AN EXPERIMENTAL INVESTIGATION

INTO THE FACTORS

CONTROLLING

ALIMENTARY GLYCOSURIA AND LACTOSURIA.

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INTRODUCTION.

The part played by carbohydrates in animal metabolism is usually considered to be that of supplying energy. This function they share with the fats, by which they can be replaced in a diet in the ratio of their heat values, the so-called "isodynamic equivalent." The task to replace the wear and tear of the body falls on the proteins of the food. If the amount of protein absorbed by an organism exceeds the amount necessary to repair tissue-waste, the excess is used as a source of energy and can be replaced by the isodynamic equivalents of fats or carbohydrates. This current conception distinguishes, then, between two fundamentally different functions of the organic food-stuffs: a specific function, namely, tissue repair, which can be fulfilled only by protein material, and a non-specific function, the supply of energy-for which either of the three groups of food-stuffs may be used.

The results of recent work, however, have made it necessary to modify this conception and to attribute a specific function, also to the carbohydrates. If the view outlined above were correct, it should be possible to withhold carbohydrates completely from a diet and to replace them either by proteins or fats.

But it is well known that in man such a diet leads in one or two days to serious disturbances in the metabolism, which manifest themselves by the excretion of oxybutyric acid and diacetic acid; in short, such a diet produces a marked acidosis. For carnivorous animals, dogs for instance, such a diet happens to be the normal diet and, therefore, produces no such disturbances.

It has also been known for some time that the nitrogen metabolism can be more efficiently diminished by the addition of carbohydrate to the diet than by the addition of equivalent amounts of fat. This so-called "protein-sparing" action of carbohydrates is seen most clearly when the condition of nitrogen hunger is established: that is, when the subject of the experiment lives on a nitrogen-free diet as for instance in the experiments of Laudergren.(1) In such a case, the excretion of nitrogen, which indicates the break-down of tissue-protein, is much lower on a carbohydrates diet than on a fat diet.

Experiments designed to demonstrate the fact that animals can maintain a nitrogenous equilibrium on a diet consisting of biuret-free products of digestion-that is polypeptides and amino acids-incidentally revealed the fact that this is possible only when an abundant supply of carbohydrates is given at the same time (2).

Although these observations suggested that the carbohydrate-metabolism was not solely concerned with the supply of energy, they were not sufficiently definite to allow of any conclusion being drawn as to the more specific functions which the carbohydrate-metabolism might subserve.

The observations of Lochhead and Cramer (3), however, on pregnant rabbits demonstrated clearly that an intimate relation exists between the growth of the foetus and the supply of carbohydrate material. Since this material does not appear as such in the foetal tissues, these authors concluded that the carbohydrates enter into the synthetic processes which lead to the building up of the protoplasm of foetal tissues. That this view which was based on observations made on foetal metabolism, applies also to the adult organism, was shown by the work of Cathcart (4) and Krause and Cramer (3).

Cathcart observed that a carbohydrate-free diet leads in man to the excretion in the urine of an abnormal end-product of nitrogen-metabolism, namely creatine. The same substance was found by Krause and Cramer to appear in the urine of diabetic patients and in dogs under the influence of phloridzin. The intimate inter-relationship which has been shown to exist between carbohydrate metabolism and protein metabolism has an important bearing on the problem of the etiology of diabetes-mellitus. Indeed, as long ago as 1894, Pavy (6) postulated that the assimilation of carbohydrates was brought about by synthesis into protein at the seat of absorption and that "the error in diabetes consists of a faulty assimilation of the sugar absorbed by the alimentary canal." His conception of the assimilation of carbohydrates has not been generally accepted, perhaps because the experimental evidence in support of it was not sufficiently convincing. But in so far as his ideas involved the conception of synthetic processes occurring between carbohydrates and proteins as an essential feature of normal metabolism, they have been confirmed by the recent work referred to above.

Since Pavy's conception assigns to the processes of assimilation of carbohydrates such an important part in the causation of diabetes, it seemed desirable to investigate experimentally some of the factors concerned in carbohydrate assimilation.

ALIMENTARY MELLITURIA.

When an excess of carbohydrate has been ingested, sugar appears in the urine. This, as Claude Bernard(7) showed, is due to an increase in the amount of sugar in the blood.

The amount of a given carbohydrate necessary to produce alimentary mellituria differs for different individuals and is dependent on a number of other conditions which will be discussed presently. For the same individual and under constant experimental conditions it is fairly constant. There is therefore a definite quantity of carbohydrate which can be assimilated without producing mellituria, and this Hofmeister (8) designated by the term "limit of assimilation." But although sugar appears in the urine as soon as the limit of assimilation is exceeded, the quantity of sugar excreted stands in no relation to the amount of carbohydrate by which the limit has been exceeded. This relation is clearly illustrated by the following table from Von Noorden's works (9).

Amount of glucose ingested:	Amount of glucose excreted:
100 grammes	0.0 grammes
150 "	0.15 "
200 "	0.26 "
250 "	0.52 "

Different carbohydrates differ considerably in regard to their respective limits of assimilation. The observations of most authors Worm-Mueller (10) Moritz(11) von Noorden(9) agree in showing that glucose is more readily assimilated than lactose while saccharose takes an intermediate position.

Starch, on the other hand, can be given in very large quantities without producing any mellituria (12). This is probably due to the fact that starch is not absorbed as such. It must be split up into maltose and glucose, before it is absorbed, and therefore the amount of carbohydrate available for absorption at any given moment is limited.

The sugar which is excreted in the urine is in most cases the same sugar as the one ingested. After the administration of lactose, glucose has been found together with galactose.

The following factors have been found to influence markedly the limit of assimilation of a given sugar in the normal organism.

1. AGE.

According to Nobecourt (13) infants have higher assimilative powers for carbohydrates weight for weight than man. He estimates them as follows:-

Milk Sugar

3.3 to 3.6	grammes per kilogram	Infants
2 to 2.5	" " "	Adults

Grape Sugar

5	grammes per kilogramme	Infants
7	" " "	Adults

2. SEX AND SEXUAL FUNCTION.

A special weakness of the carbohydrate metabolism does not exist in pregnant women. Alimentary glycosuria may, it is stated, appear somewhat more readily than otherwise. Lanz (14) states that he has frequently found sugar in the urine of pregnant women after giving 100 grammes of glucose, but only twice in quantities at all large, viz., more than 2 grammes. Zacharjhosky (15) has found that, with ordinary food, the reducing power of the urine, in the first few weeks preceding delivery, is not raised. It is said Blott (16) Fr Hofmeister (17) Kaltenbach (18) to rise after birth in connection with the secretion of milk, the sugar then excreted being lactose. This is only slight in amount unless the milk becomes reabsorbed instead of being excreted. The percentage of lactose in the urine rarely reached 0.3 to 0.5%, or an absolute amount of more than 5 to 10 grammes daily. Sinety (19) discovered that removal of the mammae both before and after delivery prevents lactosuria appearing, which would indicate that the lactose is formed only in the cells of the mammary gland.

Van Noorden (20) and Zuelzer (21) state that puerpera excrete lactose sometimes after the administration of grape sugar. They consider that puerperae generally metabolise a part of the milk sugar, reabsorbed from the mammary glands, but that the more readily decomposed glucose protects the lactose from oxidation. The first part of this explanation is opposed to the assertion of Fritz Voit (22) that milk sugar which passes into the body otherwise than through the intestine is again excreted bulk for bulk.

3. RATE OF ABSORPTION FROM THE INTESTINAL CANAL.

The limit of assimilation is lowered if the carbohydrate is given in the fasting state. This is usually explained as being due to the more rapid absorption. The fact that starch does not easily lead to an alimentary mellituria because the rate of absorption is necessarily slow has already been referred to above.

4. CONDITION OF CARBOHYDRATE-STORE IN BODY.

When the body has a large store of carbohydrate in the form of glycogen the limit of assimilation is lowered (23). Hofmeister (24), of the other hand, found that prolonged starvation also lowers the limit of assimilation, and leads to a condition in which even the ingestion of starch readily produces an alimentary mellituria. This condition Hofmeister designated by the term "starvation diabetes." But he worked, as his data show, on young dogs which are very susceptible to alimentary disturbances, and it is a well known fact that the limit of sugar assimilation for a young organism is markedly depressed by even slight disturbances. It would appear then, that the "starvation-diabetes" will have to be considered as the result of pathological conditions and should not be taken into account as a physiological factor. In pathological conditions such as Graves' disease, nervous diseases, etc., an alimentary glycosuria appears frequently even after the ingestion of small amounts of carbohydrates. In these conditions, however, we have to deal with a disordered metabolism, and the alimentary glycosuria in such cases must always be considered as a pathological phenomenon.

EXPERIMENTAL PART.

My own observations were made on dogs. Two varieties of sugars were used. viz., glucose and lactose. The animals were fed outside the cages and the urine collecetd, either every 24 hours, or in some cases it was obtained by passing a catheter. During the experiments the animals were kept in metabolism cages in the animal house of the Physiology Department of the Edinburgh University. The cages were lined with zinc and the floor sloped down to an opening at one corner of the cage, under which a receptacle was placed. Puppies were placed in small cages, with floors made up of cross pieces of wire so that all excrement dropped through this on to the bottom of the cage, which was so sloped that the urine ran to an opening in one corner, under which the receiving bottle was placed.

METHODS USED IN THE EXAMINATION OF URINES FOR GLUCOSE AND LACTOSE.

In order to ascertain the presence in the urine of a reducing sugar and to identifythe reducing sugar a series of tests must be applied under standard conditions. The routine plan adopted was as follows:- Fehling's test, Nylander's test, polarimeter, phenylhydrazine, fermentation. If every test gave a positive reaction then glucose was doubtless present. If every test was positive except the fermentation test, then lactose was present, but since the fermentation test may fail occasionally when glucose is present in small quantities the mucic acid test was carried out as a confirmatory test for lactose. The shape of the phenylhydrazine crystals gives a further indication whether glucose or lactose was the reducing agent.

Since each test is subject to numerous fallacies a trustworthy result can be obtained only by applying all the tests, and by observing the same standard conditions in each case. This is of special importance when only small amounts of reducing sugars are present in the urine as was frequently the case in my experiments. I shall therefore give in detail the methods which I have employed, and indicate briefly what conclusions can be drawn from each test.

Fehling's Test. This test is best carried out in the following manner:

Two solutions are made up.

(a) Cupric sulphate solution. 34.65 grammes of cupric sulphate are dissolved in water and made up to 500 C.C.

(b) Alkaline tartrate solution. 125 grammes of potassium hydroxide and 173 grammes of Rochelle salt are dissolved in water and made up to 500 C.C.

These solutions must be preserved in stoppered bottles and mixed when required for use. The method adopted of testing with Fehlimgs was as follows:- One took in a test tube 0.5.C.C. of each solution making 1 C.C. in all and then diluted with water to 5 C.C. Then in another test tube 1 C.C. of urine was taken. Both tubes were then heated to boiling point, and when boiling one added the urine to the Fehling's solution and allowed the mixture to stand. A positive reaction is shown by the formation of a precipitate of varying colour. If a negative reaction was obtained one tested again with 3 C.C. urine and if that failed with 5 C.C. Fehling. If albumin be present it must be removed by boiling and filtering, before applying this test. This test is only of value when it gives an undoubted negative result. If not the slightest cloudiness nor change be induced in the Fehling Solution, then one may rest assured that neither glucose nor lactose are present. A positive result however indicates nothing except the presence of some reducing agent. The method adopted tends rather to prevent any reducing agents other than a sugar operating, as such substances as conjugate glycuronates, uric acid, nucle-protein, homogentisic acid, etc., require prolonged boiling to reduce Fehling. If creatinin be present in large amount, the cupric hydroxide may be reduced to cuprous, and this in turn be dissolved by the creatinin. This will give the urine a greenish tinge, and may obscure the sugar reaction even when a considerable amount of a reducing sugar be present.

Nylanders Test.

Nylanders Reagent is prepared by dissolving 2 grammes of bismuth subnitrate and 4 grammes of Rochelle salt in 100 C.C. of a 10% solution of potassium hydroxide.

To 5 C.C. of urine in a test tube, one-tenth of its volume of Nylanders reagent was added and heated for 5 minutes over a free flame, and the tube then allowed to stand five minutes before drawing conclusions. A positive reaction is indicated by the solution darkening, or the presence of black particles in the fluid when held against a white object.

If albumen be present in the urine it must be removed by boiling and filtering before applying the test. Urines rich in indican, urochrome, uroerythrin or haemato-porphyryn, as well as urines excreted after the ingestion of large amount of certain medicinal substances, may give a reduction, simulating a true sugar reaction.

Fermentation Test.

The urine must be fresh. After it is sterilised by boiling and cooled, 20 C.C. of the urine are rubbed up in a mortar with a small piece of compressed yeast, and a little 5% tartaric acid solution added until the urine is definitely acid. Then transfer to a sacchorometer. Place in an incubator at 37° C. for 24 hours. If dextrose be present, fermentation occurs and one gets a collection of gas in the closed limb of the tube. Two controls are made, one with a solution of dextrose and the other with normal urine.

A definite fermentation is a sure indication of the presence of glucose, as no other constituent of normal or abnormal urine ferments. When dealing with urines containing only a very small percentage of glucose, one is apt to get a negative or unsatisfactory result, as sometimes the control with normal urine may show a slight trace of fermentation due to self-fermentation.

Phenylhydrazine Test.

To 5 C.C. urine add 1 C.C. of a 50% acetic acid solution and 20 drops of phenylhydrazine. Heat to boiling and keep boiling for one minute exactly. Add 5 drops of a 15% solution of caustic soda: heat a little: cool. Add distilled water to three quarters the volume of the mixture. Heat to boiling point and allow to cool. Examine in 24 hours microscopically for crystals which are characteristic if dextrose, lactose, or maltose are present, phenylglucazone, phenyllactazone, or phenylmaltazone crystals having formed. If carried out in this way this is an exceedingly delicate test, no less than 0.05% being capable of detection.

Polarimeter.

The urine must be free from protein. Since dog's urine in most cases is too highly coloured, it must be decolourised by shaking with lead acetate and filtering. A definite dextrorotation indicates the presence of either glucose or lactose. If the dextrorotation persists after fermentation the presence of lactose is indicated.

Mucic Acid Test.

Treat 100 C.C. of the urine with 20 C.C. of concentrated nitric acid (Sp. Gr. 1.4): evaporate the mixture in a broad, shallow, glass vessel on a boiling water bath until the volume of the mixture has been reduced to about 20 C.C. If lactose or galactose be present, at this point the fluid should be clear and a fine white precipitate of mucic acid should form. If the percentage of either reducing agent be low, it may be necessary to cool the solution and permit it to stand some time before the precipitate will appear.

EFFECT OF DIET ON THE ASSIMILATION OF GLUCOSE.

In two dogs the normal limit of assimilation for glucose was determined by giving the sugar together with 250 grammes of raw meat. Then the sugar was administered while the animals were fasting 24 hours, and lastly it was given with 125 grammes of lard. The urine in every case was collected for 6 hours after administration. Before all the experiments, the urine was tested to see that no sugar was present.

TABLE I.

Bitch aged 3 years weight 6750 grammes.

Date.	Grammes of glucose given.	Diet.	Fehling.	Nylander.	Polarimeter.	Fermentation.	Phenylhydrazine Crystals.
4.11.10	50	Meat	–	–	–	–	–
6.11.10	75	Meat	+	+	+	+	+
9.11.10	75	Fasting	+	+	+	+	+
11.11.10	50	Fasting	+	+	+	+	+
14.11.10	75	Lard	–	–	–	–	–
16.11.10	100	Lard	–	–	–	–	–

TABLE II.

Bitch aged 5 years weight 7850 grammes.

Date.	Grammes of glucose given.	Diet.	Fehling	Nylander.	Polarimeter.	Fermentation.	Phenyl-hydrazine Crystals.
10.11.10	100	Meat	–	–	–	–	–
12.11.10	110	Meat	+	+	+	+	+
15.11.10	100	Fasting	+	+	+	+	+
18.11.10	110	Lard	–	–	–	–	–
21.11.10	120	Lard	–	–	–	–	–

These results indicate that the lowering of the limit of assimilation in fasting animals may be due to other factors besides the greater rate of absorbtion, which is supposed to occur in fasting animals owing to the empty state of the alimentary tract. If the absorption of glucose was determined by the purely mechanical factors of fullness or emptiness of the alimentary tract one would expect to find the same limit of assimilation, whether the animal was fed on meat or on lard. But this is not the case. One would have to conclude, then, either fat delays the absorption of carbohydrate given with the fat, or that the assimilative power of the organism for carbohydrates may be increased or diminished according to the nature of the food stuffs ingested simultaneously with the carbohydrate.

It is a well established fact that fats retard the secretion of digestive juices and delay the passage of the food from the stomach to the duodenum. The higher limit of assimilation on a lard diet might therefore be satisfactorily explained as being due simply to a slower rate of absorption.

EFFECT OF DIET ON ASSIMILATION OF LACTOSE.

In order to test the matter further I carried out a series of observations on the assimilation by young dogs of lactose when given in different media. First of all milk was given until the exact amount necessary to produce lactosuria was found, and the amount of lactose in the milk given was ascertained. Thus one obtained the assimilation value of lactose in the milk. Like values were ascertained for lactose in boiled milk, whey, and dissolved in bovril and water.

The procedure was as follows:-About 9 a.m. the animals were given their test meal, their urine first of all being tested to ensure absence of lactose. They were then placed in metabolism cages, and their urine collected up till 4 hours afterwards, a sample of their urine about this time always being obtained.

Afterwards they were removed from their cages and fed. They were not allowed any food after 9 p.m. thus ensuring a 12 hours fast.

The results are given in Tables III. to VI.

TABLE III.

Male Puppy weight 800 grammes.

Date.	Diet.	Amount of lactose given.	Feh ling.	Ny lan der	Fer men ta tion	Po lari meter	Phenyl hydra zine
10.5.11	Pure Milk 50 c.c.	2.1 grams	+	+	-	+	+
11.5.11	Pure Milk 40 c.c.	1.8 grams	+	+	-	+	+
12.5.11	Pure Milk 35 c.c.	1.5	-	-	-	-	-
15.5.11	Pure Milk 40 c.c.	1.8	+	+	-	+	+
16.5.11	Boiled Milk 40 c.c.	1.8	+	+	-	+	+
17.5.11	Boiled Milk 35 c.c.	1.5	+	+	-	+	+

18.5.11	Boiled Milk 30 c.c.	1.35 grams	–	–	–	–	–
25.5.11	Bovril & Water 35 c.c.	1.5	+	+	–	+	+
26.5.11	Bovril & Water 30 c.c.	1.3	+	+	–	+	+
29.5.11	Bovril & Water 30 c.c.	1.0	+	+	–	+	+
30.5.11	Bovril & Water 20 c.c.	0.7	–	–	–	–	–
1.6.11	Whey 40 c.c.	1.8	+	+	–	+	+
2.6.11	Whey 30 c.c.	1.4	+	+	–	+	+
3.6.11	Whey 25 c.c.	1.1	–	–	–	–	–

TABLE IV.

Male Puppy Weight 1590 grammes.

Date.	Diet.	Amount of lactose given.	Feh ling	Ny lan der	Fer men ta tion	Po lari meter	Phenyl hydra zine.
10.5.11	Pure milk 75 c.c.	3.0 grams	+	+	-	+	+
11.5.11	Pure milk 65 c.c.	2.6	-	-	-	-	-
12.5.11	Pure milk 70 c.c.	2.8	-	-	-	-	-
15.5.11	Pure milk 75 c c.	3.0	+	+	-	+	+
16.5.11	Boiled milk 75 c.c.	3.0	+	+	-	+	+
17.5.11	Boiled milk 75 c.c.	2.6	+	+	-	+	+
18.5.11	Boiled milk 50 c.c.	2.1	-	-	-	-	-
25.5.11	Bovril in water to 65 c.c.	2.6	+	+	-	+	+

26.5.11	Bovril in water to 50c.c.	2.1	+	+	-	+	+
29.5.11	Bovril in Water to 40c.c.	1.6	-	-	-	-	-
30.5.11	Whey 75c.c.	3.4	+	+	-	+	+
1.6.11	Whey 65c.c.	2.9	-	-	-	-	-
2.6.11	Whey 70c.c.	3.1	-	-	-	-	-

TABLE V.

Male Puppy weight 1600 grammes.

Date.	Diet.	Amount of lactose given.	Fehling	Nylander	Fermentation	Polarimeter	Phenylhydrazine
10.5.11	Pure milk 75c.c.	3.0 grams	-	-	-	-	-
11.5.11	Pure milk 85c.c.	3.4	+	+	-	+	+
12.5.11	Pure milk 80c.c.	3.2	-	-	-	-	-
15.5.11	Pure milk 95c.c.	3.4	+	+	-	+	+

16.5.11	Boiled milk 85c.c.	3.4 grams	+	+	-	+	+
17.5.11	Boiled milk 75c.c.	3.1	+	+	-	+	+
18.5.11	Boiled milk 65c.c.	2.6	+	+	-	+	+
28.5.11	Boiled milk 60c.c.	2.4	+	+	-	+	+
26.5.11	Boiled milk 50c.c.	2.0	-	-	-	-	-
29.5.11	Bovril in Water to 50c.c.	2.0	+	+	-	+	+
30.5.11	Bovril in Water to 40c.c.	1.6	-	-	-	-	-
31.5.11	Bovril in Water to 45c.c.	1.8	+	+	-	+	+
1.6.11	Whey 85c.c.	3.4	+	+	-	+	+
2.6.11	Whey c.c.	2.8	-	-	-		
5.6.11	Whey c.c.	3.0	+	+	-	+	+

TABLE VI.

Male Puppy weight 1600 grammes.

Date.	Diet.	Amount lactose given.	Fehling	Nylander	Fermentation.	Polarimeter	Phenyl hydrazine.
10.5.11	Pure milk 75 c.c.	3.0 grams	-	-	-	-	-
11.5.11	Pure milk 85c.c.	3.4	+	+	-	+	+
12.5.11	Pure milk 80 c.c.	3.2	-	-	-	-	-
15.5.11	Pure milk 85 c.c.	3.4	+	+	-	+	+
16.5.11	Boiled milk 85 c.c.	3.4	+	+	-	+	+
17.5.11	Boiled milk 75 c.c.	3.0	+	+	-	+	+
18.5.11	Boiled milk 65 c.c.	2.6	-	-	-	-	-
25.5.11	Boiled milk 70 c.c.	2.8	-	-	-	-	-
26.5.11	Boiled milk 75 c.c.	3.0	+	+	-	+	+
29.5.11	Bovril in Water to 75c.c.	3.0	+	+	-	+	+
30.5.11	Bovril in Water to 50c.c.	2.0	-	-	-	-	-
31.5.11	Bovril in Water to 65c.c.	2.6	+	+	-	+	+
1.6.11	Whey 75 c.c.	3.4	+	+	-	+	+
2.6.11	" 65 c.c.	2.9	-	-	-	-	-
3.6.11	" 70 c.c.	3.1	-	-	-	-	-

From the foregoing tables it is readily seen that when pure milk is given a larger quantity of lactose can be assimilated than when the milk is boiled. There is a still greater fall in the limit of assimilation for lactose when it is given in Bovril. The actual amounts of lactose required in the four animals when administered in the different ways were:-

Dog.	Pure Milk.	Boiled Milk.	Bovril.	Whey.
Table III.	1.8 grams	1.5 grams	1.0 gram	1.4 grams
" IV.	3.0 "	2.6 "	2.1 "	3.4 "
" V.	3.4 "	2.4 "	1.8 "	3.4 "
" VI+	3.4 "	3.0 "	2.6 "	3.4 "

These results demonstrate clearly that the power of assimilation of the organism for a carbohydrate is dependent to a certain extent on the food stuff ingested simultaneously into the carbohydrate. The fact that even boiling milk has an effect upon the limit of assimilation indicates that the mechanism of carbohydrate assimilation is influenced by very delicate differences.

EFFECT OF PROLONGED FEEDING.

Continued feeding with glucose may in some cases have the effect of markedly lowering the limit in some cases. One bitch was fed every morning with glucose over a prolonged period. Its urine was collected six hours after the administration. When glycosuria was found, a smaller dose was given the following morning. This was first of all kept up for a period of 4 weeks. The animal was then left alone for 3 weeks, and then the experiment resumed for a further period of six weeks. The diet was the same throughout. The result is shown by the following table.

TABLE VII.

Bitch aged 3 years, weight 6810 grammes.

1st period.

Date.	Grams glucose given.	Diet.	Fehling	Nylander	Polarimeter	Fermentation	Phenylhydrazine Test.
18.11.10	75	Meat	+	+	+	+	+
20.11.10	70	Meat	-	-	-	-	-
24.11.10	70	Meat	+	+	+	+	+
28.11.10	65	Meat	-	-	-	-	-
30.11.10	65	Meat	-	-	-	-	-
2.12.10	65	Meat	-	-	-	-	-
4.12.10	65	Meat	-	-	-	-	-
7.12.10	70	Meat	+	+	+	+	+
9.12.10	65	Meat	+	+	+	+	+
15.12.10	65	Meat	+	+	+	+	+
17.12.10	60	Meat	-	-	-	-	-

TABLE VIII.

2nd period.

Date.	Grams glucose given.	Diet	Feh ling	Ny lan der	Po lari meter	Fer men ta tion	Phenyl hydra zine Test.
9.1.11	65	Meat	–	–	–	–	–
11.1.11	70	Meat	+	+	+	+	+
14.1.11	65	Meat	+	+	+	+	+
16.1.11	60	Meat	+	+	+	+	+
18.1.11	50	Meat	–	–	–	–	–
20.1.11	60	Meat	+	+	+	+	+
23.1.11	50	Meat	+	+	+	+	+
26.1.11	45	Meat	+	+	+	+	+
28.1.11	40	Meat	–	–	–	–	–
31.1.11	45	Meat	+	+	+	+	+
3.2.11	40	Meat	+	+	+	+	+
6.2.11	35	Meat	–	–	–	–	–
10.2.11	35	Meat	+	+	+	+	+
15.2.11	30	Meat	–	–	–	–	–
20.2.11	30	Meat	–	–	–	–	–

In table VII. or 1st period the limit dropped from 75 grammes to 65 grammes, and in Table VIII. or 2nd period from 70 to 35 grammes

This experiment was repeated with two other animals, in both of which the limit of assimilation remained quite constant. As an illustration the results of one experiment are given below in Table IX.

TABLE IX.

Bitch, weight 7850 grammes.

Date.	Grams glucose given.	Diet.	Feh ling	Ny lan der	Po lari meter	Fer men ta tion	Phenyl hydra zine Test
17.11.10	110	Meat	+	+	+	+	+
20.11.10	105	"	–	–	–	–	–
24.11.10	110	"	+	+	+	+	+
28.11.10	100	"	–	–	–	–	–
30.11.10	110	"	+	+	+	+	+
3.12.10	105	"	–	–	–	–	–
5.12.10	110	"	–	–	–	–	–
8.12.10	110	"	+	+	+	+	+
10.12.10	105	"	+	+	+	+	+
15.12.10	110	"	+	+	+	+	+
17.12.10	105	"	–	–	–	–	–

The conclusion to be drawn is that prolonged feeding with glucose may in certain predisposed individuals markedly lower the limit of assimilation for glucose. This fact has an important bearing on the etiology of certain cases of diabetes mellitus. It is in agreement with the clinical experience in many cases of diabetes, in which by a carefully regulated restriction of carbohydrates in the food the limit of assimilation of the patient may be gradually raised.

ASSIMILATION OF GLUCOSE IN PREGNANCY.

The fact that in lactation the administration of glucose tends to produce lactosuria has been referred to in the introduction. It was therefore of interest to determine how the female organism behaves in the latter stages of pregnancy. A pregnant animal, operated on some months before to render catheterisation easy, was taken. Two weeks before delivery large doses of glucose were administered. The urine was drawn off before the experiments and the absence of reducing sugar ascertained. The litter was born on the 7th April.

TABLE X.

Date.	Pregnant Bitch weight 7000 grammes. Grams glucose given.	Diet.	Feh ling	Ny lan der	Po lari meter	Fer men tation.	Phenyl hydra zine Test.
21.3.11	80	Meat	+	+	+	+	Phenyl glucozone & Phenyl lactazone Crystals
24.3.11	60	Meat	+	+	+	+	Phenyl glucozone & Phenyl lactazone Crystals

As confirmatory of the presence of lactose as well as glucose, several tests were made, such as the mucic acid test, examination of the urine in the polarimiter after fermentation, and after boiling the urine with dilute acid.

These results show then, that the pregnant organism if supplied with a large amount of glucose excretes lactose in the urine. Since the results of recent work tend to show that the formation of lactose is due to the activity of the mammary glands, these results would indicate that an abundant supply of glucose stimulates the cells of the mammary glands to produce more lactose. If this explanation is correct one should be able to diminish the lactosuria which sometimes occurs in pregnant women by restricting the supply of glucose.

EFFECTS OF THYROID FEEDING ON LIMIT OF ASSIMILATION.

After having determined the limit of assimilation of glucose in two dogs, they were fed with one thyroid gland and, later with two glands. Sheep's thyroid glands were used.

In each case the dogs had been operated on to render catheterisation easy and the urine was withdrawn 5 hours after administration.

TABLE XI.

Bitch weight 6730 grammes.

Date.	Amount glucose.	Amount Thyroid.	Feh ling.	Ny lan der.	Po lari meter	Fer men ta tion	Phenyl hydra zine Test.
10.2.11	40grams	nil	+	+	+	+	+
11.2.11	35 "	nil	−	−	−	−	−
13.2.11	40 "	nil	+	+	+	+	+
14.2.11	nil	1 gland	−	−	−	−	−
15.2.11	40 "	1 "	−	−	−	−	−
16.2.11	40 "	1 "	−	−	−	−	−
17.2.11	45 "	1 "	−	−	−	−	−
18.2.11	50 "	1 "	+	+	+	+	+
19.2.11	45 "	1 "	+	+	+	+	+
21.2.11	40 "	1 "	+	+	+	+	+
23.2.11	35 "	1 "	+	+	+	+	+
24.2.11	30 "	1 "	−	−	−	−	−
25.2.11	35 "	nil	−	−	−	−	−
26.2.11	40 "	nil	+	+	+	+	+
27.2.11	35 "	2 glands	+	+	+	+	+
28.2.11	50 "	2 "	+	+	+	+	+
1.3.11	25 "	2 "	−	−	−	−	−
3.3.11	25 "	2 "	−	−	−	−	−
4.3.11	30 "	2 "	+	+	+	+	+
6.3.11	30 "	nil	−	−	−	−	−
7.3.11	35 "	nil	−	−	−	−	−
8.3.11	35 "	2 glands	+	+	+	+	+

TABLE XII .

Bitch weight 7880 grammes.

Date.	Amount glucose	Amount thyroid	~~Ny~~ Feh ling	Ny lan der	Po lari meter	Fer men ta tion	Phenyl hydra zine Test.
9.1.11	100grams	nil	−	−	−	−	−
10.1.11	110 "	nil	+	+	+	+	+
12.1.11	100 "	nil	−	−	−	−	−
13.1.11	110 "	nil	+	+	+	+	+
14.1.11	110 "	nil	+	+	+	+	+
16.1.11	100 "	nil	−	−	−	−	−
17.1.11	110 "	nil	+	+	+	+	+
18.1.11	100 "	1 gland	−	−	−	−	−
19.1.11	110 "	1 "	−	−	−	−	−
23.1.11	120 "	1 "	−	−	−	−	−
25.1.11	110 "	1 "	+	+	+	+	+
26.1.11	100 "	1 "	+	+	+	+	+
27.1.11	90 "	1 "	−	−	−	−	−
28.1.11	100 "	nil	−	−	−	−	−
29.1.11	110 "	nil	+	+	+	+	+
30.1.11	100 "	1 gland	+	+	+	+	+
31.1.11	90 "	2 glands	−	−	−	−	−
2.2.11	95 "	2 "	+	+	+	+	+
3.2.11	90 "	2 "	−	−	±	±	−
4.2.11	100 "	2 "	+	+	+	+	+
6.2.11	100 "	nil	−	−	−	−	−
8.2.11	100 "	2 glands	+	+	+	+	+

In both animals the first effect of giving fresh thyroid was to raise the limit. Table XI. shows that the limit of assimilation was raised at first from 40 grammes to 50 grammes. In Table XII. where 110 grammes produced glycosuria normally, 120 failed to do so. When the feeding was continued, however, the limit was lowered, namely, in XI. from 40 to 30 grammes and in XII. from 110 tp 95 grammes. It is thus evident that glycosuria might readily result from the prolonged administration of thyroid extract.

In pregnancy thyroid feeding seems to exert a very profound influence in causing excessive glycosuria if once the limit for assimilation has been exceeded. In one bitch, 4 weeks pregnant, the limit for grape sugar was first determined and then the thyroid feeding commenced. The details of the experiment are:

TABLE XIII.

Pregnant bitch weight 8020 grammes.

Date.	Amount glucose	Amount thyroid	Feh ling	Ny lan der	Po lari meter	Fer men ta tion	Phenyl Hydra zine Test	Quan tity glucose excreted.
8.3.11	80 grams	nil	–	–	–	–	–	nil
9.3.11	90 "	nil	+	+	+	+	+	1.4 grams
10.3.11	85 "	nil	–	–	–	–	–	nil
12.3.11	90 "	nil	+	+	+	+	+	2.3 grams
13.3.11	85 "	1 gland	–	–	–	–	–	nil
14.3.11	90 "	1 "	+	+	+	+	+	8.74grams
16.3.11	80 "	1 "	–	–	–	–	–	
18.3.11	100 "	1 "	+	+	+	+	+	13.26grams
23.3.11	100 "	nil	+	+	+	+	+	2.19 "

Thus where 90 grammes of grape sugar given alone produced a glycosuria of 1.4 and 2.3 grammes, with thyroid no less than 8.74 grammes were excreted in the urine. Where 100 grammes of grape sugar under thyroid treatment gave 13.26 grammes in the urine, 100 grammes without thyroid feeding yielded 2.19 grammes.

This is of clinical importance as one might expect to produce a severe glycosuria by the administration of thyroid extract during pregnancy. The effect of thyroid feeding on the power of assimilation of the organism is in agreement with the results of recent work on the influence of internal secretionson carbohydrate metabolism.

Eppinger, Falta and Rudinger (25), experimenting on dogs found that adrenalin injections no longer produce a glycosuria after extirpation of the thyroid gland. According to their investigations the diet has but little influence upon their results, for the same data are obtained whether the animals are in a fasting condition or kept on a generous diet of sugar. The relation of the thyroid glands to sugar excretion they explain as follows:-

The pancreas produces a substance which favours the oxidation of sugar in the body. The secretion of the thyroid gland exercises a control over the internal secretion of the pancreas, holding it in check: likewise the internal secretion of the suprarenals holds in check this internal pancreatie secretion. Consequently a glycosuria results when adrenalin is injected. If the check on the pancreas exercised by the thyroids be removed, an increased function of the pancreas follows and the adrenalin is not able to depress it sufficiently to cause glycosuria.

Picks and Pineles (26) experimenting on young goats with the thyroid glands removed, also showed that no glycosuria resulted upon the administration of adrenalin, In rabbits and guinea pigs deprived of their thyroids the conditions appear to be different, thyroidectomy in these animals having no influence on the power of adrenalin to produce glycosuria.

Grey and Santelle (27) took up the problem of the relationship of the thyroid glands to the pancreas, using dogs for their experiments, and they came to the following conclusions:-

(1) After the thyroidectomy, the glycosuria produced by ether or adrenalin in the normal animal is greatly reduced.

(2) Dogs deprived of their thyroids fed on powered thyroid glands show a return of glycosuria more or less proportional to the amount ingested, when treated with adrenalin or ether.

(3) As the thyroids regenerate, the ether or adrenalin glycosuria increases similarly.

In exopthalmic goitre, spontaneous glycosuria has been observed, and moreover the administration of carbohydrates in this condition produces alimentary glycosuria (F Kraus) (28) Ludwig and Chvostech (29) and V. Noorden (30).

In myxoedema the occurrence of spontaneous glycosuria is said not to occur. V. Noorden (30) Hirochl (31) in one undoubted case of myxoedema administrated from 200 tp 500 grammes of grape sugar without producing any glycosuria. Knopflmacher (32) confirmed these findings and showed the limit of sugar assimilation sinks to the physiological level as improvement takes place under thyroid treatment.

As the results of observations on the effect of extirpation of the thyroid gland on the carbohydrate metabolism King (33) and McCurdy (34) also arrived at the conclusions that the thyroid exerts a retarding influence on the carbohydrate destroying mechanism of the body and that removal of the thyroid glands causes a rise in the assimilative limit for dextrose.

On the other hand F. P. Underhill and Tadasu Saiki (35) got marked alimentary glycosuria in dogs after complete thyroidectomy on feeding with sugar.

From this brief review of the literature it will be seen that almost all our information on the relation of the thyroid gland to carbohydrate metabolism is based, either on observation on animals deprived of their thyroids or on clinical observation on patients suffering from a defective secretion of the thyroid gland. One might argue, therefore, that the administration of thyroid gland in these cases effects simply a restoration of a pathological condition to the normal, and that beyond that the administration of thyroid gland has no further action. But my experiments show that the carbohydrate metabolism of the normal animal is also subject to the influence of the internal secretion of the thyroid gland.

SUMMARY.

I. In fully grown dogs the limit of assimilation for glucose is higher when the sugar is fed with fat, than when it is fed together with meat. It is lowest in the fasting animal. These facts suggest that the assimilation of the foodstuff is influenced to a certain extent by the nature ofother foodstuffs which are ingested at the same time.

II. This conclusion is confirmed by the fact that in young dogs the limit of assimilation for lactose varies greatly with the nature of the medium in which the lactose is given. Lactose when given in the form of milk is assimilated in greater quantity than when given dissolved in a watery solution of meat extract. There are differences even between fresh milk and boiled milk, the fresh milk representing the more favourable medium for the assimilation of lactose.

III. In one dog it has been possible to lower the limit of assimilation for glucose from 75 to 35 grammes by prolonged feeding with glucose exceeding in amount the limit of assimilation. It would therefore appear that in certain predisposed individuals, ingestion of large amounts of glucose over prolonged periods, may weaken the mechanism of assimilation, a conclusion having an important bearing on the etiology of diabetes mellitus.

IV. Feeding with thyroid gland markedly at first raises and then lowers the limit of assimilation for glucose in normal animals. This effect is especially marked in pregnant animals. This fact is an agreement with the occurrence of glycosuria in Graves' disease and with the increased tolerance for carbohydrates which has been shown to exist in thyroidectomised animals.

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