ON GLYCOSURIA CAUSED BY EXCESS OF CARBON-DIOXIDE IN THE RESPIRED AIR¹

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For the purposes of a research upon the effects on the respiratory exchanges and metabolism of varying the percentage of oxygen in the air breathed, a specially constructed apparatus was devised in which animals under experiment (usually cats) could be kept for as long a period as was desired in an atmosphere containing a constant percentage of oxygen, the carbon-dioxide produced by the animal being absorbed almost as rapidly as it was formed by a large quantity of soda-lime placed in the chamber.

One of the main objects of the enquiry being that of studying, in addition to the gaseous exchanges, the metabolism of the animal as shown by the urine; it was a routine procedure in all cases to examine the urine for abnormal constituents, and in particular for the products of incomplete oxidation, in those cases in which the percentage of oxygen was made less than that in the atmosphere.

Amongst such constituents dextrose was always tested for in the urine, and was invariably found to be absent, even when the animal had been kept in an atmosphere containing 5 to 6 per cent. only of oxygen for a period of several hours, provided that the carbon-dioxide formed during the period had been efficiently removed by the soda-lime placed in the respiratory chamber.

On one occasion, however, the soda-lime failed for some reason to act sufficiently in absorbing the carbon-dioxide, so that at the end of an experiment of 16 hours duration, the respiratory chamber was found to contain 10·3 per cent. of carbon-dioxide and 7·7 per cent.

1. The expenses of this research have been in part defrayed by a grant allotted to Professor B. Moore by the Government Grant Committee of the Royal Society, and the experiments have been carried out under his supervision,

of oxygen. During the period the animal (cat) had passed 130 c.c. of urine, which was found on analysis to contain 3.1 per cent. of sugar. A small amount of urine voided about four hours after the chamber was opened also contained sugar, but after this period the urine ceased to reduce.

This experiment led to the investigations recorded in this paper, which have had for their object the study of whether the glycosuria observed were due to deficiency of oxygen, or excess of carbon-dioxide.

Reference to the literature of the subject showed that the most closely allied observations hitherto recorded were in regard to glycosuria caused by partial asphyxiation. Experiments upon this subject have chiefly been made by observers of the French Schools, Claude Bernard, Dastre, Lépine and Boulud, who came to the conclusion that the glycosuria arose from deficiency of oxygen causing imperfect oxidation, either directly, or by leading to the formation of 'leucomaines' which interfered with the glycolysis (Lépine and Boulud). The effects of an excess of carbon-dioxide, in presence of a normal atmospheric amount of oxygen, or higher, in producing glycosuria was not tested, however, by these observers or their conclusions might have been different.

The fact that in previous experiments of our own in which the percentage of oxygen had been reduced very low (6 per cent. and under) but the carbon-dioxide had been perfectly absorbed, no glycosuria had been obtained, while here, with a somewhat higher percentage of oxygen (7.7 per cent.) in presence of a high percentage of carbon-dioxide (10.3 per cent.) glycosuria in marked degree had resulted, indicated that the glycosuria in our experiment and also that found by previous observers in partial asphyxia, arose from the presence of excess of carbon-dioxide, and not from deficiency of oxygen.

The further experiments given in detail below confirm this view, and show that glycosuria occurs in different types of animal (cat, rabbit and dog) when the percentage of carbon-dioxide in the air breathed rises to 10 to 15 per cent. (the necessary percentage varying somewhat with the animal used), and that this occurs whether the accompanying percentage of oxygen be correspondingly low, or whether the per-

centage of oxygen be maintained at or above the percentage present in the atmosphere.

Before proceeding to a description of the experiments, a brief account may be given of previous experiments upon the effects of partial asphyxiation.

Alvaro Reynoso¹ appears to have been the first to notice glycosuria under such conditions, but made no further accurate observations on the subject.

Claude Bernard² found that prolonged asphyxia destroyed the glycogen of the liver, and also that glycosuria was produced.

Dastre³ who continued Bernard's observations, experimented in a more quantitative fashion, using an apparatus by which the animal could be made to breathe at will either free air, or air from a confined space. He examined the amount of sugar in the blood, and found that this rose from 0·128 per cent. when the animal (dog) was breathing free air, to 0·253 per cent. when breathing was continued for some time in a confined space. The change was ascribed to lack of oxygen in the blood.

Schiff⁴ observed that ligature of a limb caused glycosuria and supposed that the coagulation of the blood gave rise to a ferment which increased the production of sugar in the liver.

Lépine and Boulud⁵ consider Schiff's explanation improbable, and state that in their opinion it is not the coagulation of the blood but want of oxygen which produces glycosuria by forming 'leucomaines' which interfere with the glycolysis.

These observers state, in support of their view, that injection of the blood of a partially asphyxiated animal into a normal animal (guinea pigs) produces glycosuria in the latter, and insist that this effect is due to 'leucomaines,' produced by lack of oxygen.

It will be observed that the predominant view of the previous observers is that the glycosuria in partial asphyxiation is due to lack

1. Quoted from *Comptes rendus*, Vol. LXXXIX, p. 671.

2. Quoted from *Comptes rendus*, Vol. LXXXIX, p. 669.

3. *Comptes rendus*, Vol. LXXXIX, p. 669.

4. See Lepine and Boulud, *Comptes rendus*, Vol. CXXXIV, p. 582.

5. *Comptes rendus*, Vol. CXXXIV, p. 582.

of oxygen, but throughout there is nothing to show that it might not equally well have arisen from excess of carbon-dioxide in the blood.

This is clearly the case in the earlier experiments where the animal as a whole is partially asphyxiated, for coincidently with the fall in oxygen pressure in the blood there would be a rise in pressure of carbonic acid, and dissolved carbon-dioxide.

It is no less the case in partial asphyxia of a limb produced by ligation, as in Schiff's experiment, for here there would be an accumulation of carbon-dioxide in the blood and tissue fluids of the limb and the same conditions locally as occur over the whole animal in partial asphyxia.

The same may be said about the experiment of Lépine and Boulud of injecting blood from an asphyxiated animal, for this blood would contain both an excess of carbon-dioxide, the product of the previous asphyxiation, and also an excess of sugar set free from the tissues of the asphyxiated animal. -

An important point which is shown by the experiments of the present paper is that the amount of sugar secreted in the urine is far in excess of the total amount of sugar capable of circulating in the animal's blood at any one given time, showing that the agent causing the glycosuria is capable of attacking the sugar-containing substances present in the tissues and setting free sugar from these so as to increase the percentage in the blood and lead to glycosuria.

Thus, in Expt. VII, 3, 7·7 grams. of sugar were secreted in about five hours, this, if all present in the animal's blood, would amount to about 3·8 per cent., or more than twenty times the normal amount in the blood, showing that the excess of carbon-dioxide must have led to the setting free of sugar from the sugar-containing substances of the tissues.

The view that the glycosuria is caused by excess of carbon-dioxide and not by lack of oxygen is supported by the work of Hamburger¹ on the effect of passing carbon-dioxide through whipped blood. This observer found that if this were done and then the percentage

1. Osmotischer Druck und Jonenlehre in den medicinischen Wissenschaften, Vol. I, p. 265, 1902.

of sugar in the serum after centrifugalization determined, the percentage was much higher than in the serum of blood which had not been treated with carbon-dioxide before centrifugalizing.

Hamburger ascribes the increased amount of sugar in the serum to the action of the carbon-dioxide in removing sugar from the blood corpuscles, in a similar fashion to that in which it removes alkali and increases the alkalinity of the serum, as shown by Zuntz and confirmed by Hamburger.

Experiments now being carried out in the Bio-Chemical Laboratory, University of Liverpool, by Edie and Spence, which will soon be published in detail, show, however, that there is practically no sugar in the corpuscles, and that the increased percentage of sugar found in serum after the serum or blood has been treated with carbon-dioxide is due to the setting free of sugar, probably from combination with the serum proteins, which would otherwise be lost to the analysis.

These experiments need not be referred to further here, except as an indication of the manner in which carbon-dioxide and probably other causative agents in the production of hyperglycaemia, and hence glycosuria, act in breaking down the formation of compounds in the body between the sugar and constituents of the blood and tissues.

Regarded from this point of view, the glycosuria caused by excess of carbon-dioxide in the air breathed, becomes not a mere additional example of one of the very many causative agents for glycosuria, but gives an indication also of how these many different agents may be regarded as acting in one common way, and producing glycosuria, by acting as detaching agents between glucose and the combining substances which hold it in the blood and tissues.

RESPIRATORY APPARATUS USED FOR THE EXPERIMENTS

The apparatus employed was devised to maintain a mixture of gases of approximately constant composition throughout an experiment lasting from several hours to over a day, without a constant circulation of large volumes of air, or mixed air and gas, through the respiratory chamber. Such a circulation, in an experiment lasting

for a long time, demands expensive and complicated apparatus, both for the circulation process itself and for measuring and analysing the gases passing through the chamber. Apparatus of this description lay outside our command, and also it seemed highly desirable for research work in respiration, and accompanying metabolism work, to obtain some simple form of apparatus, which would secure the ends in view. These were approximate constancy in the composition of the atmosphere of the respiratory chamber, including, therefore, some way of keeping percentages of oxygen up, and rapidly removing carbon-dioxide, without sending large volumes of nitrogen through the apparatus; some means of ready analysis of amount of oxygen used and amount of carbon-dioxide formed; and a method of collecting urine for metabolism work. The principle employed was to place an absorbent for carbon-dioxide in the chamber, outside the reach of the animal, and as the animal used up the oxygen to have it replaced by oxygen as pure as possible, drawn in automatically as the pressure fell on account of the absorption of carbon-dioxide. As it was found impracticable to obtain oxygen in large quantity above 95 to 96 per cent. pure, it was necessary to arrange a slow measured outflow from the chamber to correct for this, a previous analysis of the percentage of oxygen in the oxygen reservoir gave the rate at which the outflow was to be allowed to take place.

After a good deal of experimentation and modification, the apparatus was arranged as shown in the accompanying sketch in section (Fig. 1) and photograph (Fig. 2).

In the sketch, the respiratory chamber is shown at A. It is a cylindrical vessel made of block tin, with a conical bottom ending in a narrow tube to which a short piece of rubber tube is attached and closed during the experiment by a screw clip. This arrangement serves to collect the urine, which can be drawn off during the experiment by making the pressure in the chamber slightly above atmospheric and keeping the end of the rubber tube below the surface of the urine while it is being drawn off, or it may be collected at the end of the experiment.

The volume of the respiratory chamber was 44 litres.

The animal is placed as shown, upon a perforated circular zinc plate, which serves to retain the faeces; any particles accidentally dropping through are retained by the smaller meshed little circular plate shown by a dotted line near the draw-off tube.

At F is shown the arrangement for carrying the soda-lime, it is an annular tray of tin made to pass easily down into the chamber and is suspended from the upper rim of the chamber by three slips attached to it at equal distances and turned over at the top so as to hook over the top edge of the chamber. The tray held easily 500 grams, and was charged with about this amount in medium sized granules. The lid was attached in an air-tight fashion by means of

FIG. 1.—Sketch in section of respiratory chamber and accessory apparatus. Respiratory chamber, A; Oxygen reservoir, B; Wash bottle, C; Aspirator, D; Graduated vessel to measure outflow, E; Soda-lime holder, F; Water seal for lid, G; Glass pane for observing animal, H.

a circular water seal, as shown in the sketch. This seal is about 20 centimetres high and is filled half way up with water before the lid is put on. In the centre of the lid is a glass pane, shown at H, by which the animal can be observed during the experiment.

The gasometer B, which had a capacity of 90 litres, was charged

before the experiment with oxygen prepared by heating potassium permanganate.¹ By a side scale, the volume of oxygen contained in it could be determined at the commencement and at any time during the experiment. The suspension weights were so adjusted or weights placed on the top so that the oxygen was just about to bubble through the wash-bottle C. The main use of the wash-bottle C was to control the passage of oxygen, and to act as an index to show the rate at which the animal was using oxygen, and that the experiment was proceeding satisfactorily.

The arrangement shown by D and E was necessitated by the fact that it was not found practicable to prepare oxygen in the quantities required in a quite pure state. Oxygen prepared with moderate care from potassium permanganate, taking care to flush all the air out before starting to collect, contains 95 to 96 per cent. of oxygen, the remainder being nitrogen. If, therefore, the animal were allowed to draw over this mixture as it used up the oxygen contained in the chamber, and the carbon-dioxide was absorbed by the soda-lime, then on account of the small percentage of nitrogen drawn in, the oxygen content of the chamber would slowly fall and the nitrogen content rise as the experiment progressed. In order to correct this the aspirator, D, is turned on so as to run very slowly, and the amount run off in a given time is measured in the graduated vessel, E. It is easy to calculate the rate at which gas must be drawn into D in order to keep the percentage of oxygen in A constant, knowing the percentage of oxygen in A and in B. Suppose that B contains 95 per cent. of oxygen and 5 per cent. of nitrogen, then, if all the gas going into D were supposed to be nitrogen, it is evident that for every 1,000 c.c. leaving B, 50 c.c. of nitrogen would enter A and have to be drawn over into D to keep a constant level; but A contains oxygen as well as nitrogen and this must be allowed for. Suppose in any experiment A is to contain 20 per cent. of oxygen, then in 100 c.c. of A there are 80 c.c. of nitrogen, therefore to get 50 c.c. of nitrogen over into D, there must be run off

1. The oxygen sold in compressed condition in cylinders only contains 88 to 90 per cent. of oxygen, and is much too poor in quality for the purpose.

$50 \times \frac{100}{80}$ c.c. = 62.5 c.c. That is to say, in order to keep the percentage of oxygen in A constant under the above conditions, for every 1000 c.c. taken in from B, 62.5 c.c. must be run off into D. In general terms, if V be the volume coming from A, v, the volume run off into D, x, the percentage of oxygen in B, and a, the percentage of oxygen required to be maintained in A, then $v = V \times \frac{100 - x}{100 - a}$. It follows that with a capacity of 80 litres in B, for the maintenance of 20 per cent. of oxygen in A, a capacity of about 5 litres is required in D. For a lower required percentage in A, a slower flow into D, and for a higher percentage a more rapid flow are required.

FIG. 2.—Photograph showing respiratory chamber and aspirating apparatus for adjusting percentage of oxygen in it. The lead pipe seen passing off at the left-hand border goes to the oxygen reservoir.

Two slight modifications, not shown in the sketch were employed in actual use of the apparatus, these consisted in the insertion of a T piece on each of the short rubber joints between C and A, and D and A respectively. The first of these, between C and A, served for the insertion of a water manometer to show the pressure in A, the second, between D and A, served to draw off samples of the gas in A for analysis during the experiment. The free end of the latter

T piece was normally kept closed by a screw clip, which was opened after the gas burette had been attached, and after filling and re-emptying the burette several times so as to obtain a fair sample, the clip was re-applied and the burette withdrawn. A few minutes is enough for determining in the sample of gas so taken the percentages of carbon-dioxide and oxygen, and if the latter is different from what it ought to be, a modification is made in the rate of drawing off through D, in order to compensate.

Soda-lime was found to be the only efficient absorbent for carbon-dioxide in those experiments where it was desired to keep this low ; with soda-lime acting well, it is possible to keep the carbon-dioxide down to 0.2 per cent. and lower. Strong caustic potash solution was tried with as large a surface as possible but was found entirely inadequate.

The amount of oxygen used is easily found from the volume of B at the beginning and end, and initial and final analysis of the air in the system A + D, and a knowledge of its volume. To determine the amount of carbon-dioxide formed, the amount in A + D is found at the end of the experiment by a gas analysis, and this is added to the amount absorbed by the soda-lime, which is found by analysis of a weighed portion after thoroughly mixing to obtain an average specimen. A control analysis of the soda-lime, previous to use, must also be carried out, and the necessary deduction made.

If it is desired to work far away from the percentage of oxygen normally present in the atmosphere, it is necessary to flush the respiratory chamber out rapidly at the commencement of the experiment. If a higher percentage of oxygen than atmospheric is being used, the chamber can be flushed out with oxygen from an accessory reservoir, or part of the oxygen in B may be used for the purpose. In carrying out this operation, extra weights are placed on the reservoir and the screw clip at the bottom of the respiratory chamber is opened, while the rubber tube is kept under water in a beaker, and a rapid stream of gas is bubbled off until a sample of air taken from A shows the desired percentage composition. After this the experiment is run as above described.

For running the mixture in A down to a low percentage in oxygen, an accessory reservoir full of nitrogen is used, connected up in the same way as B is shown in the sketch, and a rapid stream sent through as above described in the case of oxygen, until a sample shows the composition desired.

After considerable experimentation it was found that the best way to prepare nitrogen in large amounts was by passing air containing ammonia vapour over palladium asbestos in a combustion tube, heated by a Bunsen burner. The air is bubbled by means of a water pump through a bottle containing *strong* ammonia, and the nitrogen washed from nitric acid or excess of ammonia by passing through wash bottles containing (a) water or dilute acid, and (b) caustic soda. The palladium asbestos acts only catalytically and is much better than copper foil which requires a good deal of adjusting, a plug of a few centimetres loosely packed is enough. When the process starts, it generates almost enough heat to go by itself, the palladium asbestos glowing a bright red. If there is not enough ammonia a good deal of nitric acid is formed, and we have found that the ammonia is almost quantitatively converted into nitric acid; but this can be obviated by using strong ammonia in the bottle through which the air is bubbled before passing over the palladium asbestos. Under such conditions all the oxygen is burnt out and one obtains both the atmospheric nitrogen and the nitrogen of the ammonia, and 80 to 100 litres can easily be made in three to four hours with a single combustion tube in use.

The respiratory apparatus is fairly efficient for the class of experiment for which it was intended, of studying metabolism and gaseous exchange when different percentages of oxygen are present in the air breathed and the percentage of carbon-dioxide is kept at a low level, and it further presents the advantage of giving the materials to be analysed in a comparatively small volume.

It is intended in the future to use it for this purpose, and to determine the lowest percentage of oxygen at which life is possible for long periods, and the effect of such low oxygen pressures upon metabolism.

But for the purpose to which the discovery of the production of glycosuria by excess of carbon-dioxide in the air breathed led of studying the effects of different percentages of carbon-dioxide, it is not possible to use the apparatus without dispensing with the special advantage of not having to ventilate with considerable volumes of gaseous mixtures.

It was necessary, therefore, to abandon the exact study of the gaseous exchanges in presence of different percentages of carbon-dioxide, as we did not possess exact gas meters, and to study only the effects of such percentages in producing glycosuria, and the level of carbon-dioxide pressure at which such glycosuria appeared.

At first an attempt was made to limit the percentage of carbon-dioxide by means of using a quantity of soda-lime presenting an inadequate surface to give complete absorption, but it was found impossible to preserve even an approximate percentage of carbon-dioxide in this way, as the soda-lime absorbed too rapidly at first and then fell off as the experiment progressed, so that the pressure of carbon-dioxide continually increased.

The plan was then followed of leaving out the soda-lime entirely and adding, at the commencement of the experiment, carbon-dioxide, generated in a Kipp by action of acid on marble, until an analysis showed that the desired percentage of carbon-dioxide had been obtained, oxygen also being fed in, initially, where it was desired to keep the percentage of oxygen at, or above, the atmospheric amount. In this way, it was possible to soon get within the respiratory chamber a mixture containing a high percentage of carbon-dioxide (10 or 15 per cent. or higher), and at the same time as high a percentage of oxygen as in atmospheric air, or higher if so desired. This state of affairs having been established, the next procedure is to maintain it so with as little variation as possible throughout the experiment. For this purpose the bottle D was removed, and the apparatus joined here to a Bunsen's water pump, while the water manometer, previously mentioned as occupying the free end of the T piece between C and A, was also removed, and a bottle similar to C, communicating with the atmospheric air in a similar way to that in which C itself

communicated with the chamber B, was inserted in its stead. Two screw-down clips were then placed, one between B and C, and the other between the new wash-bottle and the atmosphere. In this way the total amount of mixed air and oxygen fed into A could be regulated by the speed at which the water pump was worked, and accordingly the amount of carbon-dioxide removed from the chamber could be altered and made equal to the rate at which the animal produced carbon-dioxide; further, by varying by means of the two screw clips, the relative rate at which air was let in through the new wash-bottle or oxygen through the wash-bottle C, the percentage in oxygen of the mixture admitted could be varied and so that in the chamber A, so that this gave an adjustment for preserving the oxygen percentage constant at any given level. Frequent samplings of the air of the chamber are necessary and corresponding adjustments of inlet and outlet, but with care approximately constant percentages of oxygen and carbon-dioxide can be maintained.

These adaptations could be much simplified by the placing in the circuit of three good meters, one on the outlet and one each upon the air inlet and oxygen inlet, but unfortunately, these were not at our command.

DESCRIPTION OF EXPERIMENTS

The experiments may be divided into those in which the percentage of oxygen was low and the percentage of carbon-dioxide high; those in which the percentage of oxygen was low and the percentage of carbon-dioxide kept at a minimal value or practically absent; and those in which while the pressure of oxygen was kept as high as in the atmosphere or higher, the percentage of carbon-dioxide was also high.

The evidence obtained by so varying the percentages of the two gases, shows that glycosuria occurs after the carbon-dioxide passes a certain percentage, amounting to 10 to 15 per cent., whether the oxygen pressure is high or low, and that low oxygen percentage, apart from accumulation of carbon-dioxide, does not induce glycosuria.

Experiment I.—Low percentage of oxygen and high percentage of carbon-dioxide. A cat of 2.6 kilograms was placed in the respiratory chamber with an insufficient amount of

soda-lime to absorb all the carbon-dioxide. The experiment lasted for sixteen hours, and the gases of the chamber at the end of the experiment showed 7·7 per cent. of oxygen and 10·3 per cent. of carbon dioxide. During the period the animal was in the chamber, 130 c.c. of urine was passed containing 3·1 per cent. of glucose.

Experiment II.—Low percentage of oxygen, but carbon-dioxide kept at as low a value as possible. A cat of 2·2 kilograms was used, the urine being previously tested and found free from sugar. Soda-lime in sufficient amount to absorb all the carbon-dioxide produced was put in, but instead of allowing oxygen to be sucked in to the partial vacuum caused by the absorption, a supply of nitrogen was allowed to pass in until the percentage of oxygen had fallen to about 7 per cent. A mixture of air and nitrogen was now aspirated through the chamber so as to keep the oxygen in the air breathed at between 5 and 6 per cent. for about four hours.

Analyses throughout the experiment gave the following results:—

Commencement at 2.10 p.m.	0·22 per cent. CO ₂ ,	6·9 per cent. O ₂
„ 3.25 „	0·33 „ „	5·9 „ „ „
„ 4.30 „	0·45 „ „	5·0 „ „ „
„ 5.30 „	0·56 „ „	5·7 „ „ „
„ 6.30 „	0·44 „ „	5·3 „ „ „

Here, the animal was subjected to a lower percentage of oxygen than in the previous experiment for over four hours, yet, in the absence of the high percentage of carbon-dioxide, the animal suffered no inconvenience and no glycosuria whatever resulted, 50 c.c. of urine being obtained free from sugar.

Experiment III.—High percentage of oxygen accompanied by high percentage of carbon-dioxide. A cat of 2·9 kilograms, the urine of which was free from sugar, was placed in the chamber and the percentage of carbon-dioxide was raised as rapidly as possible to 18 per cent., while at the same time, the percentage of oxygen was kept up by aspirating a mixture of carbon-dioxide and oxygen through the chamber. A mixture of air and oxygen with a little carbon-dioxide was now aspirated for a period of 5½ hours. During this period the percentage of oxygen never fell below 21 per cent., that of the carbon-dioxide varied between 18 and 22·5 per cent., as shown by the following table:—

Commencement at 11.45 a.m.	20·0 per cent. CO ₂ .	21·3 per cent. O ₂
„ 12.45 p.m.	22·7 „ „ „	21·1 „ „ „
„ 2.0 „	18·1 „ „ „	25·1 „ „ „
„ 3.0 „	20·0 „ „ „	22·1 „ „ „
„ 4.15 „	18·2 „ „ „	21·2 „ „ „

Experiment finished at 5 p.m.

No urine was passed during the experiment, but next morning, at 9 a.m., 225 c.c. of urine were obtained containing 1·1 per cent. of glucose. Total amount of sugar 2·48 grams, which is far in excess of the quantity which could be normally present in the animal's blood. The next sample of urine obtained was free from sugar.

Here, the percentage of oxygen throughout the experiment was at or above the amount present in atmospheric air, and yet the high percentage of carbon-dioxide led to glycosuria.

It may be noted that the animal was anaesthetised by the carbon-dioxide throughout the experiment.

Experiment IV.—High percentage of oxygen, with high percentage of carbon-dioxide in the rabbit. In this experiment a rabbit of 2·18 kilograms was placed in the chamber with no soda-lime. A mixture of carbon-dioxide and oxygen was aspirated through the chamber so as to raise the percentage of carbon-dioxide rapidly to 17 per cent., at the same time keeping the oxygen at least up to the atmospheric percentage. Air and oxygen were then aspirated through. The experiment was kept up for 5½ hours, the animal breathing during this time 18·7 to 27·4 per cent. of carbon-dioxide, and 21·1 to 25 per cent. of oxygen as shown by the following table of analyses :—

Commencement at 12 noon	11·2 per cent. CO ₂ ,	23·7 per cent. O ₂
„ 1 p.m.,	18·7 „ „ „	25·1 „ „ „
„ 2.10 „	27·4 „ „ „	22·6 „ „ „
„ 3.30 „	24·1 „ „ „	21·1 „ „ „
„ 4.45 „	21·5 „ „ „	23·4 „ „ „
„ 5.30 „	23·1 „ „ „	21·9 „ „ „

At the termination of the experiment 32 c.c. of urine were obtained, containing 2·0 per cent. of sugar. No urine had been passed next morning nor was passed during that day, but at the end of 36 hours, 107 c.c. were found, which was free from sugar.

Although the percentage of carbon-dioxide was as high in this case as in the preceding experiment with the cat, the rabbit was not anaesthetised by the carbon-dioxide ; it sat up during the experiment and did not appear to be in any way inconvenienced by the high percentage of carbon-dioxide.

Experiment V.—High percentage of oxygen, with high percentage of carbon-dioxide in the dog. A dog of 3·2 kilograms was placed in the respiratory chamber and the experiment was carried out exactly as above described in the case of the rabbit. The percentage of oxygen was practically that in the atmosphere during the experiment, which lasted for about three hours, while carbon-dioxide varied from 17·7 to 20·8 per cent.

The animal was completely anaesthetized and took about three-quarters of an hour to come completely round after removal.

Commencement at 11.25 a.m.	19·2 per cent. CO ₂ ,	22·3 per cent. O ₂
„ 12.15 p.m.	20·8 „ „ „	19·0 „ „ „
„ 1.20 „	17·7 „ „ „	22·5 „ „ „
„ 2.15 „	20·0 „ „ „	20·1 „ „ „

No urine was present at the close of the experiment at 2.30 p.m., but at 4.30 p.m. 85 c.c. were passed which contained 1·81 per cent. of sugar. The next quantity of urine passed contained no sugar.

Experiment VI.—High percentage of oxygen, but less than adequate amount of carbon-dioxide to produce glycosuria. This experiment and the succeeding one are given to show the limit of pressure of carbon-dioxide at which glycosuria begins to be produced, which lies between 10 and 15 per cent. The percentage varies somewhat with the animal; thus in Expt. I it was obtained with slightly over 10 per cent., in another animal 10 to 12 per cent. failed to give glycosuria, but 15 per cent. or over never failed to cause glycosuria.

The animal, a cat, weighing 2.6 kilograms, was placed in the chamber with no soda-lime present, at 2.15 p.m., and a current of air and oxygen, mixed, was aspirated slowly through the chamber.

Commencement at	2.15 p.m.	0.00 per cent. CO ₂ ,	21	per cent. O ₂
„	5.15 „	5.1 „	22	„ „ „
„	6.15 „	6.9 „	20.2	„ „ „
„	8.30 „	8.2 „	15.1	„ „ „
„	9.20 „	7.9 „	17.2	„ „ „

No sugar was found in the urine as a result of the experiment.

In another experiment with this same animal, in which the carbon-dioxide reached 12.4 per cent., 120 c.c. of urine were obtained with slightly over 1 per cent. of sugar.

Experiment VII.—Amount of carbon-dioxide kept below ten per cent., but oxygen percentage allowed to fall. The animal, a cat, weighing 2.9 kilograms, was placed in the chamber, soda-lime absent, and the CO₂ formed in its own respiration allowed to increase to about 8 per cent., when a current of air was aspirated through.

At	12.25 p.m.	7.9 per cent. CO ₂ ,	9.4 per cent. O ₂
1.35 „	9.3 „	7.2 „	„ „ „
2.45 „	6.8 „	11.2 „	„ „ „
4.45 „	8.4 „	9.3 „	„ „ „
6.00 „	9.9 „	7.2 „	„ „ „

No sugar was found in the urine.

In a subsequent experiment with the same animal, in which the percentage of carbon-dioxide varied between 13.3 and 14.5 per cent., a good reduction was obtained and sugar was shown to be present by the fermentation test.

In a third experiment with the same animal, in which the carbon-dioxide throughout the greater part of the experiment was kept at the high level of 17 to 21.9 per cent., and the oxygen, although less than atmospheric value, was still very much higher than in the above negative experiment, the highest percentage of sugar in the series was obtained, viz., 5.42 per cent. in 142 c.c., followed by 97 c.c. with 1.03 per cent., giving a total amount of 8.7 grams. The animal was anaesthetized throughout by the carbon-dioxide.

For contrast with the above experiment the following details are given.

A mixture of carbon-dioxide and oxygen was first passed in and then followed by a mixture of air and oxygen.

At 11.40 a.m.	14.6 per cent. of CO ₂ ,	20 per cent. of O ₂
12.5 p.m.	17.0 „ „ „	17.6 „ „ „
1.5 „	19.5 „ „ „	17.4 „ „ „
2.0 „	19.8 „ „ „	13.8 „ „ „
3.20 „	19.5 „ „ „	12.5 „ „ „
4.35 „	21.9 „ „ „	10.2 „ „ „
5.15 „	21.4 „ „ „	9.4 „ „ „

The percentage of carbon-dioxide required to produce the glycosuria in these experiments may appear to be a high one, viz., 10 to 15 per cent., but it must be remembered that this is a percentage by volume in a gaseous mixture, and that the corresponding concentration of the carbon-dioxide regarded as dissolved to form a solution would not be very high. Thus a normal solution being the molecular weight dissolved in a litre would have a pressure of 22.3 atmospheres, and hence a partial pressure of 15 per cent. of an atmosphere, which invariably produces glycosuria, would amount to $\frac{1}{22.3} \times \frac{15}{100}$ normal = $\frac{1}{148}$ N.

The production of glycosuria by the presence in the blood of such a small amount of weak acid like carbonic acid, suggested the idea that the sugar in diabetes might possibly be detached from combination with the proteid of the cells, by the agency of the weak organic acids such as β -oxy-butyric, and diacetic acid, which are commonly present in the blood in that disease.

It is exceedingly difficult to test this hypothesis experimentally, because it is necessary to anaesthetize in order to collect the urine, and to get the acid injected intravenously, and anaesthetization with chloroform, ether, or other anaesthetic, gives rise to a copious glycosuria, in presence of which it becomes almost impossible to judge what effect is being produced by the acid injection. As far as it was possible to judge the effect was negative, no increase of glycosuria resulting, but in some cases an apparent diminution. The question may, however, be one of dosage, and a *small* percentage of dilute organic acid, in the absence of other causative agent for glycosuria, might have a different result to a larger dose when glycosuria is already present. In connection with the anaesthetizing action of

the carbonic acid in the case of the cats and dog, it is interesting to note that all anaesthetics which have been tested to that end have been shown to produce glycosuria, such as ether, chloroform, nitrous oxide, morphia, etc.—there is no known exception to the rule.

If it be remembered that all the anaesthetics, as shown by Moore and Roaf,¹ possess the property of adsorbing and combining with the tissue proteins and in this way limiting the activity of bioplasm and its power of combining with bodies for the purposes of metabolism, as also with inorganic constituents, an easy explanation becomes apparent for the general effect of all anaesthetics in producing glycosuria.

There is little doubt that the carbohydrate of the cell is retained by a process of combination or adsorption with the protein of the cell. If, now, the protein is simultaneously offered any anaesthetic for combination and has a stronger attraction for this, in all probability the carbohydrate previously held in combination will be set free, and the percentage of sugar in the blood will be raised and lead to glycosuria.

The action of carbon-dioxide, ether and chloroform upon the carbohydrate of the serum, blood, and tissues, as an agent in producing glycosuria is at present being investigated by Moore, Edie, and Spence, and will be described in a separate paper.

SUMMARY

1. The glycosuria found after partial asphyxiation, is not due as hitherto supposed to lack of oxygen, but to the high percentage of carbon-dioxide in the respired air.

2. The presence of ten to fifteen per cent., by volume, of carbon-dioxide in the respired air leads to glycosuria, and this occurs even if the percentage of oxygen be over that present in the atmospheric air.

3. A low percentage of oxygen alone (less than six per cent.) unaccompanied by excess of carbon dioxide never produces glycosuria.

4. The high percentage of carbon-dioxide necessary to produce glycosuria causes also in the cat and dog, complete anaesthesia; in the

1. *Proc. Roy. Soc.*, Vol. LXXIII, p. 382, 1904; and B, Vol. LXXVII, p. 86, 1905. See also Edie, *Thompson Yates and Johnston Labs. Reports*, Part i, p. 195, 1905.

rabbit, glycosuria appears before anaesthesia, whether a higher percentage of carbon-dioxide would also produce anaesthesia in this animal was not observed.

5. Carbon-dioxide, accordingly, falls into the general rule that all anaesthetics produce glycosuria.

6. Since it is known that anaesthetics combine with proteins, this suggests that the glycosuria is due to combination occurring between anaesthetic and protein, and causing the setting free of carbohydrate from previous combination with protein.