled) fasting level to a great height, whence it falls slowly and very slightly (fig. 16, solid line).

(b) A common type, in which the normal curve is replaced by a flat curve approaching at times a straight line, usually remaining above the fasting level at one hour (fig. 16, dotted lines). Varying degrees of this type are seen, and four are shown. These curves are found in a variety of conditions, including

FIG. 16.—High (abnormal) intravenous glucose tolerance curves, contrasted with a normal (double line) : (a) diabetic (solid line) : (b) non-diabetic impairment of tolerance (see text) (dotted lines).

carbohydrate deprivation and impaired absorption, intoxication, infection, and liver disease.

(c) The 'humped' curve (fig. 17, dashed lines). Here there is a more or less rapid change in the rate of fall of the blood sugar at from twenty to fifty minutes, usually about thirty minutes, the curve becoming convex. This striking type is seen at times in carbohydrate deprivation and in absorptive disease or disorder.

Curves of a smaller area than normal are uncommon and we have seen them in only four conditions—in the normal subject after high carbohydrate diet (fig. 18, solid line) ; in Frohlich's syndrome, when the curve is sometimes similar ; in a case of suprarenal neuroblastoma, in which the fasting level was high but the tolerance great, presumably from hyper-adrenalinism (dashed line) ; and in a case of hepatomegaly thought to be due to compensatory hypertrophy after subacute necrosis (dotted line). In this connexion it is

FIG. 17.—‘ Humped ’ (abnormal) intravenous glucose tolerance curves, contrasted with a normal (double line).

FIG. 18.—Abnormally low intravenous curves contrasted with a normal (double line): (a) high-carbohydrate diet in a normal subject (solid line); (b) suprarenal tumour (dashed line); (c) hepatomegaly (?) hypertrophic (dotted line).

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interesting to note the statement in Crawford's paper (1938*a*) that there is a gradual prolongation of tolerance curves in children as the age increases. It is possible that this merely reflects the tendency of younger children to take a higher carbohydrate diet. Curves performed with patients on standard ward diet reveal no such tendency.

The interpretation of intravenous curves is a relatively simple matter, for they represent only one process—the removal of sugar from the blood. Admittedly, while the blood-sugar level is above renal threshold there may be, and there sometimes is, a glycretic contribution to this ; but the later portion of the curve represents 'tolerance' alone, and this is at once shown as normal, sub-normal, or supernormal.

The only type of curve calling in itself for further comment is the 'humped' variety. Here there is patently a change of tolerance during the test, and the implication is that that change is due to the test dose. It probably represents, in fact, a sensitization of insulin, and is of the same general nature as the 'Staub-Traugott phenomenon' (Himsworth, 1933–4–5). It is of some interest that this type of curve has been seen only in the relatively less chronic conditions of starvation or malabsorption.

The combination of oral and intravenous tests

This combination is of particular interest, in that it gives information regarding absorption which is not available from either source alone. In fact, apart from symptomatology, it provides the first method available of demonstrating a deficient absorption of digested carbohydrate, since the quantitative analysis of faeces for carbohydrate residues is not practicable. In the following table there are summarized the findings made by using both tests in a variety of conditions.

TYPE OF CONDITION	CLINICAL INSTANCES	ORAL CURVE	INTRAVENOUS CURVE
Hypoinsulinism . . . Carbohydrate deprivation.	Diabetes mellitus. Starvation. ¹ Anorexia nervosa. ² Experimental diets. ³ Coeliac disease. ⁴ Sprue. ⁵	'Diabetic.' High with delayed fall or typical 'lag.' 'Flat.'	'Diabetic.' High with delayed fall ; may be 'humped.' High ; may be 'humped' in some.
Deficient absorption of carbohydrate.	Chr. idiop. steatorrhoea. ⁶ Mesenteric tuberculosis. ⁷ Chr. intestinal dyspepsia. ⁸		
Infection. Intoxica- tion.	Tonsillitis, arthritis, etc. ⁹ Diphtheria. ¹⁰ Alcoholism. ¹¹	High.	High.
Liver disease . .	All grades, acute and chronic, from cat- arrhal jaundice to cirrhosis and necrosis, except post-necrotic hypertrophy. ¹²	High, unless gross intestinal dis- turbance, when it may be flat or grotesque.	High 'flat' type.

¹ See fig. 2. ² Ross, 1938. ³ See fig. 1. ⁴ Ross, 1936*a*. ⁵ Fairley, 1936. ⁶ Vaughan, 1936. ⁷ Ross, 1936*b*. ⁸ Ross, 1936*c*. ⁹ Pemberton, 1920. ¹⁰ Begg and Harries, 1935. ¹¹ Unpublished cases. ¹² Jacobi, 1936–7 ; Ross, 1936*a*.

It will be seen that, with the aid of the combined tests, defective absorption may be detected. If insulin-sensitivity is also measured, diabetes mellitus can with certainty be separated from other common conditions giving a high intravenous and oral curve.

Summary

- (1) The history of tolerance investigations is briefly reviewed.
- (2) General considerations affecting any test of tolerance are considered.
- (3) Oral tolerance tests are discussed, with special consideration of 'flat' curves and their significance.
- (4) Intravenous tests are considered in detail, and a technical description is given.
- (5) Intravenous curves are described and their significance indicated.
- (6) The use of combined oral and intravenous tests is indicated, particularly in the demonstration of defective absorption.

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