STUDIES ON GLYCOSURIA: (A) CONSIDERATIONS OF MODES OF PRODUCTION OF GLYCOSURIA, AND THE CARBOHYDRATE, OXYGEN AND CARBON-DIOXIDE EQUILIBRIUM IN THE TISSUE-CELL. (B) RESPIRATION UNDER LOW OXYGEN PERCENTAGES AND UNDER HIGH CARBON-DIOXIDE PERCENTAGES IN THE RESPIRED AIR. (C) ANAESTHETIC GLYCOSURIA. (D) FEEDING EXPERIMENTS WITH PANCREAS AND INTESTINAL MUCOSA IN PANCREATIC GLYCOSURIA¹

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**SECTION A. CAUSATION OF GLYCOSURIA AND CARBOHYDRATE
EQUILIBRIUM**

The separate means by which sugar in the urine, or glycosuria, can be induced are very numerous, and at first sight seem most dissimilar and heterogeneous, so that the study of this signal of a disordered carbohydrate balance becomes most perplexing from the chaos due to a multiplicity of apparently unconnected causes.

1. Many drugs produce glycosuria, amongst which may be particularly mentioned curare, morphine, strychnine, amyl-nitrite, and phloridzin, as well known and frequently corroborated; but a careful testing of drugs would probably reveal dozens of others.

2. All general anaesthetics induce glycosuria in varying degree, and this has been especially noted in the case of the commonly used anaesthetics, ether and chloroform; but it has also been found in nitrous ether anaesthesia.

3. Partial asphyxiation by coal gas causes glycosuria; as also does carbon-monoxide alone.

1. The expenses of this Research have been defrayed from a grant from the Government Grant Committee of the Royal Society.

4. Breathing an atmosphere rich in carbon-dioxide (over 13 per cent.) even in presence of an ample amount of oxygen for respiratory purposes has been shown by Edie¹ to produce glycosuria in marked degree.

5. Many nervous lesions and stimulations occasion glycosuria. The most noted of these is the piqûre of Claude Bernard, but many other procedures such as section of the vagi lead to glycosuria. It is noteworthy here that all those nervous lesions which produce glycosuria as a result, either cause a partial asphyxia from interference with the nervous respiratory mechanism or produce a local vaso-dilation and venous condition of a large area.

6. Glycosuria appears under conditions of senility of tissues, and with local gangrene.

7. Temporary ligation of the abdominal aorta in rabbits is followed by glycosuria.

8. Stimulation of certain parts by adrenalin, such as painting the pancreas or liver, leads to glycosuria.

9. The specially severe form of glycosuria known as pancreatic diabetes follows complete removal of the pancreas.

This long array of causes of glycosuria is by no means exhaustive of the sources narrated in the literature, nor does it enter into details; but it is a sufficiently heterogeneous list to demonstrate the apparent multiplicity of routes by which the symptom, or condition, of glycosuria may be reached.

The problem, apart altogether from its relationship to disease, is a most important one in bio-chemistry, because it lies deep at the root of the explanation of carbohydrate transmission and storage in plants and in animals.

In later years it has become clearly realized that protein, fat and carbohydrate are woven together into one organic whole in the bioplasmic molecule.

A great colloidal molecule or system of molecules is elaborated in the cell, with protein molecules probably playing the central rôle, but these are in a kind of molecular union with fats and carbohydrates. The fats and carbohydrates are by no means free, unless present in great excess, yet they are in such a condition that on a change of equilibrium they can be rapidly broken loose and set free as complete fatty or carbohydrate molecules. No fat may be visible to the microscope in a well-fed liver which contains a high percentage as shown by extraction with ether;

1. This Journal, Vol. I, p. 455, 1906.

but interfere with the nervous-supply, or blood-supply, of the organ, and fat droplets at once begin to show in the cells.¹ Similarly, it has been shown that a liver showing no obvious glycogen to microscopic observation in its living cells, on death of the bioplasm, and disruption of the bond of union between protein and carbohydrate may yield much glycogen, or if left to itself after death yields much sugar, far more than could be present in solution at the plasma percentage. Hence, living tissue contains within it in water-clear form, invisible to the microscope in discrete particulate phase, and lost to ordinary chemical tests, both fat and carbohydrate in forms insoluble in water when set free from the bioplasm. It is the sheer excess of glycogen or starch only which appears as cell granules. Such granule formation is a plethora of stored-up food after all the cell protein is saturated and satisfied. The working allowance, in generous amount, is in union with the protein, and may rise and fall during metabolism without any discrete phase with granules separating out.

Also in the blood plasma itself, the fat and carbohydrate are carried round in union to large extent with protein. A perfectly clear serum or plasma with no emulsion visible in it may contain as much as one per cent. of fat in various forms; an amount which could not be dissolved by the water and salts of the plasma, and which is kept in solution by the other organic constituents of the serum. Experimental evidence has also accumulated to show that all the sugar of the blood is carried by the plasma, the corpuscles containing practically none of it, and it has been proven that the sugar is held in a feeble kind of union most probably with the serum proteins.

In any case, it has been shown in this laboratory by Edie and Spence² that the amount of sugar dialysing away from the serum into saline is increased by the addition of carbon-dioxide or of anaesthetics to the serum. Edie has moreover shown, as stated, that breathing a high excess of carbon-dioxide leads even in presence of excess of oxygen to marked glycosuria.

These now well known facts as to union of protein in labile delicate equilibrium with fats and carbohydrates, have been recited because they appear to us to furnish a common basis on which many, if not all, of the common causes of glycosuria may be correlated and somewhat better understood.

1. Bainbridge and Leathes, *Bio-Chemical Journal*, Vol. II, p. 25, 1907.

2. This Journal, Vol. II, p. 34, 1907.

The secretion of sugar by the kidney may arise from one of two primary factors, viz., a variation in the amount of sugar in free solution in the plasma in the direction of increase so that there is hyperglycaemia; or, secondly, an increased sensitivity of the renal secreting epithelium, so that sugar secretion follows with a lower than normal amount of sugar in the blood or hypoglycaemia, this leading in turn to a disturbance of the carbohydrate metabolism of the tissue cells and diffusion out of sugar from the cell to restore the sugar content of the plasma, so giving rise to a continued secretion.

The glycosuria of phloridzin is the only form which appears to be accompanied by a hypoglycaemia, and hence to be due to heightened renal sensitivity; all other forms so far as they have been investigated in this regard are associated with hyperglycaemia, and hence with something which has its seat at the tissue cells, disturbing the equilibrium in such a direction that sugar dissociates off unless the osmotic pressure of sugar in the plasma is kept higher than the renal cells are able to withstand without secreting sugar out into the urine.

In attempting to obtain a connected picture of the various types of glycosuria and to correlate their very heterogeneous causes, we are hence driven back to study the conditions of equilibrium of carbohydrate metabolism in the cell and how this may be disturbed by various movements of the concentrations of the different soluble products concerned in the maintenance of equilibrium.

The sugar which under the abnormal conditions of glycosuria appears in the urine, would under normal conditions be oxidized to carbon-dioxide and water.

As a preliminary step to such oxidation the sugar molecule must first be built in as an intrinsic part of the bioplasm, disappearing meanwhile as free carbohydrate. During the time that the carbohydrate is in labile union with the bioplasmic molecule, oxygen is also taken up by the bioplasm from the plasma, and the carbohydrate molecule being rendered catalytically unstable by the action of the rest of the bioplasmic molecule, just as an enzyme renders its substrate unstable, union with the oxygen becomes possible and carbon-dioxide is given off.

Now in this delicately balanced equilibrium of (1) the catalyst or bioplasmic molecule, (2) the carbohydrate, (3) the oxygen and (4) the carbon-dioxide formed in the reaction, variations of the concentrations, or osmotic pressures, of any member of the system will lead to corresponding alterations in rate of oxidation.

If we take first the central figure of the reacting system, viz., the bioplasm, it is obvious that the power of this for acting as a receptor for carbohydrate will vary with the pressure of carbohydrate in the plasma and with the pressures of other dissolved substances which might possibly replace the carbohydrate in the same position (or vacant niche) in the bioplasmic molecule. If, for example, the molecule of an anaesthetic has a stronger affinity for the bioplasm than the carbohydrate, then dependent upon the concentration of the anaesthetic in the plasma, a certain amount of anaesthetic will enter into union with the bioplasm and to that extent the bioplasm will be placed *hors de combat* for oxidation of carbohydrate. Thus part of the glycosuria of anaesthesia may arise in this way from direct interference with the bioplasm and so preventing it from acting as an energy machine for oxidizing the carbohydrate.

Next we may take the concentration of the substrate itself, namely, the carbohydrate, in the plasma. Here it is obvious that other things being equal the higher the pressure of carbohydrate in the plasma, the more will pass into union with the bioplasm, and hence be in a position for chemical manipulation. But a limit soon will be reached if more and more is poured in; as, for example, from very excessive carbohydrate alimentation or intravenous injection. Part of this excess is dealt with by being converted into higher carbohydrates and fats for reserve food-storage by certain of the tissue cells, and, as we have seen, all cells have wide limits of quite invisible storage before visible droplets or granules of fat or glycogen appear. But if a certain upper limit is surpassed we reach 'alimentary glycosuria.'

The third factor in the equilibrium is the osmotic pressure of the dissolved oxygen, and this might seem at first sight the most important factor, but experimentally it is found that here there is a very large factor of safety, before the ordinary oxidation processes of the cell suffer from lack of oxygen.

Experiments on the lowest amount of oxygen necessary to support life have shown that in some species of animals this can be done on as low as four to five per cent. of oxygen in the atmosphere instead of the normal twenty-one per cent. of atmospheric air.

We have specially tested whether sugar appears in the urine under such conditions where animals breathe for a prolonged period the lowest amount of oxygen compatible with life.

Our experiments have been made with cats, rats, and rabbits, and we have found that *provided the carbon-dioxide formed in respiration is not*

allowed to accumulate in the atmosphere breathed by the animals, there is neither glucose in the urine nor any of those other products of incomplete oxidation, such as oxalates, which have been mentioned by other observers.¹

In the experiments which we have carried out, instead of keeping the animals only for a period of an hour or so, as was formerly done,² the animals have been kept for over forty hours on five to six per cent. of oxygen, without any evil after effects, and *with absolutely no glycosuria*.

The fourth factor in the rate of carbohydrate oxidation, namely the osmotic pressure of the carbon-dioxide in the tissue-cell, is one which, in our opinion, has not yet received that attention which it deserves.

The form of chemical reaction most nearly allied to that of living cells is the enzymic action.

Here a colloidal catalyst, or chemical energy transformer, enters into a labile union with a substrate usually of an organic nature, and having united with it alters its chemical equilibrium, or balance of chemical potentials, in such a way that it is open to attack by a third body, such as an alkali or acid, and becomes hydrolysed, oxidised, or reduced, as the case may be. Now we know experimentally, and can easily understand on the general fundamental principles of chemical equilibrium, and kinetics of chemical reactions, that any accumulation of the products of reaction causing an increase in osmotic pressure and hence an increased chemical potential of these produced constituents, will act as a resistance upon the reaction, and with further increase finally bring it to a standstill with the different chemical potentials balanced against one another at certain definite osmotic pressures. The same thing has been shown conclusively for certain simple living organisms, such as the yeast plant, where the simpler constitution of the organism allows the reaction to be more definitely followed, and there is little doubt that this fundamental law of chemical kinetics holds for all living cells.

Returning now to the kinetic considerations of the production of glycosuria, as a result of hyperglycaemia, we readily see that the rate of utilization of sugar in the organism by oxidation, and hence the power of the various tissue cells to remove food sugar from the circulation and so prevent hyperglycaemia, may depend as much upon removal of carbon-dioxide as upon the presence of oxygen. In short, the reaction may be embarrassed and slowed as much from failure to remove carbon-dioxide

1. See Araki, *Zeitsch. j. physiol. Chemie*, Bd. XV, S. 335, 1891.

2. See v. Terray, *Arch. j. d. ges. Physiol.*, Bd. LXV, S. 393, 1897.

from the *rear* as from failure to supply oxygen in the *front* of the reaction.

Further, in the complex organism of the higher animals with its generous provision of a luxury of haemoglobin for carrying oxygen to the tissue, so that at any moment the oxygen pressure is at least four-fold what it needs to be for carrying on operations, the situation is actually reached that the rapid removal of carbon dioxide is even more exigent than the supply of oxygen.

For, although both arterial and venous blood carry more carbon-dioxide than oxygen, this is not the determining factor in respect to relative power of the blood as an oxygen-carrier and as a carbon-dioxide-carrier. The efficiency in this regard is determined by the variation in amount of dissolved gas, per unit variation in partial pressure of the gas. Now, round about the physiological working limits, on account of the chemical constitution of haemoglobin, there is a huge uptake or output of oxygen for a small variation of partial pressure of oxygen, which indicates the blood as a most excellent carrier of oxygen at these particular limits, keeping a very short *range* in the tissues of osmotic pressures of oxygen however the external partial pressure of oxygen may vary. There exists no such definite short range in the case of carbon-dioxide, for although various substances in the blood enter into labile union with carbon-dioxide and so act as carriers, there has none developed in the process of evolution with the definiteness of haemoglobin for oxygen.

Accordingly, if the partial pressure of carbon-dioxide in the lungs be varied by any procedures there is no very strong regulation of pressures, and the osmotic pressure of carbon-dioxide in the tissues will rise and fall fairly rapidly in a way which oxygen osmotic pressure never can do, until the very verge of asphyxiation is reached.

The same effects which follow variation of partial pressures in the lungs will follow upon impeded circulation, or stasis, in organs or gangrenous tissues. As the blood becomes more venous, the oxygen osmotic pressure only slightly decreases as it is pumped off from the reservoir in the haemoglobin, so that even in a very congested tissue the oxygen pressure may only be slightly below the normal, and oxidation processes are not embarrassed on this account. It is, however, quite otherwise with the carbon-dioxide pressures. In normal venous blood returning from an organ, practically all the special facilities possessed by proteins for holding carbon-dioxide in labile union are already used up. Blood cannot hold over fifty parts by volume of carbon-dioxide dissolved without the osmotic

pressure of dissolved carbon-dioxide mounting straight up in a purely physical fashion.

This fact has not hitherto been realised with any great clearness, but it is the key to the explanation of many important problems, including many of the forms of glycosuria with which we are here particularly concerned.

One must always be careful to distinguish an asphyxia due to excess of carbon-dioxide, from an asphyxia due to deficiency of oxygen. The former sets in under chronic conditions before the latter, and alters in a gradual way the whole life of the cell; the latter does not set in until there is nearly complete deprivation of oxygen, and then comes on in an acute way with rapid death.

Leaving the subject of glycosuria for a moment, we may perhaps be allowed to point out the important bearings of this point of view upon certain fundamental bio-chemical problems. There is, firstly, the chemical regulation of the rhythm of respiration following carbon-dioxide variations much more sensitively than the oxygen variations, as has been shown by Haldane and his co-workers. The bio-chemical basis for this is very clearly shown by the above reasoning. For any moderate variation in partial pressure of oxygen in the lungs, such, say, as a fall to 10 per cent. instead of 20 per cent., will practically not affect the chemical equilibria in the nerve cells of the medulla or any other tissues of the body; on the other hand, a doubling of the percentage of carbon-dioxide in the lungs will lead almost to a simple doubling of the carbon-dioxide pressure in the medullary cells and elsewhere. Such a doubling must affect seriously the whole life-processes of the cells, for oxidation cannot go on if the products of oxidation cannot be got rid of from the catalyst which is inducing the oxidation. There is thus, it will be seen, not the slightest need to invoke the aid of secondary products of incomplete oxidation, such as oxalates, lactates, etc.; such bodies are truly secondary, and to cite them as the causes of increased respiratory activity in defective oxidation is most assuredly putting the cart before the horse. At most, they can only be contributory as part of a vicious circle; but they are not primary products of the oxidation reaction which is being embarrassed. The carbon-dioxide, which is a product of the reaction, is the primary cause by its accumulation and heaped-up osmotic pressure of the main oxidizing reaction being embarrassed. Other subsidiary possible reactions, of which carbon-dioxide is not a product, or is a less abundant product, thereupon commence to run, with increased comparative velocity, and here arise the pathological

products such as lactates, etc. But, remove the excess of carbon-dioxide, and all the rest falls away, as the reaction returns to its normal channels. Short of this excessive embarrassment by greatly increased carbon-dioxide pressure, there are no less interesting effects, especially to the pathologist, produced by chronic action of a slight excess of carbon-dioxide. Many of the chronic tissue changes induced by back-pressure and venous engorgement may be ascribed to increased pressure of carbon-dioxide. In work previously carried out by some of us upon the growth of artificially fertilized echinus eggs, we found that it was essential to guard against accumulation of carbon-dioxide, and that *small* amounts of carbon-dioxide artificially added gave rise to increased and pathological cell-divisions. From this point of view it is interesting to note that parts subject to chronic venous congestion are the seats of election for growth of tumours, both benign and malignant, in both sexes.

More than this inimical amount of carbon-dioxide stops cell-division in the rapidly growing organism entirely, as we found in our experiments upon echinus eggs; and it is similarly found, as, for example, by Waller in the case of muscle and nerve, that very minimal increases in carbon-dioxide stimulate to increased activity, but that more decreases and finally abolishes activity.

Returning now to the more immediate question of glycosuria and its production, we find experimentally, as mentioned above, that animals can be kept at the lowest amount of oxygen in the air compatible with life, for a period of nearly two days, without any appearance of sugar in the urine; if, on the other hand, they are kept for a like period of time in an artificial atmosphere containing at least 20 per cent. of oxygen, but in which by diminishing the nitrogen, etc., the amount of carbon-dioxide has been raised to over 14 per cent. by volume, then glycosuria is the result, varying in amount with the type of animal.

The explanation of this has been traced out above in that even at the lowest level there is enough oxygen for oxidation, and so long as the carbon-dioxide is removed oxidation can go on practically unembarrassed in the quiet animal, fresh oxygen in sufficient amount being brought round by means of the haemoglobin from an atmosphere containing about 5 per cent. of oxygen. If now, even with over 20 per cent. of oxygen available, there is also a partial pressure of 15 per cent. of carbon-dioxide in the cell then this almost completely locks the carbon-dioxide arising from oxidation into the cell catalyst, and although there is abundance of oxygen, oxidation of carbohydrate must cease.

In addition to this action there is probably also the purely physico-chemical effect seen in the experiments of Edie and Spence, whereby the carbon-dioxide simply detaches carbohydrate from protein, probably taking its place by direct substitution.

These experimental findings of the action of carbon-dioxide in producing glycosuria, and the physical chemistry underlying them, lead us onward to a common understanding of many of the forms of glycosuria due to drugs, anaesthetics, and interferences with nervous mechanisms, or blood supply.

Taking first nervous disturbances, we find that the lesions are such as would lead in large areas to a venous condition with increased carbon-dioxide pressure. For example, the puncture of the fourth ventricle in Claude Bernard's experiment disturbs the vagus nucleus, and leads by inefficient ventilation of the lungs to a high level of carbon-dioxide pressure throughout the body. Again, the operation of double vagotomy leads to a similar result.¹

Also in two experiments not hitherto recorded, carried out by Moore and MacLean, in Liverpool, upon previously anaesthetized animals decerebrated by Sherrington's method, it was found that although the administration of anaesthetic was only kept up until decerebration was completed, that is, for a period of under half an hour; yet glycosuria persisted until the cessation of renal secretion, three or four hours later. It is, of course, impossible to decide clearly whether the continued glycosuria was due to the preliminary short anaesthetization or whether decerebration alone is capable of causing glycosuria; but here, although spontaneous respiration persists, there is no doubt that the condition is more venous than in the intact animal.

The continued glycosuria defeated the object of the experiments, which was to test the effects of removal of the pancreas afterwards, followed by intravenous injection of pancreatic extract.

The glycosuria in rabbits following temporary occlusion of the abdominal aorta may also arise from increased carbon-dioxide pressure during the tissue asphyxiation. The diabetes of gangrene also follows a

1. The original view of Bernard as to the cause of puncture diabetes, was that it was due to increased blood flow through the liver. In more recent times it has been suggested that this and other forms of glycosuria, and associated hyperglycaemia are due to asphyxia. (See Araki, *Zeitsch. f. physiol. Chemie*, Bd. XV, S. 335, 1891, Macleod, *Amer. Jnl. of Physiology*, 1907 *et seq.*) The main factor advanced here by previous workers has, however, been *deficiency of oxygen*, and not *increase of carbon-dioxide*, this we think is shown to be wrong by our negative results with excessively low oxygen pressures and positive results with high carbon-dioxide pressures. We suspect that certain of these results may have been due to carbon-dioxide and anaesthetic variations unsuspected by the authors. (See pp. 337-350 of this paper).

highly venous tissue condition. Partial general asphyxiation also leads to glycosuria.

The leading drugs which have been shown to cause glycosuria all possess an action which from different causes leads to an accumulation of carbon-dioxide in the circulation. Thus, curare induces paralysis of the respiratory system. So does morphia, and an overdose of morphia will by embarrassing the respiration lead to venous congestion and enhanced carbon-dioxide pressure in the tissues. Other narcotics, such as sulphonal, trional, and veronal, tested in this laboratory, upon animals, even in considerable doses did not lead to glycosuria, but these hypnotics do not cause such a generally venous condition as morphia. Amyl-nitrite, another drug which induces glycosuria, probably owes its effect, in part at least, to the general vaso-depression and venous condition which it induces. General lowering of blood-pressure alone is not sufficient without venous engorgement, for glycosuria does not occur after suprarenal removal, although the arterial system is under very low pressure. There is, however, in suprarenal inadequacy none of that flushing which follows nitrite administration due to venous congestion with high carbon-dioxide pressure in the tissues.

The glycosuria accompanying general anaesthetization, is probably due in part to the direct physico-chemical action of the anaesthetic in detaching carbohydrate from the protein in the bioplasm, in part to the condition of partial asphyxiation which the anaesthetic induces, and in part to the anaesthetic itself interlocking in the cell catalyst and arresting chemical action just as would carbon-dioxide, which is itself, from and on account of this action, a great natural anaesthetic.

Most of the apparently disconnected causes of glycosuria can accordingly be shown to be connected with an increased pressure of carbon-dioxide in the tissues, or the presence of bodies such as anaesthetics which act similarly to carbon-dioxide.

In the section on respiration under low oxygen pressures, the work of Araki is referred to, in which this author introduces a similar common explanation of (*a*) glycosuria due to reduced oxygen, and (*b*) glycosuria due to nervous lesions and many different drugs. The essential difference between our view and this of Araki, more recently revived by Macleod, is that we attach chief importance to carbon-dioxide increase rather than oxygen diminution.

Whether there is any similar condition after pancreatic removal would be exceedingly difficult to determine, since anaesthetization alone

leads to glycosuria. In fact, all experimental procedures are hampered by this unfortunate condition, and it has wrecked more than one scheme which we have laid for practical work on glycosuria.

So little stress is laid upon it by other workers who have obtained marked glycosuria after other experimental procedures, such as intravenous injections, that we venture to think there is a danger point here concerning which experimenters should be warned. At the commencement of our work we did not intend to work upon anaesthetic glycosuria. We had the usual amount of Hospital Ward information that after a severe and prolonged surgical operation a patient's urine might contain a little sugar, especially after chloroform anaesthesia. But we certainly did not suspect that an animal's urine within an hour after the induction of ether anaesthesia might usually contain 4 or 5 per cent., and occasionally had as much as 11 per cent., and that chloroform anaesthesia gave similar results with somewhat lower figures.

Since we had found that carbon-dioxide, a weak acid, had the physico-chemical property of detaching sugar from protein, we were led to suppose that an increased acidosis or more acid condition of the blood, such as usually obtains in diabetes, might similarly detach sugar and be the root cause of the glycosuria. On starting to experiment we at first thought our conjecture was correct, for on adding intravenously dilute acid (acetic, or β -oxy-butyric) we promptly obtained with the urine a marked reduction. However, in the next experiment, and within half an hour or so from the commencement of the experiment, *and before any acid had been added*, the urine was loaded up with sugar. This onset of anaesthetic glycosuria had misled us in the earlier experiment, and it has proved our *bête noir* ever since in all our work on glycosuria. Ruschhaupt¹ appears to have been the first to notice that ether administration in rabbits led to glycosuria, and the subject was afterwards worked out in detail by Seelig,² who reached the following conclusions:—

1. Ether inhalation in strong dogs always causes glycosuria.
2. Glycosuria is present throughout anaesthesia, even if this is kept up for seven hours.
3. The glycosuria disappears rapidly, clearing up in three to four hours.

1. *Arch. j. expl. Path. u. Pharm.*, XLIV, p. 140.

2. *Zeitsch. j. inn. Medizin*, XXIV, p. 221, 1903.

4. The urine of strong animals contained as much as 8 to 10 per cent., there being a lower percentage in weak or poor animals.

5. There was apparently hyperglycaemia accompanying the glycosuria.

Seelig further found that when ether inhalation was accompanied by intravenous oxygen injection there was no glycosuria; but that after glycosuria had set in it could not be removed by oxygen injection. This result, which would evidently connect glycosuria of ether inhalation with oxygen deficiency, has recently been confirmed by Macleod. It is however, difficult to conceive how ether anaesthesia would lead to a lower oxygen pressure than 5 per cent. in the respired air, which we have found never to lead to glycosuria, and also in certain of our own experiments with ether inhalation, we performed positive ventilation of the lungs with air, and there was certainly no oxygen defect which respiration in increased amount could avoid. We are hence driven to assume that there must be some other explanation for the non-appearance of glycosuria when oxygen is administered intravenously. The explanation may possibly be a physico-chemical one, implying not that the anaesthetic glycosuria is due to oxygen deficiency in the ordinary sense of the word; but the high partial pressure of oxygen produced by the venous administration may restore the balance so that the tendency to glycosuria, induced by such factors as ether pressure and carbon-dioxide pressure may be neutralized. There is, in fact, a combat between carbohydrate and oxygen on the one side, and ether and carbon-dioxide on the other for the valencies of the biogen molecule. If now oxygen pressure be heaped up, the scale may be turned away from glycosuria. That mere defect of oxygen alone can ever produce glycosuria is impossible, as far as we can see from our respiration experiments with low oxygen pressures.

SECTION (B).—EXPERIMENTS ON THE RESULTS OF RESPIRATION AT (*a*) LOW OXYGEN PARTIAL PRESSURES, AND (*b*) HIGH CARBON-DIOXIDE PARTIAL PRESSURES

A very large number of observers have studied the effects of respiration under low oxygen pressures from Mayow and Lavoisier onwards, and the greater number by far of these make no reference either to the presence of reducing substances in the urine or the presence of such substances as show incomplete oxidation, such as lactates and oxalates.

The few earlier observers who studied the condition, as to glucose,

of the blood and urine after partial asphyxia always worked upon animals breathing in a confined space under such conditions that oxygen partial pressure fell and carbon-dioxide partial pressure rose simultaneously, and yet they all with one accord state that the glycosuria or hyperglycaemia is due to lack of oxygen, disregarding the other factor altogether.

This view is persisted in, as has already been indicated, down to the present day, no all-round consideration having been accorded to all the factors concerned in the equilibrium and kinetics of the oxidizing reaction in the tissues.

Alvaro Reynoso,¹ as far as we have been able to trace the matter, was the first to find glycosuria after partial asphyxiation, then Claude Bernard² proved that prolonged partial asphyxiation removed the glycogen of the liver, the removal being accompanied by glycosuria. Dastre³ next investigated the matter, going into it more quantitatively. He found that an animal (dog) breathing free air had 0.128 per cent. of sugar in its blood; but when it breathed and re-breathed air for some time from a confined space the percentage in the blood rose to 0.253 per cent. The change was ascribed to lack of oxygen, but obviously the animal would at the same time be breathing a high percentage of carbon-dioxide, as in certain of our experiments recorded below.

Schiff⁴ showed that ligation of a limb caused glycosuria, and gave us an explanation that the coagulation of the blood gave rise to a ferment which increased the production of sugar in the liver. Lepine and Boulud⁵ ascribe such glycosuria to the production of 'leucomaines,' which interfere with glycolysis.

In our own opinion, the experiments below recorded furnish a much simpler explanation than either, viz., that the high carbon-dioxide pressure caused by the ligaturing of the limb causes the direct production of free glucose in the limb-tissues.

The only author we have been able to find who obtained both sugar and lactates in the urine after respiration of low percentages of oxygen, in absence, *as he supposed*, of any increased amount of carbon-dioxide, was Araki;⁶ another observer who found lactates and oxalates in the urine, after keeping animals in a cage which was being rapidly ventilated by a mixture of gases containing a very low percentage of oxygen, and no carbon-dioxide, was Paul v. Terray,⁷ but this observer found no sugar in

1, 2, 3. Cited from Dastre, *Comptes rendus*, LXXXIX, p. 669.

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

6, 7. *Loc. cit.*

the urine from his animals. It must be mentioned, however, that v. Terray's animals were treated for a much shorter period than those of Araki, so that sugar may not have had time to develop.

It is a most remarkable thing that though both these authors had their eyes open to the fact that the results of other workers were vitiated by the accumulation of carbon-dioxide at the same time that oxygen diminished, so that the two factors could not be dissociated, and although both of them arranged apparatus on their air current to deal with carbon-dioxide presence or accumulation, yet neither of them, as far as can be judged from their recorded papers, took any precaution to test whether their arrangements were efficient for the purpose intended.

That such arrangements were inefficient in point of fact is, we think, the explanation of the glycosuria obtained by Araki alone, and the presence of incompletely oxidized products such as oxalates and lactates obtained by both observers.

No analyses are given by either author of the air actually being breathed by the animals; but there is no doubt that there would be an accumulation of carbon-dioxide in both cases, and more particularly in the method of Araki. For v. Terray only sent the air through the chamber once, while Araki established a circuit, the air being, as he supposed, deprived of its carbon-dioxide while outside the chamber, by means of bubbling through strong potash before being returned to the chamber.

It is for this reason, we believe, that Araki obtained glycosuria, while v. Terray, like ourselves, obtained none.

The only effectual way, as we learnt by experience, is to deprive the air of its carbon-dioxide *in the actual respiratory chamber itself*, by an enormous surface of a powder such as soda-lime. Placing even a very large surface of strong caustic potash solution in the chamber itself is quite ineffectual, and merely bubbling the air through wash-bottles of strong caustic as it goes round in a pumped circuit is still worse. We have no hesitation from our own experience in stating that the air in the chamber used by Araki must have contained a high percentage of carbon-dioxide.

The method of placing a large tray or gallery of soda lime in the respiratory chamber has, moreover, the advantage in respiratory work, that rapid circulation is not required, with all its inherent difficulties, and the analytical errors which it introduces.

While in v. Terray's experiments, there would be no return of air containing carbon-dioxide to the respiration chamber, there is the serious

defect that the animal is breathing carbon-dioxide out into the chamber all the time, and usually such chambers are not large compared to the size of the animal inside. Hence, unless a veritable torrent of air is sent from the reservoir there must soon accumulate 2 or 3 per cent. of carbon-dioxide in the chamber. Even with a good water pump working full power, the rate of flow of air through the chamber would not keep the carbon-dioxide down below such a percentage.

In our own experiments, the percentage of carbon-dioxide was nearly always below 1 per cent., often considerably below that figure, and we have never found in the urine of animals, even when kept much longer than either Araki or v. Terray kept them, and at the lowest percentages of oxygen compatible with life (*viz.*, 4 to 5 per cent. in rabbits), any abnormalities in the way of products of decreased oxidative activity, such as sugar or oxalates. When, however, the carbon-dioxide percentage exceeds about 13 per cent., even in presence of 20 per cent. of oxygen, there has invariably followed in our experiments marked glycosuria with a considerable percentage of sugar.

For comparison with the absence of sugar after low oxygen percentage alone, we have included certain of the experiments showing production of glycosuria by carbon-dioxide.

We also include an experiment in illustration of the above remarks to demonstrate how inefficient the placing of a strong solution of caustic potash in the chamber, or bubbling the chamber air through caustic potash, is in removing carbon-dioxide.

The apparatus used for these experiments was the same as that used before by Edie for the experiments on glycosuria induced by high carbon-dioxide percentages, and hence need only be briefly described.

The animal is enclosed, with food and with a tray of circular shape containing soda-lime, in a space shut off by a water-seal, and draws in its own oxygen, from a supply made from potassium permanganate by heating and kept in a large reservoir. As the oxygen cannot without special difficulty be made more than 95 to 96 per cent. pure, an aspirator is set flowing at a correspondingly slower rate, so as to prevent nitrogen accumulation and keep the oxygen percentage constant, or nearly so, throughout the experiment. Initially, either nitrogen is flushed through the chamber until the oxygen percentage falls to the proper limit, or the animal is allowed to automatically use up oxygen, and as the carbon-dioxide is taken up by the soda-lime, air is allowed to be sucked into the

chamber until the percentage is shown in a sample drawn off and analysed in a gas burette, to have reached the desired low level.

The apparatus is shown in section in the accompanying sketch, and a general photograph of it as fitted up is also shown.

In certain experiments, instead of allowing the animal to suck in oxygen, and adjusting for the nitrogen percentage in the oxygen supply by a correspondingly slower exhaust into an aspirator,¹ it was arranged

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.

that there should be fed in an atmosphere of a known composition, and the gases of the respiration chamber were analysed at intervals during the experiment.

Experiment I.—Rabbit of 2.73 kilograms placed in cage with soda-lime at 9.40 a.m. The animal was allowed to suck in air automatically till percentage of oxygen fell to desired low percentage of about 6 per cent., which is near the lowest possible limit for a long experiment in the rabbit,

1. The mode of use of the aspirator is very simple. Supposing the oxygen fed in is 95 per cent. pure, and the internal air is to be kept adjusted at 7 per cent. of oxygen. Then every 100 c.c. of air sucked in introduces 5 c.c. of nitrogen which must be got rid of again; but if air inside has 93 per cent. of nitrogen, to get rid of 5 c.c. there must obviously be $5 \times \frac{100}{93} = 5.4$ c.c. run off by means of aspirator or, roughly, aspirator must work at about $\frac{1}{25}$ of rate of oxygen current, which can readily be arranged by a little manipulation.

and can barely be supported by some animals. This animal, however, stood an amount of barely over 4 per cent. for a considerable period, that is, about one-fifth only of the normal atmospheric amount. It is to be remembered, however, that the animal was perfectly quiet in the cage, with no muscular work save that of breathing.

The results of analyses of the air of the respiratory chamber at various periods in the experiment were as follows:—

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.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
9.40 a.m.	...	Commencement	...	Atmospheric
10.45 a.m.	...	1 hr. 5 min.	...	0.8 per cent. carbon dioxide 15.3 „ oxygen
1 p.m.	...	3 hrs. 20 min.	...	0.9 per cent. carbon dioxide 6.1 „ oxygen
1.30 p.m.	...	3 hrs. 50 min.	...	0.8 per cent. carbon dioxide 5.2 „ oxygen
3 p.m.	...	5 hrs. 20 min.	...	1.2 per cent. carbon dioxide 4.2 „ oxygen
4.45 p.m.	...	7 hrs. 5 min.	...	1.1 per cent. carbon dioxide 5.0 „ oxygen
6.30 p.m.	...	8 hrs. 50 min.	...	0.9 per cent. carbon dioxide 4.1 „ oxygen
8.30 p.m.	...	10 hrs. 50 min.	...	0.7 per cent. carbon dioxide 4.3 „ oxygen

The result of the experiment, through which the animal came quite well, was that 92 c.c. of urine were obtained at the end of the experiment, containing no sugar. Next morning a further 35 c.c. of urine were obtained also quite free from sugar. Oxalic acid was also absent from the urine. This experiment therefore clearly demonstrates that keeping the oxygen percentage at the lowest possible value compatible with life does not produce a trace of glycosuria, provided the carbon-dioxide percentage is not allowed to rise. In a subsequent experiment in which the carbon-dioxide pressure was allowed to mount up this same animal had glycosuria on a much higher percentage of oxygen. (See Experiment III.)

Experiment II.—The same animal and the same method used as in previous experiment.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
10 a.m.	...	Commencement	...	Atmospheric
12 noon	...	2 hrs.	...	0.9 per cent. carbon dioxide 11.8 „ oxygen
2.15 p.m.	...	4 hrs. 15 min.	...	0.7 per cent. carbon dioxide 5.1 „ oxygen
3.45 p.m.	...	5 hrs. 45 min	...	1.0 per cent. carbon dioxide 4.3 „ oxygen
6.30 p.m.	...	8 hrs. 30 min.	...	0.5 per cent. carbon dioxide 4.0 „ oxygen
8.45 p.m.	...	10 hrs. 45 min.	...	0.4 per cent. carbon dioxide 3.8 „ oxygen
9.15 a.m. (next morning)	...	23 hrs. 15 min.	...	0.7 per cent. carbon dioxide 4.5 „ oxygen

At the end of the experiment 155 c.c. of urine were obtained free from sugar. The next quantity of urine obtained was also free from sugar and oxalic acid.

Experiment III.—In this third experiment the same rabbit was used as in Experiments I and II, but without soda-lime to keep down the carbon-dioxide in the respiration chamber, and at the same time the gaseous mixture admitted to the chamber was forced through and not drawn in automatically by the animal. Moreover, the reservoir contained as mixed gases nitrogen, oxygen, and carbon-dioxide, blended in such proportions that the air in the chamber being breathed by the animal was rich in carbon-dioxide, but also so rich in oxygen that the percentage of this gas never fell below that in ordinary atmospheric air. As a result we can see in this experiment the effects of carbon-dioxide alone, dissociated from any possible effects due to reduction of oxygen. The

results should be compared with those of Experiments I and II, where oxygen alone was down to the extreme limit of viability, but without much accumulation of carbon-dioxide.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
10.15 a.m.	...	Commencement	...	—
10.45 a.m.	...	0 hrs. 30 min.	...	9.4 per cent. carbon dioxide 29.6 „ oxygen
12 noon	...	1 hr. 45 min.	...	14.7 per cent. carbon dioxide 26.9 „ oxygen
1.35 p.m.	...	3 hrs. 20 min.	...	22.0 per cent. carbon dioxide 22.5 „ oxygen
2.40 p.m.	...	4 hrs. 25 min.	...	26.5 per cent. carbon dioxide 21.6 „ oxygen
4.35 p.m.	...	6 hrs. 20 min.	...	26.4 per cent. carbon dioxide 22.7 „ oxygen
5.30 p.m.	...	7 hrs. 15 min.	...	27.9 per cent. carbon dioxide 21.4 „ oxygen

Experiment stopped at this period (7h. 15m.). No urine found in cage, but next morning 183 c.c. were found, giving a good reduction and showing on quantitative estimation 0.44 per cent. reckoned as dextrose. No oxalic acid found.

Experiment IV.—A different rabbit was used for this experiment weighing 1,726 grams. The animal was put in respiratory chamber with soda-lime. Nitrogen from a gas reservoir was then passed through, till the percentage of oxygen had fallen below 10 per cent., when connection was made with the oxygen reservoir, so that oxygen was sucked in as the expired carbon-dioxide was absorbed by the soda-lime. The aspirator bottle was adjusted so as to balance off small amount of nitrogen taken in with oxygen, and so keep percentage of oxygen in the respiratory chamber approximately the same at the low percentage.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
3.30 p.m.	...	Commencement	...	—
3.50 p.m.	...	0 hr. 20 min.	...	0.0 per cent. carbon dioxide 8.8 „ oxygen
8.50 p.m.	...	5 hrs. 20 min.	...	0.1 per cent. carbon dioxide 7.6 „ oxygen
8.55 a.m. (next morning)	...	17 hrs. 25 min.	...	0.2 per cent. carbon dioxide 7.8 „ oxygen
5 p.m.	...	25 hrs. 30 min.	...	0.0 per cent. carbon dioxide 7.6 „ oxygen
8.45 a.m. (second morning)	...	41 hrs. 15 min.	...	0.7 per cent. carbon dioxide 7.2 „ oxygen
3.45 p.m.	...	48 hrs. 15 min.	...	0.2 per cent. carbon dioxide 7.5 „ oxygen

The animal was not removed from the respiratory chamber during this period. It had moist bran in the chamber with it, which it ate at intervals. The urine was drawn off from the funnel-shaped bottom of the cage by putting on a little positive pressure inside and opening the clip on the rubber outflow tube.

In the first twenty-four hours 75 c.c. of urine were obtained, and in the next twenty-four hours 85 c.c. were found. Both lots were free from sugar, and also from acetone and oxalates. In the forty-eight hours the animal used up 50·45 litres of oxygen, and gave out 40·21 litres of carbon-dioxide, yielding a respiratory quotient of approximately 0·8.

The low value kept through this prolonged experiment for carbon-dioxide, and the constant low value for oxygen, make it, we think, a good test experiment for prolonged effects of low oxygen pressure. The animal appeared to suffer no serious injury from this prolonged stay in only about one-third of the atmospheric amount.

If deficiency of oxygen alone at such levels as are at all likely to obtain in diabetic piqûre, vagotomy, or morphia or similar drug poisoning, were at all likely to produce glycosuria, that condition ought to have appeared in this experiment, but there was not a trace of it actually found.

Experiment V.—In this experiment a cat of 2·73 kilos was used. The animal was put in the respiratory chamber with soda-lime in the tray. It was allowed to breathe air at first until the percentage of oxygen was reduced to about 11, after which the chamber was put in connection with the oxygen reservoir.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
10.15 a.m.	...	Commencement	...	—
11.15 a.m.	...	1 hr.	...	0·2 per cent. carbon dioxide 15·2 „ oxygen
12.45 p.m.	...	2 hrs. 30 min.	...	0·3 per cent. carbon dioxide 9·3 „ oxygen
2.40 p.m.	...	4 hrs. 25 min.	...	No carbon dioxide 10·8 per cent. oxygen
3.50 p.m.	...	5 hrs. 35 min.	...	No carbon dioxide 9·7 per cent. oxygen

The experiment was stopped at this stage, as the animal had considerable dyspnoea. Next morning about 20 c.c. of urine were obtained free from sugar and oxalates.

In another experiment on a cat weighing 2·4 kilograms, carried out in

the same manner as above described, no urine was obtained, the animal becoming asphyxiated, although the percentage of oxygen throughout was much higher than that borne successfully by the rabbit.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
10 a.m.	...	Commencement	...	—
11 a.m.	...	1 hr.	...	0.3 per cent. carbon dioxide 15.6 .. oxygen
12 noon	...	2 hrs.	...	0.3 per cent. carbon dioxide 10.9 .. oxygen
1.45 p.m.	...	3 hrs. 45 min.	...	No carbon dioxide 12.4 per cent. oxygen
3.30 p.m.	...	5 hrs. 30 min.	...	No carbon dioxide 11.4 per cent. oxygen

This experiment and the last show clearly that in the absence of carbon-dioxide cats become asphyxiated when the percentage of oxygen in the air breathed is reduced to about half the normal figure; whereas in the case of the rabbit existence is possible at a much lower limit of oxygen.

Experiment VI.—A rabbit of 1.7 kilograms was put in the respiratory chamber with soda-lime, and the experiment carried out exactly as above.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
10.30 a.m.	...	Commencement	...	—
12.30 p.m.	...	2 hrs.	...	No carbon dioxide 14.5 per cent. oxygen
4.15 p.m.	...	5 hrs. 45 min.	...	No carbon dioxide 8.4 per cent. oxygen
5 p.m.	...	6 hrs. 30 min.	...	No carbon dioxide 4.6 per cent. oxygen
6.30 p.m.	...	8 hrs.	...	No carbon dioxide 3.0 per cent. oxygen
6.45 p.m.	...	8 hrs. 15 min.	...	No carbon dioxide 4.9 per cent. oxygen
9.30 a.m.	...	23 hrs.	...	No carbon dioxide 3.5 per cent. oxygen

The rabbit became asphyxiated during the night, the percentage of oxygen being then 3.5. At 6.30 p.m. the animal was exhibiting marked dyspnoea, the percentage of oxygen having dropped as low as 3, owing to an accidental constriction in one of the rubber connections between the chamber and the oxygen reservoir.

About 10 c.c. of urine were obtained by cutting open the rabbit's bladder. This urine was free from sugar.

Experiment VII.—A rabbit of 1·85 kilograms was used in this case, the details of the experiment being the same as before.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
10 a.m.	...	Commencement	...	—
12.15 p.m.	...	2 hrs. 15 min.	...	No carbon dioxide 12·7 per cent. oxygen
1.30 p.m.	...	3 hrs. 30 min.	...	No carbon dioxide 9·7 per cent. oxygen
3.15 p.m.	...	5 hrs. 15 min.	...	No carbon dioxide 4·1 per cent. oxygen
3.45 p.m.	...	5 hrs. 45 min.	...	No carbon dioxide 13·3 per cent. oxygen
5.30 p.m.	...	7 hrs. 30 min.	...	No carbon dioxide 5·4 per cent. oxygen
7 p.m.	...	9 hrs.	...	No carbon dioxide 10·0 per cent. oxygen
9.30 a.m.	...	23 hrs. 30 min.	...	No carbon dioxide 7·3 per cent. oxygen

At the end of this experiment the rabbit was found asphyxiated. It had passed 100 c.c. of urine, free from sugar and oxalates. The urine in the animal's bladder was also free from sugar.

At 3.15 p.m., when the percentage of oxygen had dropped to 4·1, the animal showed dyspnoeic breathing, and was nearly asphyxiated. Hence it was thought advisable to increase the percentage of oxygen by washing out the chamber with a current of that gas until the animal had fully recovered. The experiment was then continued as usual until 6 p.m., when the animal was again partially asphyxiated.

Experiment VIII.—A rabbit weighing 1·76 kilograms was used for this experiment, which was carried out as already described.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
10.30 a.m.	...	Commencement	...	—
2.15 p.m.	...	3 hrs. 45 min.	...	No carbon dioxide 4·8 per cent. oxygen
2.45 p.m.	...	4 hrs. 15 min.	...	No carbon dioxide 10 per cent. oxygen
4.15 p.m.	...	5 hrs. 45 min.	...	No carbon dioxide 6·6 per cent. oxygen
6.30 p.m.	...	8 hrs.	...	No carbon dioxide 6·3 per cent. oxygen
11 a.m.	...	24 hrs. 30 min.	...	No carbon dioxide 8·3 per cent. oxygen

At the end of the experiment the rabbit was asphyxiated. It had passed about 150 c.c. of urine, containing neither sugar nor oxalates. At 2.15 p.m., the percentage of oxygen having dropped below 5, the animal began to be asphyxiated, for which reason it was relieved by a current of oxygen being rapidly passed through the chamber for a few minutes.

This was also done at 6.30 p.m.

In this experiment again we see that an animal which has been exposed to a percentage of oxygen sufficiently low to produce asphyxia cannot immediately afterwards breathe such a low percentage with safety.

Experiment IX.—Six rats were put in the respiratory chamber, and a mixture of carbon-dioxide and oxygen was used in order quickly to produce an atmosphere containing a high percentage of carbon-dioxide, with not less than the normal proportion of oxygen.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
11 a.m.	...	Commencement	...	—
11.25 a.m.	...	25 min.	...	9 per cent. carbon dioxide 24.2 „ oxygen
12 noon	...	1 hr.	...	20.3 per cent. carbon dioxide 23.4 „ oxygen
2.15 p.m.	...	3 hrs. 15 min.	...	21.4 per cent. carbon dioxide 25.3 „ oxygen
3.30 p.m.	...	4 hrs. 30 min.	...	21.1 per cent. carbon dioxide 27.3 „ oxygen

All of the rats were completely anaesthetised by the carbon-dioxide, and three succumbed during the course of the experiment.

Next morning 15 c.c. of urine were obtained from the surviving animals, and this contained 0.72 per cent. of sugar.

Experiment X.—In this experiment twelve rats were used in the manner described last.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
11.30 a.m.	...	Commencement	...	—
12.30 p.m.	...	1 hr.	...	14.9 per cent. carbon dioxide 29.1 „ oxygen
2.15 p.m.	...	2 hrs. 45 min.	...	18.2 per cent. carbon dioxide 29. „ oxygen
3.45 p.m.	...	4 hrs. 15 min.	...	28.2 per cent. carbon dioxide 23.3 „ oxygen
4.45 p.m.	...	5 hrs. 15 min.	...	25.6 per cent. carbon dioxide 23.4 „ oxygen

All the rats were anaesthetised in this experiment. At the end 10 c.c. of urine were obtained, free from sugar. This had probably been passed soon after the commencement of the experiment, when the animals became excited by the sudden inrush of carbon-dioxide. Next morning 14 c.c. of urine had been passed, containing 0·84 per cent. of sugar.

Experiment XI.—A cat weighing 2·6 kilograms was used in this case, and a mixture of carbon-dioxide and oxygen used to bring up the percentage of the former gas as rapidly as possible.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
11.20 a.m.	...	Commencement	...	—
12.10 p.m.	...	50 min.	...	3·3 per cent. carbon dioxide 22·5 „ oxygen
2.45 p.m.	...	3 hrs. 25 min.	...	20·3 per cent. carbon dioxide 26·7 „ oxygen
4.45 p.m.	...	5 hrs. 25 min.	...	20·5 per cent. carbon dioxide 24·8 „ oxygen
6 p.m.	...	6 hrs. 40 min.	...	18·4 per cent. carbon dioxide 25·5 „ oxygen

At this time 60 c.c. of urine were obtained, free from sugar. Next morning there were 62 c.c. containing 3·64 per cent. of sugar.

This shows that even in presence of 25 per cent. of oxygen a high proportion of carbon-dioxide in the air breathed produces very marked glycosuria in cats.

Experiment XII.—It has already been mentioned that we have found the attempt to absorb carbon-dioxide by means of potassium hydrate solution unsatisfactory, even when a large surface of solution was exposed to the products of respiration. This experiment shows how rapidly carbon-dioxide can accumulate in such a case.

A cat weighing 2·66 kilograms was put in the respiratory chamber, and in the bottom of the latter 350 c.c. of a 30 per cent. solution of potassium hydrate was placed, contained in a shallow flat-bottomed glass dish, about seven inches in diameter. The animal was allowed to breathe air until the percentage of oxygen in the chamber had fallen to about 16 by the accumulation of nitrogen, at which point the apparatus was put in connection with the oxygen reservoir, and the experiment continued

as in those cases where it was intended to keep the animal breathing a diminished proportion of oxygen, free from carbon-dioxide.

Clock time		Time Interval from beginning of experiment		Composition of chamber air
9.45 a.m.	...	Commencement	...	—
12 noon	...	2 hrs. 15 min.	...	10.5 per cent. carbon dioxide 8.4 .. oxygen
12.40 p.m.	...	2 hrs. 55 min.	...	12.4 per cent. carbon dioxide 6.6 .. oxygen

It will be seen that in three hours the carbon-dioxide produced by the animal accumulated in the chamber to over 12 per cent., this representing nearly the total quantity which an animal of this size would produce in that time. This shows that potassium hydrate, employed in the above-mentioned manner at least, is of practically no use as an absorbing agent for carbon-dioxide.

In the evening of the same day the cat was again put in the chamber, another freshly-made solution of potassium hydrate being used in the same way as before. In the course of two hours the carbon-dioxide had accumulated to 7 per cent., and the experiment was stopped. Next morning 120 c.c. of urine were obtained, with 1.1 per cent. of sugar.

It will be seen that cats are much more sensitive to diminution in the proportion of oxygen in the air breathed than rabbits, and at the same time a considerably higher percentage of sugar is generally obtained in the urine, when the animals are made to breathe air containing a high proportion of carbon-dioxide.

SECTION C. ANAESTHETIC GLYCOSURIA

Some incidental account of the literature of glycosuria following anaesthesia by ether has already been given (p. 336). In the following brief outline of the literature of glycosuria due to chloroform anaesthesia we have borrowed a great deal from the valuable paper of W. H. Thompson¹ on 'Anaesthetics and Renal Activity.'

The presence of a reducing body in the urine after chloroform narcosis appears to have been noticed even in the earlier days of anaesthesia; but, whether the reducing body is actually glucose, in most cases would appear even now hardly to be settled.

1. *Brit. Med. Jour.*, p. 613, March, 1906.

Hegar and Kaltenbach¹ early experimented on the subject, and seem to have been the first to come to the conclusion that the reducing body was not glucose. Zweifel² found traces of chloroform in such post-anaesthetic urine, which of course would alone reduce Fehling's solution, but also confirmed the presence of another reducing substance, which he stated was not either uric acid or sugar. Kast³ stated that the reducing property of the urine after chloroform anaesthesia was lost on removal of an organic chlorine compound. This author thought that carbohydrates are not increased, and that the reducing body is in all probability analogous to uro-chloralic acid (tri-chlor-ethyl-glycuronic acid) derived by oxidation from the chloroform and subsequent combination with the glycuronic acid. Nachod⁴ attributes the reduction to an increase of kreatinin.

Thompson⁵ found osazone crystals in five cases out of seven after chloroform anaesthesia; but states that in the majority of cases the crystals were unlike those of glucosazone in appearance, and gave melting points in impure conditions ranging about 130° C. In two cases there were undoubtedly glucosazone crystals present as well; the appearance was typical, and after fermentation with yeast they were no longer obtainable. The fermentation test gave unsatisfactory and inconclusive results in the other cases. No quantitative determinations of reducing power are recorded in this paper, which deals with many other aspects of renal activity after anaesthetics.

Thompson made examinations for osazone crystals after ten cases of ether inhalation in animals, and only obtained typical glucosazone crystals in two cases in which morphine had also been administered. Only four out of eight of the other animals gave crystals, and these were different from glucosazone crystals and resembled those seen after chloroform. The quantity, judged roughly, was smaller than after chloroform anaesthesia, and the frequency of occurrence also less in the case of ether. The substance yielding the crystals was probably the same in both cases.

1. *Virchow's Arch.*, Bd. XLIX, S. 43, 1869.
2. *Berl. klin. Wochensch.*, S. 245, 1874.
3. *Berl. klin. Wochensch.*, S. 377, 1888.
4. *Arch. f. klin. Chirurg.*, Bd. LI, S. 646, 1895.
5. *Loc. cit.*

In the experiments recorded below, the reducing substance was without doubt glucose, both after ether inhalation and chloroform inhalation. The substance was present in large amount and gave a quite typical reduction of Fehling altogether unlike creatinin, uric acid and similar bodies. The crystals, obtained in abundance, were undoubtedly glucosazone crystals; both by microscopical appearance, and by the fact that on careful purification by re-crystallization from hot alcohol and drying, they gave a melting point of 205° C. to 210° C., which agrees with that of glucosazone.

The animal used for these experiments was the dog, which received no other anaesthetic or drug than that being investigated; and the anaesthesia was kept complete during the experiment, at the expiration of which the animal was killed. As pointed out above, we came only incidentally to the study of anaesthetic glycosuria, our first objective being the effect of acidosis on production of glycosuria. Hence, in the earlier experiments, there are intravenous injections of dilute organic acids, as noted in the schedules (column three of each table); but the experiments are not invalid on this account, since, first, there is no rise in amount of glycosuria after the acid injection, showing that increased acidosis apparently has no effect upon glycosuria, and, secondly, exactly similar results are shown by the later experiments where intravenous injections of acid were not made, or were made much later in the experiment with no appreciable effect upon the glycosuria.

In case where the secretion of urine was scanty or failing, isotonic or hypotonic saline intravenous injections were made. It is remarkable how comparatively little of such intravenously injected saline reappears in the urine, although there is undoubtedly an appreciably increased diuresis. Incidentally we may mention that we often noticed very much increased secretion into intestine and peritoneal cavity after such saline injections.

The urine was collected by a cannula inserted into the bladder; the injections were made into the jugular vein.

The sugar determinations were made by precipitating the cuprous oxide in considerable excess of Fehling's solution, filtering into a Gooch crucible, and determining as cupric oxide by gravimetric analysis. This method we have found gives much more accurate results than a Fehling's volumetric analysis in any of the usual modifications, and when a large number of determinations are to be made, does not take a very great deal more labour.

Experiment I.—Dog of 5 kilograms. Ether anaesthesia. Animal under anaesthetic at 11-50 a.m.

Clock_time	Time interval from start of experiment	Amounts injected of N/10 lactic acid +0.45 per cent. sodium chloride	Amount of urine excreted during each interval	Weight of dextrose excreted during each interval	Percentage of dextrose during each interval
a.m. p.m.	Minutes				
11.57—12.12	0—15	nil	0.7 c.c.	nil	nil
p.m. p.m.					
12.12—12.27	15—30	46 c.c.	1.15 c.c.	0.006	0.56
12.27—12.42	30—45	54.5 c.c.	1.2 c.c.	0.005	0.43
12.42—12.57	45—60	85.8 c.c.	1.8 c.c.	0.043	2.49
12.57— 1.12	h. m. h. m. 1 0 — 1 15	115 c.c.	2.6 c.c.	0.055	2.12
1.12— 1.27	1 15— 1 30	111 c.c.	4.0 c.c.	0.071	1.79
1.27— 1.42	1 30— 1 45	111 c.c.	7.3 c.c.	0.126	1.73
1.42— 1.57	1 45— 2 0	111.5 c.c.	5.8 c.c.	0.098	1.68
1.57— 2.6	2 0 — 2 9	65.5 c.c.	0.8 c.c.	0.015	1.93
2.7 — 2.22	2 10— 2 25	nil	1.6 c.c.	0.024	1.50
2.22— 2.37	2 25— 2 40	nil	7.2 c.c.	0.087	1.22
2.37— 2.52	2 40— 2 55	„	5.8 c.c.	0.198	1.71
2.52— 3.7	2 55— 3 10	„	5.8 c.c.		
3.7 — 3.22	3 10— 3 25	„	3.8 c.c.	0.142	1.82
3.22— 3.37	3 25— 3 40	„	4.0 c.c.		
3.37— 3.52	3 40— 3 55	„	3.9 c.c.	0.111	1.85
3.52— 4.7	3 55— 4 10	„	2.1 c.c.		
4.7 — 5.7	4 10— 5 10	„	9.8 c.c.	0.068	0.70
5.7 — 6.7	5 10— 6 10	„	7.1 c.c.	0.082	1.15
Totals and averages : Total period		Total volume injected	Total urine excreted	Total weight of dextrose	Average percentage of dextrose
6 h. 10 m.		700 c.c.	76.4 c.c.	1.133 grams	1.49

NOTES.—At 2.6 p.m. flow of urine was stopping so injection of dilute lactic acid was discontinued.

The animal later became oedematous and ascitic fluid was secreted in large amount. These injections of dilute acid fluid were not the cause of the glycosuria as shown by subsequent experiments.

Experiment II.—Dog of 4.43 kilograms. Ether anaesthesia. Cannulae in bladder and external jugular vein. Urine commenced to be collected at 12.45 p.m.

Clock time		Time interval from start of experiment	Amount injected of N/5 hydro- chloric acid	Amount of urine excreted during each interval	Weight of dextrose excreted during each interval	Percentage of dextrose during each interval
p.m.	p.m.	minutes				
12.45	—1.15	0—30	nil	1.4 c.c.	nil	nil
1.15	—1.45	30—60	50 c.c.	1.1 c.c.	nil	nil
		h. m. h. m.				
1.45	—2.15	1 0 —1 30	50 c.c.	1.0 c.c.	0.018	1.83
2.15	—2.45	1 30—2 0	50 c.c.	3.0 c.c.	0.009	0.33
2.45	—3.15	2 0 —2 30	nil	1.6 c.c.	0.089	5.56
3.15	—3.45	2 30—3 0	„	2.0 c.c.	0.052	2.62
3.45	—4.15	3 0 —3 30	„	1.5 c.c.	0.004	0.25
4.15	—4.45	3 30—4 0	„	1.5 c.c.	0.002	0.13
4.45	—5.15	4 0 —4 30	„	2.4 c.c.	0.024	0.99
5.15	—5.45	4 30—5 0	„	2.3 c.c.	0.025	1.08
5.45	—6.15	5 0 —5 30	„	2.9 c.c.	0.034	1.18
6.15	—6.45	5 30—6 0	50 c.c.	3.9 c.c.	0.027	0.70
6.45	—7.15	6 0 —6 30	nil	4.4 c.c.	0.040	0.91
7.15	—7.45	6 30—7 0	50 c.c.	4.0 c.c.	0.039	0.98
7.45	—8.15	7 0 —7 30	30 c.c.	4.1 c.c.	0.014	0.34
Totals and averages :		Total period	Total volume injected	Total urine excreted	Total weight of dextrose	Average percentage of dextrose
		7 h. 30 m.	280 c.c.	37.1 c.c.	0.377 grams	1.02

NOTES.—From 2.45 to 3.15 p.m. onwards, the animal required scarcely any anaesthetic to keep under deep anaesthesia. About 8 p.m. it was noticed that the stomach was enormously distended by gases. A post-mortem examination showed nothing abnormal in naked-eye appearances.

Experiment III.—Dog of 10·22 kilograms. Ether anaesthesia. Cannulae in bladder and external jugular vein. Fluid injected was N/2 acetic acid. Collection of urine commenced at 11.54 a.m.

Clock time	Time interval from start of experiment	Amount injected of N/2 acetic acid	Amount of urine excreted during each interval	Weight of dextrose excreted during each interval	Percentage of dextrose during each interval
a.m. p.m.	minutes				
11.54—12.24	0 30	nil	1·4 c.c.	nil	nil
p.m. p.m.					
12.24—12.54	30—60	100 c.c.	0·8 c.c.	slight trace	
12.54—1.24	h. m. h. m.				
	1 0 — 1 30	nil	3·6 c.c.	Reduction increasing in amount all the time but precipitate of cuprous oxide is very fine and can- not be filtered off.	
1.24—1.54	1 30—2 0	„	3·4 c.c.		
1.54—2.24	2 0 — 2 30	„	3·5 c.c.		
2.24—2.54	2 30—3 0	„	4·2 c.c.		
2.54—3.24	3 0 — 3 30	„	7·4 c.c.	0·344	4·66
3.24—3.54	3 30—4 0	„	7·0 c.c.	0·306	4·37
3.54—4.24	4 0 — 4 30	„	7·0 c.c.	0·303	4·33
4.24—4.54	4 30—5 0	„	6·0 c.c.	0·291	4·85
4.54—5.24	5 0 — 5 30	„	4·3 c.c.	0·174	4·05
5.24—5.54	5 30—6 0	„	4·0 c.c.	0·135	3·37
5.54—6.24	6 0 — 6 30	„	3·6 c.c.	0·118	3·27
6.24—6.54	6 30—7 0	„	2·8 c.c.	0·045	1·60
6.54—7.24	7 0 — 7 30	„	1·9 c.c.	0·042	2·23
7.24—7.54	7 30—8 0	„	4·6 c.c.	0·103	2·24
7.54—8.24	8 0 — 8 30	„	3·8 c.c.	0·074	1·94
8.24—8.54	8 30—9 0	„	2·6 c.c.	0·056	2·14
8.54—9.24	9 0 — 9 30	„	2·6 c.c.	0·052	2·26
9.24—9.54	9 30—10 0	„	3·6 c.c.	0·074	2·07
9.54—10.24	10 0 —10 30	„	2·6 c.c.	0·062	2·40
10.24—10.54	10 30—11 0	„	2·8 c.c.	0·030	1·08
10.54—11.24	11 0 —11 30	„	1·8 c.c.	0·037	2·06
11.24—11.54	11 30—12 0	„	1·9 c.c.	0·029	1·54
p.m. a.m.					
11.54—12.24	12 0 —12 30	„	2·8 c.c.	0·050	1·80
a.m. a.m.					
12.24—12.54	12 30—13 0	„	4·6 c.c.	0·051	1·12
Totals and averages :	Total period	Total volume injected	Total urine excreted	Total weight of dextrose	Average percentage of dextrose
	13 hrs.	100 c.c.	94·6 c.c.	2·376 grams	2·51

NOTES.—The duration of ether anaesthesia here is for the remarkably long period of thirteen hours. It was noticed again that much less anaesthetic was required as the experiment progressed. The glycosuria persisted throughout the whole interval.

Experiment IV.—Dog. Ether anaesthesia. Cannulae in bladder and external jugular vein. Collection of urine commenced at 11.15 a.m.

Clock time	Interval from start	Amount of urine excreted during each interval	Weight of dextrose excreted in urine	Percentage of dextrose in urine
a.m. a.m.	minutes		grams	
11.15—11.45	0—30	3.0 c.c.	0.128	4.27
p.m.				
11.45—12.15	30—60	2.0 c.c.	0.092	4.62
p.m.	h. m. h. m.			
12.15—12.45	1 0 —1 30	1.2 c.c.	0.029	2.45
12.45—1.15	1 30—2 0	1.0 c.c.	0.035	3.51
1.15—1.45	2 0 —2 30	0.2 c.c.		
1.45—2.15	2 30—3 0	1.3 c.c.	0.036	2.43
Totals and averages	Total period	Total urine excreted	Total glucose excreted	Average percentage of dextrose in urine
	3 hours	8.7 c.c.	0.320 grams	3.70

NOTES.—The post mortem examination in this experiment revealed little of interest. The kidneys and liver appeared sclerosed, and the pancreas was very pale. The lungs were normal.

Experiment V.—Dog. Ether anaesthesia. Cannulae in bladder and external jugular vein. Commenced to collect urine at 1 p.m.

Clock time	Interval from start	Amount of urine excreted during each interval	Weight of dextrose excreted in urine	Percentage of dextrose in urine
p.m. p.m.	h. m. h. m.		grams	
1.0 —1.30	0 0 —0 30	4 c.c.	0.078	1.94
1.30—2.0	0 30—1 0	8.2 c.c.	0.479	5.84
2.0 —2.30	1 0 —1 30	6.7 c.c.	0.764	11.40
2.30—3.0	1 30—2 0	6.2 c.c.	0.645	10.40
3.0 —3.30	2 0 —2 30	6.0 c.c.	0.697	11.62
3.30—4.0	2 30—3 0	6.2 c.c.	0.581	9.38
4.0 —4.30	3 0 —3 30	5.0 c.c.	0.429	8.59
4.30—5.0	3 30—4 0	5.5 c.c.	0.467	8.50
Totals and averages:	Total period	Urine excreted	Total dextrose in urine	Average percentage of dextrose in urine
	4 hours	47.8 c.c.	4.140 grams	8.66

This experiment is remarkable for the high percentage of dextrose found in the urine throughout the whole period. The presence of dextrose was proved by examination of the osazone crystals prepared from the urine.

Experiment VI.—Dog of 5.01 kilograms. Chloroform anaesthesia. Cannula in bladder. Commenced to collect urine at 12 noon.

Clock time	Interval from start	Amount of urine excreted during each interval	Weight of dextrose excreted in urine	Percentage of dextrose in urine
p.m.	h. m. h. m.		grams	
12.0 —12.30	0 0 —0 30	7 c.c.	0.071	1.01
p. m.				
12.30— 1.0	0 30—1 0	0.2 c.c. }	0.519	3.53
1.0 — 1.30	1 0 —1 30	14.5 c.c. }		
1.30— 2.0	1 30—2 0	11.4 c.c.	0.254	2.23
2.0 — 2.30	2 0 —2 30	5.2 c.c.	0.095	1.82
2.30— 3.0	2 30—3 0	5.4 c.c.	0.138	2.55
3.0 — 3.30	3 0 —3 30	11.1 c.c.	0.251	2.26
3.30— 4.0	3 30—4 0	6 c.c.	0.136	2.27
Totals and averages :	Total period	Total urine excreted	Total dextrose in urine	Average percentage of dextrose in urine
	4 hours	60.8 c.c.	1.464 grams	2.41

NOTES.—In this case again the presence of dextrose in the urine was proved by an examination of the osazone prepared from it. A post mortem examination showed that the animal's stomach was greatly distended with gas, which contained 29.7 per cent. of carbon-dioxide, and 1.1 per cent. of oxygen. Otherwise nothing abnormal was observed.

Experiment VII.—Dog of 8.62 kilograms. Ether anaesthesia. Cannula in bladder. Urine commenced to be collected at 12.15 p.m.

Clock time	Interval from start	Amount of urine excreted during each interval	Weight of dextrose excreted in urine	Percentage of dextrose in urine
p.m. p.m.	h. m. h. m.		grams	
12.15—12.45	0 0 —0 30	1.1 c.c.	—	—
12.45— 1.15	0 30—1 0	0.2 c.c. }	0.034	7.55
1.15— 1.45	1 0 —1 30	0.2 c.c. }		
1.45— 2.15	1 30—2 0	0.05 c.c. }	0.362	4.31
2.15— 2.45	2 0 —2 30	0 }		
2.45— 3.45	2 30—3 30	8.4 c.c.	0.206	5.90
3.45— 4.15	3 30—4 0	3.5 c.c.		
Totals and averages :	Total period	Total urine excreted	Total dextrose in urine	Average percentage of dextrose in urine
	4 hours	13.45 c.c.	0.602 grams	4.47

NOTES.—The excretion of urine was extremely slow at first in this experiment, consequently at 2.45 p.m. 200 c.c. of 0.5 per cent. sodium chloride solution were injected intravenously. No abnormal features were observed on a post mortem examination being made. The animal had been kept without food for a day previous to the commencement of the experiment.

Experiment VIII.—Dog of 9·65 kilograms. Ether anaesthesia. Cannula in bladder. Commenced to collect urine at 11.30 a.m.

Clock time	Interval from start	Amount of urine excreted during each interval	Weight of dextrose excreted in urine	Percentage of dextrose in urine
a.m. p.m.	h. m. h. m.		grams	
11.30—12.30	0 0 —1 0	4·7 c.c.	0·052	1·11
p.m.				
12.30—1.30	1 0 —2 0	4·6 c.c.	0·081	1·76
1.30—2.30	2 0 —3 0	5·6 c.c.	0·087	1·56
2.30—3.30	3 0 —4 0	4·7 c.c.	0·085	1·80
3.30—4.30	4 0 —5 0	1·8 c.c.	0·056	3·11
4.30—5.30	5 0 —6 0	4·0 c.c.	0·061	1·52
5.30—6.0	6 0 —6 30	6·1 c.c.	0·085	1·39
6.0 — 6.30	6 30—7 0	4·4 c.c.	0·071	1·62
Totals and averages :	Total period	Total urine excreted	Total dextrose in urine	Average percentage of dextrose in urine
	7 hours	35·9 c.c.	0·578 grams	1·61

NOTES.—The dog used in this experiment had been fed principally on carbohydrates, but had received no food during the 24 hours preceding the experiment.

As the secretion of urine was very slow, 200 c.c. of water were run into the oesophagus at noon, and another 100 c.c. at 4.30 p.m.

SECTION D.—FEEDING EXPERIMENTS WITH EXTRACTS OF PANCREAS, INTESTINAL MUCOSA, AND LIVER IN PANCREATIC GLYCOSURIA

In two successful experiments in which the pancreas had been removed in dogs under deep anaesthesia, the animals were given along with their food (*a*) pancreatic extract, (*b*) pancreatic extract to which extract of intestinal mucosa (duodenum) had been added, treated as in preparing secretin, and (*c*) a combination of extracts of pancreas, duodenum and liver. In the first of these two experiments the sugar output fell to zero, after a marked drop, so soon after administration of pancreas *plus* duodenum *plus* liver extract had been begun, as to appear a consequence of it. But next day sugar was present, although in diminished amount, and the third day the output of sugar was back to its original amount, and remained there in spite of administration of the combined extracts until the animal died three days later. No such result could be repeated with the second dog, and we cannot furnish any

explanation of this seemingly chance variation in sugar output, with entire absence for one whole period of twenty-four hours.¹

In the first dog acetone and di-acetic acid were absent throughout the entire period; in the case of the second animal they were just as invariably present in large amount. Although the feeding results proved negative as regards any effect upon the glycosuria, the course of the diabetes and determinations not only of output of sugar, but that of urea, ammonia, acidity, and total organic acids of the urine, phosphates, etc., are sufficiently interesting to quote the two experiments in tabular form.

The amount of urine is given in c.c., and the total daily output of (a) sugar (gravimetric), (b) total urea (Dupre), (c) ammonia (Schlössing), (d) acidity and total organic acids (Edie & Whitley), (e) total phosphoric acid (Neumann), and total nitrogen (Kjeldahl) are given in grams.

1. Forsbach has recently recorded diminution of output of sugar after administration of a pancreatic hormone prepared by Zuelzer's method (*Deutsch. med. Wochensh.*, 1909).

TABLE I

Experiment I.—Dog of 4.43 kilograms had pancreas completely removed under chloroform anaesthesia, weight of pancreas 14.74 grams. Glycosuria immediately followed on operation, from which the animal made a good recovery, but succumbed to diabetes and wasting after an interval of eighteen days.

Date 9 a.m.	Urine c.c.	S.G.	Total Sugar	Total Urea (Dupre)	Total NH ₃	As HCl Total Acidity	As HCl Total organic acids	Total P ₂ O ₅	Acetone	Di-acetic Acid (Kjeldahl)	Total N (Kjeldahl)	Weight of dog in kilograms
Thurs., Dec. 7	80	1040	9.53	—	0.071	0.14016	0.226	—	0	0	—	4.43
Fri., " 8	365	1045	25.86	15.33	0.216	0.14921	0.418	1.102	0	0	6.26	—
Sat., " 9	285	1051	22.46	12.88	0.377	0.38691	0.214	0.756	0	0	7.25	4.20
Sun., " 10	305	1060	28.57	16.17	0.481	0.71693	0.283	1.381	0	0	7.57	—
Mon., " 11	230	1056	20.74	12.54	0.374	0.48187	0.341	0.962	0	0	6.00	3.97
Tues., " 12	315	1058	29.06	18.74	0.405	0.70135	0.050	1.304	0	0	8.75	—
Wed., " 13	400	1056	33.41	22.60	0.634	0.87600	0.117	1.632	0	0	11.41	3.74
Thurs., " 14	510	1048	36.83	24.23	1.110	1.06106	1.113	1.945	0	0	11.71	—
Fri., " 15	350	1048	25.63	20.30	0.612	0.88148	0.473	1.521	0	0	9.47	3.63
Sat., " 16	480	1050	28.81	24.38	0.444	1.05105	0.829	2.186	0	0	10.90	—
Sun., " 17	315	1054	21.50	16.69	0.401	1.24100	0.533	1.658	0	0	8.36	—
Mon., " 18	520	1049	38.57	23.40	0.647	0.72353	0.801	1.739	?	0	11.39	3.30
Tues., " 19	220	1052	8.45	18.26	0.456	0.64722	0.567	1.154	0	0	8.88	2.95
Wed., " 20	180	1048	0.85	15.21	0.482	0.56765	0.752	1.143	0	0	7.36	—
Thurs., " 21	215	1050	6.45	15.80	0.459	0.69058	0.662	1.503	0	0	7.54	3.07
Fri., " 22	555	1050	36.43	28.86	1.121	1.12227	1.450	2.384	0	0	14.40	3.07
Sat., " 23	445	1051	29.77	20.03	0.675	1.02650	0.942	1.983	0	0	10.52	3.07
Sun., " 24	370	1055	26.42	20.72	0.475	0.69886	0.160	1.023	0	0	9.49	2.73
Mon., " 25	50	1061	3.06	3.68	0.122	0.13505	0.026	0.361	0	0	1.70	Dead

Secretin

2 doses
night

TABLE II

Experiment II.—In a dog of 7.2 kilograms the pancreas was removed under deep chloroform anaesthesia, glycosuria followed, and the period of survival was fourteen days, details are given in Table II, as under:—

Date 9 a.m.	Urine c.c.	S.G.	Total Sugar	Total Urea (Dupre)	Total NH ₃	As HCl Total acidity	As HCl Total organic acids	Total P ₂ O ₅	Acetone	Di-acetic Acid	Total N (Kjeldahl)	Weight of dog in kilograms
Jan. 9	110	1057	0	9.9	0.163	0.108	0.453	0.890	0	0	4.93	—
" 10	240	1064	0	34.32	0.595	0.856	0.964	1.514	0	0	17.04	7.27
Operation : complete removal of pancreas under deep anaesthesia. Weight of pancreas 21.15 grams. Weight of dog 7.27 kilograms												
" 11	400	1040	13.31	15.60	0.359	1.146	0.252	1.773	0	0	8.56	7.05
" 12	600	1074	53.99	44.10	0.889	1.730	1.486	2.934	Trace	0	20.60	7.27
" 13	660	1057	55.64	39.29	0.867	1.157	1.080	2.202	Strong	Present	18.19	6.93
" 14	490	1075	54.79	33.81	0.801	0.734	1.102	1.840	V. strong	Strong	15.85	6.70
" 15	475	1076	51.18	34.91	1.205	0.843	1.555	2.019	Intense	V. strong	15.43	6.59
" 16	580	1060	47.55	33.64	1.578	1.039	1.964	2.253	"	Intense	15.66	6.36
" 17	460	1066	40.12	27.37	1.185	0.901	1.168	1.808	"	"	12.78	6.36
" 18	510	1055	37.84	27.80	0.988	1.037	0.920	1.934	V. strong	V. strong	12.79	6.25
" 19	420	1076	38.81	30.87	1.054	1.387	1.650	2.322	Intense	Strong	14.16	5.91
" 20	295	1079	32.57	19.18	0.957	0.697	1.526	1.591	"	V. strong	8.58	5.80
" 21	235	1083	24.93	21.50	0.763	0.690	0.989	1.214	"	Moderate	9.97	5.57
" 22	275	1087	30.72	26.26	0.765	0.756	0.880	1.357	"	Slight	12.40	5.45
" 23	220	1085	25.49	18.59	0.748	0.686	1.175	1.196	"	Trace	8.79	5.34
" 24	510	1053	27.23	—	—	—	—	1.701	Weak	"	—	5.11
" 25	500	1051	32.11	—	—	—	—	1.737	None	None	—	5.0