CXLVI. A THERMAL STUDY OF A POSSIBLE SOURCE OF ENERGY IN ANAEROBIC METABOLISM WITH SOME NOTES ON THE PROPERTIES OF SORBITOL.

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INTRODUCTION.

IN order to study the energy-producing mechanism in the living cell under anaerobic conditions, it is first necessary to ascertain, as far as possible, what reactions take place. The only one which has so far been established is the glycolytic formation of lactic acid, although several others have been suggested as the possible source of energy. The evidence for the existence of these reactions in the living organism is being examined in each case, in order to determine whether any amongst them may be of importance in anaerobiosis. The production of valeric acid by *Ascaris lumbricoides* has been dealt with by one of the authors [Slater, 1925] in a previous communication, and the present work deals with the second reaction investigated.

Amongst the sources of anaerobic energy which have been suggested, the auto-oxidation and reduction of dextrose after the manner of the Cannizzaro reaction [Mathews, 1921] is perhaps the best supported by collateral evidence; but, unfortunately, no direct proof is available for the existence of such a process in the animal body.

It has long been known that the small quantities of fatty aldehydes existing in the animal organism can be converted into the corresponding acids. Further, it has been shown that isolated tissues have the power to bring about this reaction. It was at first assumed that the only factors involved were the aldehyde and the free oxygen reacting under the influence of aldehydease [Dakin, 1922].

The work of Dony-Hénault and Van Duuren [1907], amongst others, showed that free oxygen is not essential to the reaction, and that in certain circumstances its presence actually retards the formation of the acid. Parnas [1910] and Battelli and Stern [1910] were able to prove that the formation of the

fatty acid is not a simple oxidation, but a hydrolytic auto-oxidation and reduction of the same type as benzaldehyde undergoes when shaken with strong aqueous potash. This change may be represented by the equation:

The last named workers further demonstrated that the reaction was dependent on an enzyme "aldehydemutase," which appeared to occur largely in the liver. Parnas [1910], calculating from the known heats of combustion of the materials involved, showed that the reaction is, in the case of the fatty aldehydes considered, exothermic, and hence a potential source of energy.

Owing to the highly reactive character of dextrose in the presence of small quantities of oxygen, it has not yet been possible to show whether the aldehyde group of the molecule acts in this way or not. The difficulty is increased by the possible changes which may take place in the end-products of the reaction—gluconic acid and *d*-sorbitol.

If, however, it can be demonstrated that the reaction in the case of dextrose is exothermic, additional weight will be lent to the evidence already available in support of the hypothesis; whilst if, on the other hand, the reaction should prove to be endothermic, further investigation on the subject can be abandoned.

In order to calculate the heat exchanges in the reaction it is necessary to know the heat of combustion of gluconic acid, dextrose and *d*-sorbitol in solution. The value for this constant has been determined in each case, the actual figures being:

Dextrose	676.08 Cals. per g. mol.
Gluconic acid	618.59 " "
Sorbitol	732.44 " "

The energy change is therefore equal to

$$\frac{(2 \times 676.08) - (618.59 + 732.44)}{2}$$

or 0.57 Cal. per g. mol.

It is immediately obvious that although such a reaction would be exothermic, the heat available is so small that compared with the breakdown of glycogen to lactic acid it is negligible.

There appear, therefore, to be good grounds for rejecting the suggestion that under anaerobic conditions dextrose reacts in this way, with the production of the necessary energy for the maintenance of life.

DETERMINATION OF THE HEAT OF COMBUSTION OF DEXTROSE.

The heat of combustion of dextrose has been previously determined by Stohmann and Langbein [1892], who obtained the figure 3743 g. cals. per g., and by Amery and Benedict [1911], whose value was 3739 g. cals. per g. Although these values were in excellent agreement, some doubt remained as to the state of hydration of the dextrose used. The heat of combustion was therefore determined on a carefully purified sample of dextrose, and an average

value of 3739 g. cal. per g. was obtained. As the value is in agreement with the previous determinations, the details of the work are given as briefly as possible.

The purification of the dextrose and its combustion.

A sample of commercially pure dextrose was crystallised several times by Soxhlet's method from methyl alcohol and water, and after preliminary drying in air was heated at 80° until a constant weight was reached.

The material so obtained was burnt in the Mahler-Cook bomb used by one of the authors for the determination of the heat of combustion of glycogen [Slater, 1924], the same calibration figure being employed. Collodion was used to fire the dextrose, and this is allowed for in the calculations. Four determinations are given below.

Heat equivalent of bomb = 760.5 g. cal.

Heat of combustion of collodion taken as 2780 g. cal. per g.

	Weight of glucose (g.)	Weight of collodion (g.)	Weight of water in calorimeter (g.)	Rise in temperature (° C.)	Heat of combustion (g. cal. per g.)
(1)	0.9994	0.0356	2489.7	1.178	3734.1
(2)	1.0039	0.0704	2488.8	1.218	3746.5
(3)	1.0004	0.0197	2485.2	1.167	3731.5
(4)	1.0371	0.0263	2481.5	1.221	3743.9

Average value = 3739.0 g. cal. per g.

Heat of solution of dextrose.

The method used for the determination was the same as that for glycogen [Slater, 1924]. The water equivalent of the flask found by electrical calibration was 16.9 g. cal. per ° C. The following table gives the results of five determinations.

	Weight of dextrose (g.)	Total heat equivalent (g. cal.)	Change in temperature (° C.)	Heat of solution (g. cal. per g.)
(1)	1.0030	166.9	-0.105	-17.5
(2)	1.0001	168.7	-0.095	-16.0
(3)	1.0018	167.6	-0.099	-16.6
(4)	1.0015	167.2	-0.101	-16.9
(5)	0.9992	169.3	-0.112	-18.7

Average value = -17.1 g. cal. per g.

From these results the heat available from 1 g. of dextrose in solution is 3756.1 g. cal., or 676.08 Cals. per g. mol.

DETERMINATION OF THE HEAT OF COMBUSTION OF GLUCONIC ACID.

Gluconic acid has not been obtained in a pure state, the best preparation consisting of a syrup of unknown water content. An indirect method had therefore to be adopted. After some preliminary experiments and a general consideration of the possible methods, it was decided to use the following four reactions:

By summing these equations we get

Preparation of the calcium gluconate.

A specimen of commercially pure calcium gluconate (Kahlbaum) was purified after the manner described by Blanchetière [1923], and finally carefully dried to constant weight at 50°. This sample was shown to lose weight equivalent to 1 molecule of water for each molecule of salt on drying to constant weight at 120°, and to contain the correct amount of calcium.

% loss on drying	Found 4.04	Theory 4.02
% calcium	Found 8.85	Theory 8.92

Determination of the heat of combustion of calcium gluconate.

The production of calcium hydroxide in the combustion provided a difficulty as the carbon dioxide formed combined with it to a limited extent forming calcium carbonate. It was found, however, that, under constant conditions as to the weight of material used and as to the pressure of oxygen in the bomb, the percentage of calcium hydroxide converted was relatively constant. Thus determinations made by titrating the carbonate present gave values between 16 % and 18 %. The heat exchanges in the conversion of the hydroxide to carbonate are known and are given by the equation

The weight of calcium gluconate taken in each combustion was approximately one gram, yielding 0.165 g. of hydroxide. If 17 % of this material is converted into carbonate, the heat evolved will be 11 g. cals. It is obvious, therefore, that small variations in the amount of carbonate found will not materially affect the result, and 11 g. cals. is therefore applied as a correction to the average result of the separate determinations. The combustions were made exactly as in the case of dextrose.

Heat equivalent of the bomb = 760.5 g. cals.

Heat of combustion of collodion taken as 2780 g. cals. per g.

	Weight of calcium gluconate (g.)	Weight of collodion (g.)	Weight of water in calorimeter (g.)	Rise in temperature (° C.)	Heat of combustion (g. cals. per g.)
(1)	1.0009	0.0327	2477.2	0.839	2626.1
(2)	1.0005	0.0255	2479.2	0.830	2616.7
(3)	1.0003	0.0220	2493.1	0.823	2615.7
(4)	1.0042	0.0198	2491.0	0.829	2629.2
(5)	1.0007	0.0248	2501.1	0.827	2626.5
(6)	1.0019	0.0366	2501.0	0.835	2616.5

The average of these figures is 2621.8, from which must be subtracted the 11 g. cal. due to the formation of carbonate, giving the final value for the heat of combustion as 2611 g. cal. per g. or 584.86 Cals. as the value of C in equation (3).

Determination of the heat of neutralisation of gluconic acid.

Solutions of calcium hydroxide and gluconic acid were prepared, the former by shaking pure calcium oxide with water free from carbon dioxide and filtering the undissolved material, and the latter by decomposing a solution of calcium gluconate quantitatively with oxalic acid. The solutions so obtained were adjusted to equivalent strengths, the calcium hydroxide solution containing 1.2861 g. per litre, and the gluconic acid 6.808 g. per litre.

100 cc. of the calcium hydroxide solution were placed in a flask with a Bechmann thermometer and allowed to come to thermal equilibrium in a constant temperature bath. In a vacuum flask in the same bath 100 cc. of the gluconic acid solution were adjusted to the same temperature, and the solutions rapidly mixed. The rise in temperature was noted and corrections for cooling made. The heat equivalent of the vacuum flask, stirrer and thermometer had previously been determined by electrical calibration.

Although this method for determining the heat of neutralisation is open to some criticism, it gave uniform results, and in the opinion of the authors is sufficiently accurate for the present purpose, the maximum deviation from the mean being less than 2 g. cal. per g. The results obtained are given in the table below. In each experiment 0.6808 g. of gluconic acid was neutralised by 0.1286 g. of calcium hydroxide. The total heat equivalent of the calorimeter and its contents was in each experiment 213.4 g. cal.

	Rise in temperature (° C.)	Heat of neutralisation per g. of gluconic acid (g. cal.)
(1)	0.133	41.69
(2)	0.140	43.88
(3)	0.129	40.44
(4)	0.138	43.26
(5)	0.130	40.75

This gives a mean value of 42 g. cal. per g. of gluconic acid, or a value for A in equation (1) of 8.23 Cals.

Determination of the heat of solution of calcium gluconate.

The determination of the heat of solution of calcium gluconate proved a somewhat difficult operation. The low solubility and the slow rate of solution tended to introduce considerable errors. Ultimately it was found most satisfactory to use only small quantities of salt, and to carry out the whole operation at 40°. Otherwise the technique was as described for dextrose. The results obtained in this way were fairly concordant, but were not so uniform as the others under consideration in the present investigation. The extent of the

variation, however, was not sufficiently large to affect materially the final result. The figures obtained are given in the following table.

	Weight of calcium gluconate (g.)	Fall in temperature (corrected) (° C.)	Total heat equivalent of calorimeter (g. cal.)	Heat of solution of calcium gluconate (g. cal.)
(1)	0.0500	0.040	133.6	-106.9
(2)	0.0502	0.039	133.6	-103.8
(3)	0.0501	0.041	133.4	-109.2
(4)	0.0488	0.041	134.2	-112.7
(5)	0.0450	0.035	133.7	-104.0

This gives an average value of -107.6 g. cal. per g., and for B in equation (2) a value of 24.10 Cals.

Heat of solution of calcium hydroxide.

This value has been determined by Thomsen [1882] to be 2.79 Cals. per g. mol.; hence D in equation (4) is equal to 1.395 Cals.

Heat of combustion of gluconic acid in dilute solution.

The sum of the heat exchanges in equations (1), (2), (3) and (4) gives a total value of 618.59 Cals. per g. mol., or 3151 g. cal. per g. for the heat available when gluconic acid in solution is oxidised completely to carbon dioxide and water.

HEAT OF COMBUSTION OF *d*-SORBITOL.

Preparation and purification of sorbitol.

The literature dealing with the chemical and physical properties of sorbitol is indefinite and lacking in concordance. A general examination of the properties of sorbitol was therefore made, and its synthetic preparation attempted, before a sample was finally selected for combustion purposes. The factors of greatest importance in the present work are the ash and moisture content.

Properties of sorbitol and some of its derivatives.

Moisture content. Sorbitol unlike mannitol does not crystallise readily from water or alcohol-water mixtures, and when crystals are obtained they do not appear to have a definite water content. The information in the literature is as follows. Vincent and Delachanal [1889] obtained needles of indefinite composition from 95 % alcohol. Boussingault [1872] and Hitzermann and Tollens [1889], working with sorbitol from natural sources, isolated a monohydrate melting between 55° and 60° . Fischer [1890], using synthetic sorbitol, obtained a half hydrate by drying over sulphuric acid at room temperature for 3 days; melting-point 75° . Nanji and Paton [1924] obtained what appeared to be anhydrous sorbitol by precipitation with absolute alcohol. This material was very hygroscopic.

General behaviour of sorbitol exposed to air and dehydrating agents. The sample used in the following experiments was purchased from Kahlbaum, and

appeared to be of good quality, except in respect of moisture content. A portion of this was dried at 110° over phosphorus pentoxide *in vacuo*, using the apparatus of Harden and Young [1902]. The loss at constant weight was 3.4 %, as compared with the half hydrate 4.5 %, and the monohydrate 9.0 %. The anhydrous material so obtained was very hygroscopic, and it was therefore concluded that the original sample was a mixture of hydrates. On drying over sulphuric acid at room temperature, the loss at constant weight after 10 days was almost identical, viz. 3.5 %.

On exposure to air, the sorbitol lost or gained weight according to the moisture content of the air. The maximum water content absorbed was 33 %, the sorbitol dissolving at approximately 11 %, and recrystallising when the moisture content fell below this value.

It was concluded that sorbitol forms a series of hydrates which are stable over only small ranges of vapour pressure, and consequently that it cannot be isolated in a stable form.

In order to show that the material obtained on drying was anhydrous sorbitol, samples were taken simultaneously for drying and ultimate analysis. The following results were obtained:

Loss on drying	...	...	...	...	...	...	%
Carbon found	...	...	...	...	...	...	4.36
Carbon required if the residue is anhydrous sorbitol	...	...	...	...	...	...	38.18
"	"	"	"	"	"	"	37.83
"	"	"	"	"	"	"	39.80
"	"	"	"	"	"	"	36.02

Although the agreement is not good owing to the nature of the experiment, it appeared to warrant the assumption that anhydrous sorbitol results on drying.

Methods of identification and purification. The variations in the melting-point found in the literature were confirmed, it being early evident that unless the moisture content could be stabilised, melting-point determinations were of no value. The optical rotation was also examined as a possible test of purity, but this constant is too small to be of use.

It was therefore decided to prepare some of the derivatives of sorbitol which might serve as a means of identification, and possibly of purification. The dibenzal, which was first prepared by Meunier [1891], was made and examined. The method used was that of Meunier with some slight modifications in the extraction. A mixture is made of a thick aqueous syrup of sorbitol, 50 % sulphuric acid and benzaldehyde in the ratio 1 : 1 : 0.8, from which the dibenzal separates as a paste on standing. The reaction mixture is diluted with water and the paste filtered off and boiled with a dilute solution of sodium carbonate. The addition of the carbonate prevented the decomposition of the dibenzal by traces of acid which cannot be removed by washing with water on the pump. The solid is filtered off when the smell of benzaldehyde is no longer detectable, and washed with water. If it is coloured it is advisable to wash with a little alcohol, and then with ether. The dibenzal is dried in an oven

at 80°, higher temperatures causing decomposition, and should then be a white odourless amorphous powder. Attempts to find a solvent suitable for recrystallisation proved abortive. The melting-point is markedly affected by small traces of benzaldehyde, and by the slow decomposition below the melting-point. The highest value obtained was 179–184°. Meunier claims to have isolated two forms, the one melting at 163° and the other at 200°. It has been found impossible to prepare the second form, and it appears probable that it may have been impure tribenzal mannitol which melts at 213–217°.

Hexa-acetylsorbitol was prepared exactly as described by Tutin [1925], and specimens obtained both from natural and synthetic sorbitol melted sharply at 99° after recrystallisation from ethyl acetate. This derivative proved to be by far the most satisfactory material both for the identification and the purification of sorbitol.

It is obviously a matter of great difficulty to show the presence of small quantities of other hexitols in a specimen of sorbitol, but fortunately for the present work the effect of such impurities on the heat of combustion would be exceedingly small, as they themselves have a heat of combustion approximately the same as sorbitol.

The dibenzal can be obtained in the best yields and was used as a rough assay of the sorbitol present in any product under investigation.

For purposes of purification it was found best to prepare first the dibenzal, which was then decomposed by dilute sulphuric acid in the presence of a little benzaldehyde; the acid was removed by baryta and the benzaldehyde by steam distillation. The product was a syrup, which was free from impurities other than the hexitols. To remove these the hexa-acetyl compound was prepared and repeatedly recrystallised from ethyl acetate. When a product with a sharp melting-point had been obtained, it was decomposed by the method described by Tutin [1925]. Unfortunately, this lengthy process resulted in very poor yields, and was of value only for the preparation of small quantities of material of high purity.

Reduction of dextrose.

The reduction of dextrose by metallic reducing agents has been carried out by numerous workers, with varying degrees of success. It is unfortunate that in many cases the yields are not given, and also, owing to the difficulty of identifying sorbitol, the state of purity of the final product is often in doubt.

The method most generally known is that of Fischer [1890] in which the reduction is carried out with sodium amalgam, the solution being frequently neutralised by the addition of sulphuric acid, so that it is first faintly acid and then faintly alkaline. Meunier [1891], on the other hand, uses a concentrated solution of dextrose and sodium amalgam, the reaction mixture being allowed to become strongly alkaline. In neither case is the yield of sorbitol given.

Neuberg and Marx [1907] carried out the reduction by means of metallic calcium, the solution being kept nearly neutral by means of a stream of carbon

dioxide. No definite yield is given. More recently Nanji and Paton [1924] have used aluminium amalgam for the reduction, the solution being rendered alkaline by ammonia. They claim to have obtained a 60 % yield, but give no data as to the purity.

On considering these methods it was decided to try first that of Fischer, as in the other cases the dextrose was reduced in a definitely alkaline medium, with the obvious possibility of the formation of the mixture of the substances known to be yielded by dextrose in such a solution, together with the reduction products of these substances.

It is necessary to point out, at this stage, that the disappearance of the reducing power of the dextrose cannot be taken as a measure of the sorbitol formed. The only satisfactory method is to weigh the sorbitol as the dibenzal. Although this may not give the total sorbitol formed, it does give the more important value, the amount of sorbitol which can be extracted.

The method described by Fischer was carefully repeated, the solution being frequently neutralised. After several hours' treatment with amalgam no reduction had taken place. The experiment was repeated with exactly similar results. It was found, however, that if the neutralisation was less frequent, the dextrose began to disappear. This might have been due to the production of sorbitol or to the alkaline decomposition of the dextrose.

In order to test this a reduction was attempted in which the solution was maintained throughout alkaline in reaction, acid being added from time to time in quantities sufficient to bring the solution back to p_H 8-9. The solution consisted of 45 g. of dextrose in a litre of water, the sodium amalgam ($2\frac{1}{2}$ %) being added in quantities of 100 g. as it was used up. The experiment was carried out at room temperatures and with only occasional shaking. When 1000 g. of amalgam had been added over a period of 3 days, the reducing power of the solution had fallen to 10 %. After the solution had been evaporated *in vacuo* and extracted with absolute alcohol, a yield of dibenzal was obtained corresponding to 30 % formation of sorbitol.

The by-products were examined and it was found that mannitol had been formed in considerable quantities (5-10 %), and a certain amount of syrupy and gummy material, which appeared to be a mixture containing some sorbitol, mannitol and unchanged dextrose. Meunier's method was also tried, but the by-products formed in this case rendered the separation of the dibenzal difficult, and the yield was consequently low. The method of Neuberg and Marx proved unsatisfactory owing to the large bulk of carbonate precipitated, from which the whole of the sorbitol is only removed after prolonged washing. This difficulty in washing away sorbitol was found to apply to other bulky precipitates.

Reduction with aluminium amalgam as recommended by Nanji and Paton was only moderately successful, yields of not more than 10 % being obtained when the sorbitol was extracted and weighed as dibenzal. It is interesting to note in this connection, that the ammonia which Nanji and Paton believe to

play an important part in the reduction, may be replaced with equally good results by a sufficient quantity of caustic soda to reproduce a similar hydrogen ion concentration. It should be stated, however, that the aluminium used in the present experiment may not have been so good as that used by Nanji and Paton, as it appeared to react more slowly. Three different samples of aluminium were tried, but none of them proved entirely satisfactory.

The best yield obtained was that from the modification of Fischer's method. It is probable that Fischer actually allowed his reaction mixture to become more strongly alkaline than he states, and in this way obtained reduction.

It is unfortunate that the conditions essential to the reduction of dextrose are also those which produce a large number of by-products and an impure end-product. In the present work, where it is essential that pure material should be combusted, sorbitol prepared by reduction is useless, unless it has first been converted into the hexa-acetyl derivative and carefully purified by repeated recrystallisation from ethyl acetate.

The combustion of sorbitol.

After it had been decided that the reduction of dextrose would not yield sorbitol of sufficient purity for the combustion in the necessary quantities, the question of its extraction from natural sources, or its purchase, was considered. Owing to the seasonal difficulties and the low yields obtained either from mountain ash berries or from apples, it was found better and cheaper to use the commercial product.

A very pure sample was fortunately obtained, which was ash-free and showed no reducing power. As far as could be ascertained it had been extracted from natural sources. When a portion of the material was converted into the hexa-acetyl derivative, the product from the ethereal solution after once recrystallising from ethyl acetate melted sharply at 98°. It was concluded from this, that the original material did not contain any notable impurity capable of forming an acetyl derivative under the conditions of acetylation employed.

In order to overcome the difficulty of the irregular moisture content a quantity of about 15 g. was thoroughly powdered and mixed, and placed in a well stoppered bottle. The moisture content was determined by drying and the material used for the combustion was put in the crucible as quickly as possible and weighed in a stoppered tube. The combustion was carried out as in the case of dextrose and calcium gluconate, it being necessary, however, to use a larger quantity of cotton to fire the sorbitol. In order to obtain the accurate correction for the cotton used, its heat of combustion was determined.

Heat of combustion of cotton used for ignition.

Heat equivalent of the bomb = 760.5 g. cals.			
	Weight of cotton (g.)	Water in calorimeter (g.)	Rise in temperature (° C. corrected)
(1)	0.6317	2504	0.750
(2)	0.8030	2501.5	0.955

Heat of combustion
(g. cals./g.)
3875.3
3878.7

Mean value = 3877 g. cals. per g.

Heat of combustion of sorbitol.

Heat equivalent of the bomb = 760.5 g. cal.

Correction for the moisture content of the sorbitol = 4.36 %.

	Weight of sorbitol (uncorrected) (g.)	Weight of water in calorimeter (g.)	Rise in temperature (corrected) (° C.)	Weight of cotton (g.)	Heat of combustion (g. cal./g.)
(1)	0.5840	2500	0.716	0.0266	3995.3
(2)	0.5981	2503	0.726	0.0200	4003.7
(3)	0.6366	2500	0.790	0.0366	3997.4
(4)	0.7120	2504	0.867	0.0295	3988.5
(5)	0.7957	2502	0.976	0.0330	4016.1
(6)	0.6426	2501	0.812	0.0485	4003.3

Mean value = 4000.7 g. cal. per g.

Heat of solution of sorbitol.

The determination was made in the manner previously described, precautions being taken to prevent the sorbitol absorbing moisture before it was dropped into the water. The same sample of sorbitol was used as that from which the material for the heat of combustion had been drawn.

	Weight of sorbitol (uncorrected) (g.)	Fall in temperature (° C.)	Equivalent of calorimeter + water (g.)	Heat of solution (g. cal./g.)
(1)	0.2498	0.052	115	-24.9
(2)	0.1829	0.040	114	-26
(3)	0.3779	0.073	115	-22.8

Moisture content of sorbitol = 4.36 %.

Mean value = 24.5 g. cal. per g. of anhydrous sorbitol present in the moist sample.

Heat of combustion of sorbitol in solution.

From the two determinations we have the heat of combustion of sorbitol in dilute solution equal to 4025 g. cal. per g. of anhydrous sorbitol dissolved, or 732.44 Cals. per g. mol.

SUMMARY.

(1) The heats of combustion of dextrose, gluconic acid and sorbitol in dilute solution have been determined.

(2) It has been shown that the auto-oxidation and reduction of dextrose to gluconic acid and sorbitol produces only a small amount of heat, and hence can be neglected as a source of anaerobic energy.

(3) The chemical and physical properties of sorbitol and two of its derivatives have been examined.

(4) The conditions for the reduction of dextrose to sorbitol by metallic reducing agents have been investigated.

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