LXIII. STUDIES IN CARBOHYDRATE METABOLISM.

I. THE UTILISATION OF DIHYDROXYACETONE BY THE ANIMAL BODY AND A METHOD FOR ITS ESTIMATION.

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(*Received March 11th, 1926.*)

The possible use of dihydroxyacetone in the treatment of diabetes was suggested, apparently first of all, by Emil Fischer, and this has been followed by observations upon its clinical use by various workers, particularly in Germany [Isaac and Adler, 1924], and in Canada. Rabinowitch [1925], for example, has shown that it possesses considerable antiketogenic power and has employed it successfully in the treatment of diabetic coma.

For a long time discussion has centred around the claims of three compounds containing three carbon atoms as possible intermediaries in carbohydrate metabolism: methylglyoxal, glyceric aldehyde and dihydroxyacetone. It is impossible to discuss in detail here the respective claims of each of these, but reference may be made to the discussion by Dakin [1922].

In this connection the rather interesting fact may, however, be mentioned that dihydroxyacetone, when perfused through the liver [Embden, Baldes and Schmitz, 1912], can give rise to small amounts of *d*-lactic acid and to *d*-glucose, whereas glyceric aldehyde, under the same conditions, gives rise to large amounts of inactive lactic acid containing excess of the *l*-isomeride, and to *d*-sorbose, both of which are foreign to the animal body. In addition, glyceric aldehyde has been found [Sansum and Woodyatt, 1916] to damage the kidneys of rabbits, whereas dihydroxyacetone appears to be as innocuous as glucose itself. Moreover, *dl*-glyceric aldehyde when administered to diabetics increases the glycosuria [Wells, 1920], whereas dihydroxyacetone is almost completely utilised. Again, the tolerance for glyceric aldehyde, when injected intravenously in the healthy individual, is much less than that for glucose or for dihydroxyacetone. It has also been shown [Wind, 1925] that dihydroxyacetone is oxidised in neutral phosphate solution with great ease; for example, it absorbs atmospheric oxygen about 20–30 times as rapidly as

does fructose, which again is more easily oxidised than glucose. Further, as is well known, it reduces Fehling's solution in the cold and some preliminary experiments have shown us that at p_H 7·3 its rate of oxidation by permanganate is very considerably greater than that of glucose.

It seemed to us that one possible way of distinguishing between various suggested intermediaries is provided by the consideration that any intermediary ought to be a substance which, when administered to animals suffering from convulsions and coma due to insulin hypoglycaemia, will relieve the condition. It is not, of course, suggested that the converse is the case, since the animal body may well be able to utilise certain substances, for example mannose, which presumably do not occur naturally in it.

On the other hand, a substance might prevent hypoglycaemia by being converted into glucose, and in this case one would expect that in all its reactions it would behave similarly to glucose, only showing possibly a delayed action.

In spite of these reservations it seems of great importance definitely to establish whether or not dihydroxyacetone is able to relieve the symptoms of insulin hypoglycaemia in animals.

The following experiments demonstrate quite definitely that dihydroxyacetone produces prompt and effective recovery from insulin coma and that the recovery is at least as rapid as with glucose.

The mechanism by which this recovery is brought about has occupied our attention and is very difficult to determine conclusively. It may be, as suggested above, that the dihydroxyacetone is directly oxidised, thereby providing the necessary energy for cellular metabolism, or it may be that it is very rapidly converted into glucose which then causes recovery, perhaps owing to its restoring the necessary glucose "tension" in the tissues as suggested by Noble and Macleod [1923] with reference to other substances which relieve insulin hypoglycaemia. The evidence, on the whole, we think, accords best with the first of these assumptions. This evidence, which will be discussed later, is supported by the results obtained by Rabinowitch and others, who claim that the substance is antiketogenic and utilisable by the diabetic organism, and by the experiments and clinical observations described below.

Experimental.

The dihydroxyacetone used in the following experiments is that sold by Meister, Lucius, Brüning & Co. under the name of "oxantin." The solution in water reduces Fehling's solution and alkaline picric acid solution in the cold and gives no precipitate with phloroglucinol and sulphuric acid. The latter observation indicates the absence of glyceric aldehyde. A 50 % aqueous solution does not rotate the plane of polarisation of polarised light. It leaves no ash on ignition. When mixed with phosphorus pentoxide and gently heated it yields methylglyoxal, as stated by Meisenheimer [1912] and by Fischer and Taube [1924, 1926.]

I. *Detection and estimation of dihydroxyacetone in blood.*

It appeared to us to be of importance in connection with the present work to develop a method which would enable us to detect and to estimate approximately any dihydroxyacetone present in small quantities in blood. In this way it seemed that we might be able to obtain information as to the rate at which dihydroxyacetone is utilised in the animal body. If this substance actually forms one of the intermediates in carbohydrate metabolism it might be expected that it would be utilised very rapidly. We believe that we have obtained evidence, using the method about to be described, which shows, at least, that when injected it disappears from the blood almost immediately.

Two colour tests for dihydroxyacetone are mentioned in the literature. One is the reddish brown colour given by an alkaline solution of sodium picrate in the cold, due to the reduction of the picric acid. Compared with the colour given by an equal quantity of creatinine, it is found to have only two-fifths of the intensity. A method based on this reaction would not be very sensitive.

The other reaction, which appears to be more sensitive and which therefore we have adopted as a basis of the present method, is the colour given by sulphuric acid and a phenol. A marked coloration is given by 0·10 mg. of dihydroxyacetone to which 5 cc. of sulphuric acid containing phenol have been added. Further, the colour appears to be a permanent one and it has been found possible to make up standard solutions in sealed tubes with which the tint corresponding to an unknown may be compared. It is, of course, necessary to remove all protein from the blood to be tested and also water. On this account methyl alcohol was used as a protein precipitant as this could be readily removed *in vacuo* at a temperature not exceeding 50°. The details of the method are as follows.

Blood (0·4 cc.) is thoroughly shaken up with methyl alcohol (9·6 cc.) and allowed to stand for 2 hours. The precipitated proteins are then filtered off. The filtrate (5 cc.) is placed in a comparator tube and the methyl alcohol completely removed *in vacuo* at 50°. A solution of phenol in sulphuric acid is prepared as follows. Phenol (20 g.) is warmed up with water (1 cc.) until it melts and concentrated sulphuric acid is then added slowly with cooling until the total volume is 250 cc. This solution possesses a faint pink tinge which, however, cannot be observed in the comparator tube. It appears to keep for several weeks without deterioration. This solution (5 cc.) is added to the comparator tube and the colour, which is fully developed after 2 hours at room temperature, is then compared with a series of standard solutions prepared as follows. Varying amounts of a methyl alcoholic solution of dihydroxyacetone (0·2 %) are introduced into a series of comparator tubes. The alcohol is then removed as above, and the phenol-sulphuric acid solution (5 cc.) added to each. The tubes are then hermetically sealed. The tints appear to be permanent.

In order to determine whether the method used could safely be applied to blood and also to find out whether dihydroxyacetone when added to blood

remains unchanged for a short time and is not immediately altered, the following experiment was carried out. Varying amounts of dihydroxyacetone were added to aliquot portions of human blood and the percentage then estimated colorimetrically. The results were as follows:

Amount added mg.	Amount found mg.
0·14	0·14
0·16	0·16
0·18	0·18
0·20	0·18
0·50	0·50
1·00	1·00
1·50	1·50
2·00	2·00

As in the normal blood of rabbit, man and cat a coloration is obtained corresponding to about 0·065 % of dihydroxyacetone, the tubes in the above experiment were balanced in the comparator with one prepared from 0·4 cc. of normal blood without addition of dihydroxyacetone. The nature of the substance giving this colour is being investigated.

II. *Animal experiments.*

In the following experiments rabbits were injected subcutaneously with insulin after preliminary starvation for 24 hours, and they were left untreated until complete coma had ensued and there were no signs of spontaneous recovery. As many animals recover and relapse repeatedly, it is occasionally difficult to judge when this stage has been reached, but in the majority of instances this is not so. In this respect five animals which were treated either with pyruvic acid or with lactic acid acted as controls. In only one case did spontaneous recovery take place.

The blood-sugar was determined by MacLean's method in a sample usually obtained from the ear, but, on occasions when this was impossible, by heart puncture.

The estimation of dihydroxyacetone was carried out by the method described above. In Table I we have given the actual figures obtained without subtracting the value corresponding to the colour developed in normal blood.

The following table sums up the relevant facts.

Table I.

Rabbit	Weight g.	Dose of insulin units per kg.	Time for coma hours	Blood-sugar in coma %	Blood-dihydroxy-acetone in coma %	Injection. g. per kg.		Time for recovery mins.	Blood-sugar on recovery %	Blood-dihydroxy-acetone on recovery %	Remarks
1	2000	20	5·5	—	—	Dihydroxyacetone	2	10	—	—	Relapse in 2 hrs.
2	1215	5	4·0	0·054	—	,,	4·8	12	0·065	0·20	Recovery complete and permanent
3	1275	10	5·5	0·039	—	,,	0·75	5	0·042	0·07	Relapse in 2 hrs. 45 mins.
4	1167	20	5·0	0·042	0·09	,,	2·60	10	0·06	0·20	Relapse in 1 hour
5	1911	30	4·5	0·056	—	,,	0·75	10	0·025	0·10	Relapse in 3 hrs.
6	1625	10	4·7	0·022	0·05	,,	0·70	7	0·025	0·05	Recovery complete and permanent
7	(*a*) 2220	10	4·25	0·027	0·05	Glucose	0·70	7	0·056	0·05	Relapse in 120 mins.
	(*b*) ditto 6 days later	15	4·85	—	—	Dihydroxyacetone	0·70	7	—	—	Relapse in 84 mins.
8	(*a*) 1610	15	5·85	—	—	,,	0·70	6	—	—	Relapse in 34 mins.
	(*b*) 1670	15	3·33	0·032	—	Glucose	0·70	3	0·032	—	Relapse in 36 mins.
9	1400	15	4·33	0·044	—	Dihydroxyacetone	0·70	12	0·025	0·07	Relapse in 48 mins.

We have not included in the above table certain striking experiments referred to in the discussion in which recovery took place as a result of injection of dihydroxyacetone although the animals, which had previously been treated, with negative results with other substances such as sodium pyruvate or sodium lactate, were at one time in a moribund condition and in fact were kept alive by artificial respiration.

The relapses after treatment, which were frequent, are accounted for by the large doses of insulin used and were found to occur under similar conditions after treatment with glucose. No difficulty was experienced in bringing about complete and permanent recovery with a further dose of dihydroxyacetone. Altogether 12 rabbits were treated with dihydroxyacetone and recovery took place in every case except one in which a large dose of sodium citrate (10 g. in 20 cc.) had been previously injected. Death in this case was evidently due to oedema of the lungs and alkalosis.

Similar results have been obtained in an experiment in which twenty mice were used instead of rabbits. Here, dihydroxyacetone proved to be quite effective in causing rapid recovery from hypoglycaemic convulsions and coma.

These results seem to us to leave no doubt as to the power of dihydroxyacetone to remove the symptoms of insulin hypoglycaemia.

The practically equal efficiency of dihydroxyacetone and glucose is strikingly shown by Exp. 8 in which the animal received a small dose of dihydroxyacetone and relapsed in 34 minutes. Three days afterwards, after the same dose of insulin, recovery was produced by an equal dose of glucose and relapse took place after 36 minutes.

Similarly in Exp. 7, using 10 units of insulin per kg., relapse occurred with glucose in 120 minutes, whilst using 15 units of insulin it occurred in 84 minutes after a similar dose of dihydroxyacetone.

III. *Comparison between rate of utilisation of glucose and of dihydroxyacetone by muscle.*

It has been shown elsewhere [Lambie, 1926] that when the liver is excluded from the circulation of a decerebrated and eviscerated cat, there occurs a sharp fall in the concentration of the sugar in the circulating blood, and that this fall may be counterbalanced, and the blood-sugar level kept approximately constant, by the continuous injection of glucose at a uniform rate of about 0·15 g. per kg. per hour. In the present experiment the technique described fully in the above-mentioned communication was used. A cat was decerebrated after brief etherisation and artificial respiration applied. Cannulae were inserted into each jugular vein and into one carotid artery. After rapid evisceration, the portal and renal vessels were ligated so as to exclude the liver and kidneys. Glucose transfusion was begun into one of the jugulars, while samples of blood were taken from the carotid cannula, 10, 25, and 45 minutes after commencing the injection. As shown in Table II, the blood-

sugar remained at an approximately constant level. The glucose transfusion was then stopped and immediately dihydroxyacetone was perfused at the same uniform rate through the other jugular cannula. Another sample of blood was taken 3 to 4 minutes after the change-over and further samples 15 and 35 minutes later. During the period with dihydroxyacetone a fall was observed in the blood-sugar level. At the same time dihydroxyacetone determinations showed that no significant change had taken place in the concentration of this substance in the blood. It is clear that the dihydroxyacetone injected was utilised almost immediately and at a much greater rate than glucose. It may be mentioned that experiments with other sugars, for example laevulose, show that these are not utilised at a greater rate than glucose. It is also evident that during the period with dihydroxyacetone, the total utilisation of carbohydrate is increased, for not only is the dihydroxyacetone completely used up but also a considerable amount of glucose disappears. This result cannot be explained on the assumption that dihydroxyacetone was first converted into glucose because in that case no fall in the total glucose in the blood would occur.

Table II. *A decerebrated and eviscerated cat was used; liver excluded from circulation and renal vessels ligated.*

Time					
3.0	Glucose transfusion. 0·15 g. per kg. per hour				
3.10	Blood 1	Glucose	0·357 %	Dihydroxyacetone	0·065 %
3.25	„ 2	„	0·354	„	0·065
3.45	„ 3	„	0·360	„	0·065
	Glucose transfusion stopped and dihydroxyacetone 0·15 g. per kg. per hour transfused				
3.50	Blood 4	Glucose	0·355 %	Dihydroxyacetone	0·065 %
4.5	„ 5	„	0·340	„	0·055
4.25	„ 6	„	0·299	„	0·075

Observations on man.

In order to elucidate the above results the following experiments were performed on man. In general they are confirmatory of those of Rabinowitch. In the first place estimations were made of the sugar and dihydroxyacetone in the blood of a normal individual after ingestion of 50 g. of dihydroxyacetone by the mouth. The blood-sugar curve is given in Table IV. For comparison, the curve obtained in the same individual after taking 50 g. of glucose is also given in Table III. Only a very slight rise in the reducing power of the blood is observed after dihydroxyacetone and this may well be accounted for by the formation of small amounts of hexoses in the alkaline secretion of the intestine, a suggestion put forward by Rabinowitch who, however, definitely attributed the rise to glucose. It is difficult, however, to exclude the possibility that other hexoses may be formed under these conditions, and the slight increase in the reducing power of the urine after ingestion of dihydroxyacetone may be partly due to the presence of such hexoses.

Table V indicates the blood-sugar curve of a diabetic man after ingestion of 50 g. of dihydroxyacetone. The rise in blood-sugar is much smaller than

would be observed after taking a similar amount of glucose and it is quite clear from this curve and similar ones given by Rabinowitch that rapid conversion into glucose in the blood-stream or in the tissues and organs does not take place. The small rise may well be due, again, to formation of hexoses in the intestine and, naturally, since this subject was diabetic, the resulting rise in the blood-sugar is rather greater than in a normal individual.

Table III. *Curves of sugar and dihydroxyacetone in blood of normal man after ingestion of* 50 *g. of glucose.*

Time	Blood-sugar %	Blood-dihydroxy-acetone %
9.55 a.m. (before glucose)	0·110	0·06
10.0 a.m. (glucose taken 50 g.)	0·160	0·065
10.30 a.m.	0·160	0·065
11.0 a.m.	0·126	0·060
11.30 a.m.	0·094	0·060
12.0 noon	0·082	0·060
12.30 p.m.	0·091	0·065

Table IV. *Curves of sugar and dihydroxyacetone in blood and reducing power of urine, after ingestion of* 50 *g. of dihydroxyacetone by a normal man.*

Time	Blood-sugar %	Blood-dihydroxy-acetone %	Urine sugar %
9.55	0·106	0·065	0·048
10·0	(50 g. dihydroxyacetone)		
10.30	0·114	0·070	0·132
11.0	0·098	0·075	0·171
11.30	0·102	0·085	0·145
12.0	0·106	0·075	?
12.30	0·111	0·080	0·145

The reducing power of the urine was determined by Benedict and Osterberg's method for sugar in normal urine.

The urine, after dihydroxyacetone, gave no reduction with Fehling's solution in the cold, but slight reduction occurred on heating.

Table V. *Curve of blood sugar in diabetic after* 50 *g. dihydroxyacetone by mouth.*

Time	Blood-sugar %
9.55	0·183
10.0	50 g. dihydroxyacetone
10.30	0·218
11.0	0·180
11.30	0·175
12.0	0·180

A further indication that dihydroxyacetone is not converted rapidly into glucose by the tissues is furnished by the fact that this subject, after administration of dihydroxyacetone, excreted no sugar detectable by Fehling's test in the 24 hours' urine.

Another diabetic gave the following results.

A. B., a pregnant female diabetic on a constant diet of 2000 calories and 20 units of insulin, was excreting 1 g. glucose per diem and acetone. With 15 units insulin she excreted approximately 10 g. glucose. With 20 units insulin plus 30 g. dihydroxyacetone the urine contained no acetone, and the sugar excretion did not exceed 2 g. Further investigations are being carried out on the utilisation and antiketogenic action of dihydroxyacetone from a quantitative point of view. Owing to the fluctuation in tolerance of individuals it is very difficult to obtain conclusive results.

Discussion.

In reviewing the results of the above experiments, it is convenient to consider each group separately in the first place and to discuss the possible explanations of the phenomena observed. In the case of the experiments on rabbits and mice, three possible explanations of the recovery from hypoglycaemic coma under dihydroxyacetone suggest themselves. The dihydroxyacetone might be condensed into glucose in the animal body and the glucose so formed would relieve the symptoms just as glucose does when injected as such. This view is made feasible by the observation that dihydroxyacetone is converted into glucose when perfused through the glycogen-poor liver [Embden, Schmitz, and Wittenberg, 1914], and that it causes an increased excretion of glucose in the phloridzinised dog [Ringer and Frankel, 1914]. Moreover, it has been suggested by Macleod that those sugars relieve insulin hypoglycaemia which are readily convertible into glucose, *e.g.* mannose, fructose, and possibly maltose.

There are, however, several difficulties to be considered with regard to this view. In the first place, the action of dihydroxyacetone on hypoglycaemic rabbits and mice appears to be as rapid as that of glucose and the quantities required to relieve the symptoms are approximately the same, namely 0·7 g. per kg. under the conditions of the above experiments.

If, then, this explanation were the correct one, it would imply an almost quantitative and very rapid conversion of dihydroxyacetone into glucose by the animal organism. This would mean that the blood-sugar would always be raised as it is after an injection of glucose. In three cases, however (Exps. 5, 6 and 9), it may be observed that recovery occurred when the blood-sugar was as low as 0·025 %, whereas, after the administration of glucose the blood-sugar is practically always at or above the convulsion-level of 0·04 %. Only in one case was it below this, namely in Exp. 8, when the reading of blood-sugar was 0·032 %. Naturally the variability of the convulsion-level in different animals and the experimental error in the sugar determinations have to be taken into account. In some cases where very large doses of dihydroxyacetone had been injected the apparent blood-sugar level rose to 0·065 %. In these cases, however, a correction has to be made as the concentration of dihydroxyacetone present in the blood, namely 0·135 %, which is the 0·2 %

found, minus 0·065, is itself sufficient to cause considerable reduction. In fact, it has been found that this concentration of dihydroxyacetone gives, by MacLean's method, reductions corresponding roughly to 0·03 % of glucose. When this is subtracted from the value obtained (0·06 %), the actual concentration of glucose in the blood is found to be 0·035 % and therefore no great emphasis can be laid on the apparent high value obtained.

It appears, therefore, that recovery with dihydroxyacetone usually occurs when the blood-sugar is at a lower level than that at which convulsions occur or that at which recovery takes place after the administration of glucose.

The next explanation that may be offered of the action of dihydroxyacetone is that it may be used directly. This possibility is particularly attractive in view of the experiments of Wind [1925], who has demonstrated the remarkable ease with which dihydroxyacetone is oxidised in neutral phosphate solution by gaseous oxygen, and this marked tendency to be oxidised is also illustrated by the rapid reduction of permanganate at 37° and p_H 7·3.

The remaining possibility is that dihydroxyacetone is condensed or otherwise changed into some particularly reactive sugar, which is also formed from glucose in the ordinary course of metabolism. It is, of course, difficult to exclude this explanation, but until definite evidence for the existence of such a compound is forthcoming it seems best to enquire how far the simpler hypothesis of direct oxidation will explain the facts.

The experiment on the cat shows how dihydroxyacetone is immediately removed from the circulating blood, and this at once excludes the hypothesis that dihydroxyacetone is rapidly converted into glucose in such a preparation, otherwise the blood sugar would remain level. Even the possibility that it might be converted into glucose in the cells seems improbable, otherwise the blood-sugar would remain level in the above experiments.

Taking this experiment alone, the disappearance of dihydroxyacetone might be explained as being due merely to its rapid diffusion into cells without utilisation; but this would not, of course, explain the permanent recovery obtained with rabbits and mice in which direct utilisation or conversion into glucose must take place. Here again, the hypothesis that it is rapidly oxidised seems the most feasible.

The experiments on man appear to show clearly that in the intact individual dihydroxyacetone is not rapidly and quantitatively converted into glucose. If it were, one would expect the blood-sugar curve in the normal individual after ingestion of 50 g. of dihydroxyacetone to be very similar to that after an equal amount of glucose. Again, if it were converted into glucose it should cause a marked excretion of sugar and a marked rise in the concentration of sugar in the blood in diabetes. Such a result could of course be explained on the assumption that the liver can store dihydroxyacetone more readily than it can glucose. But this hypothesis does not explain the results of the experiment with the cat in which the liver was excluded from the circulation. The possibility cannot, however, be excluded that, particularly

in a normal individual, rapid storage may take place. But it is difficult to agree that this would occur readily in the diabetic, as the formation of glucose would be the first step in the synthesis of glycogen, and glucose does not form glycogen in the diabetic.

On reviewing all the results, it is seen that although special explanations might apply to each separate set of experiments, yet all the phenomena are readily explained on the simple assumption that dihydroxyacetone is very easily and possibly directly oxidised and utilised by the animal organism. This simple hypothesis, we think, explains all the facts.

Any dogmatic conclusion would at present be premature. Further experiments with other intermediary metabolites might confirm the rough hypothesis above formulated and these are being carried out together with quantitative observations upon the utilisation and antiketogenic power of dihydroxyacetone.

In conclusion, reference may be made to a paper which has recently appeared and which has just come to our notice. In this paper Campbell [1926] describes a method for the estimation of dihydroxyacetone in blood and applies the method to the determination of dihydroxyacetone in the blood after the ingestion of 100 g. of the substance. During the first half hour a rapid rise in the blood-dihydroxyacetone from 0·0 to 0·07 % was found and this was followed by a rapid drop. The blood-sugar simultaneously rose during the first half hour but after that it usually showed a definite fall. This seems to support the view that the disappearance of dihydroxyacetone from the blood is not due to its conversion into glucose, as in that case one would expect some indication of a rise in the glucose concentration rather than a fall. The fact that Campbell does obtain a rise in the blood-dihydroxyacetone after the ingestion of dihydroxyacetone is not necessarily inconsistent with our results as Campbell used double the quantity, namely 100 g. as compared with the 50 g. that we have used. In some of our experiments with rabbits, where very large quantities of dihydroxyacetone were injected, a marked rise occurred in the concentration of the substance in the blood. Naturally, although dihydroxyacetone is utilised rapidly by the tissues, there is a limit to the rate at which utilisation can take place. The very small rises in blood-sugar which Campbell obtained after ingestion of 100 g. glucose are worthy of remark.

Summary.

1. Dihydroxyacetone is able to cause recovery of rabbits and mice from the symptoms of insulin hypoglycaemia, the amount and time required for its action being approximately the same as in the case of glucose.

2. Dihydroxyacetone is more rapidly removed from the blood stream by the muscles than is glucose or laevulose, and unless excessive amounts are given the removal is practically complete.

3. This apparently ready utilisation of dihydroxyacetone by the tissues is also observed in certain diabetic individuals.

4. The facts accord with the hypothesis that dihydroxyacetone is directly utilisable by the normal animal organism and need not first be converted into glucose.

5. A method is given for the detection and estimation of dihydroxyacetone in small amounts of blood.

We desire to express our thanks to Mr W. Leiper for carrying out the blood sugar determinations in connection with this research and to the Department of Scientific and Industrial Research for a personal grant to one of us (R. H. S.).

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