

## ON THE GASEOUS EXCHANGE FOLLOWING THE ADMINISTRATION OF DIHYDROXYACETONE

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In recent papers (1, 2, 3) the behavior of the total metabolism and the respiratory quotients of normals and diabetic patients, before and after administration of glucose or dihydroxyacetone, has been reported. It was shown that, in both normals and diabetics, the total metabolism after dihydroxyacetone administration, and also the respiratory quotient, rises more than is the case after glucose has been administered. Rabinowitch (4) had previously published a respiratory quotient curve in a mild diabetic after administration of 100 grams of dihydroxyacetone by mouth. A rise of the respiratory quotient from the basal level of 0.706 to 0.817 was obtained in two hours. Early in our own clinical investigations on such cases we encountered the same condition as is shown in Mason's report: namely, that the more severe the degree of the diabetes the greater the dissimilarity between the results on normal individuals and on the patient. Indeed, the severest diabetics show no rise in respiratory quotient whatever following dihydroxyacetone administration.

In our work a number of other facts were encountered which seemed inconsistent with the viewpoint that dihydroxyacetone was more readily metabolized than glucose itself, and we accordingly transferred the investigation to animals in which an unequivocal answer might be expected. It seems justifiable to expect that in diabetic patients (who are part-normals) the results of any experimental investigation would also be part normal and only the most clear cut evidence on the severest cases should be accepted in evaluating the use of the triose for therapeutic purposes. This absolutely essential criterion of therapeutic value has sometimes been neglected. On the other hand, the results of our experiments on animals are entirely incompatible with the view that the triose can be metabolized in any other way than

through glucose, which it has been shown requires insulin in its metabolism. It has been shown that if the triose normally appears in the blood the amount must be exceedingly minute (5); that, when administered, it disappears from the blood coincident with the appearance of extra glucose (6, 7); that it cures insulin hypoglycemia (6), which only certain hexoses can do; that when administered to depancreatized dogs on a fixed diet and insulin, the same amount of glucose is excreted in the urine as when glucose itself is fed (7); that the fasting depancreatized dog excretes the same amount of extra glucose as is equivalent to the weight of triose fed (7); that the respiratory quotient of a fasting depancreatized dog is not affected by the administration of the triose (7); and that in the eviscerated animal the dihydroxyacetone does not cure the hypoglycemia nor disappear from the blood as does glucose but, in the absence of the liver, remains unchanged, and that most of the substance can be obtained from the muscles as the unchanged triose (9).

These investigations, however, did not include any evidence as to the behavior of the total metabolism of a fasting *normal* dog when given glucose or dihydroxyacetone. The protocols later presented are of interest in supplying this information under experimental conditions, which have not been quite duplicated in man and from which further deductions may be drawn. These observations were undertaken in the period November 1 to 16, 1926. From the December number of the Proceedings of the Society for Experimental Biology and Medicine we learned that Himwich, Rose and Malev (10) had presented a preliminary note on a somewhat similar experiment on December 15, 1926. Since, so far as we know, no more extensive report by these authors has appeared in the past year it seems desirable to place these observations on record.

Himwich, Rose and Malev, using a trained dog, injected 10 grams of glucose or dihydroxyacetone dissolved in warm water subcutaneously and collected the expired air at short intervals through a leakproof mask into a spirometer whence samples were collected over mercury and analyzed by the Haldane-Henderson apparatus. Prompt increases in the respiratory quotient occurred, the latter rising, in fact, over 1.0 in all experiments with dihydroxyacetone and in one case to 1.31. In the glucose experiments the respiratory quotient increased,

though not so rapidly, nor to such high levels as in the dihydroxyacetone experiments. These results lead them to conclude that dihydroxyacetone is more readily available than glucose when administered subcutaneously.

More recently Cathcart and Markowitz (11) have discussed this question in their paper on respiratory quotients. They show the fundamental weakness of several of the conceptions of the significance of the respiratory quotient and support the more reasonable conception of the respiratory quotient as the algebraic sum of the whole series of metabolic transformations taking place at the time rather than the mere oxidation of certain proportions of protein, carbohydrate and fat. Furthermore, their evidence effectively disposes of the short period respiratory experiment as a means of investigating the combustion of food substances administered to the animal except under very strictly limited conditions.

The plan of the experiments to be reported is as follows: Two normal dogs in good physical condition received no food, except water, for two days and were then placed in the respiratory cabinet and their respiratory quotients and gaseous exchange recorded. Twenty-five grams of the food stuff, glucose or dihydroxyacetone, were then administered by mouth and the observations on gaseous exchange and respiratory quotient continuously recorded, using periods of thirty minutes to one hour, for three to five hours after feeding. In both dogs the first pair of observations was repeated, reversing the order of administration of the carbohydrates. Eight experiments on two normal dogs were thus obtained, four with glucose and four with dihydroxyacetone. The apparatus used was described by Macleod (12). As the readings of volume, weights, and corrections for temperature, pressure, humidity, etc., have been checked it appears unnecessary to repeat them. The values given below are the corrected values for the oxygen intake and  $\text{CO}_2$  output. As there is no warrant for determining the non-protein respiratory quotient from the nitrogen excretion in such experiments these determinations have been omitted. Similarly, since the ratio of oxygen intake to carbon dioxide output does not, under these conditions, represent a combustion quotient it is not possible to estimate the heat production from the so-called respiratory quotient and the

total gaseous exchange in any one period and, therefore, figures on heat production per hour have been omitted. It should also be stated that the behavior of the animals was very satisfactory. The

TABLE 1

Period	O <sub>2</sub> absorp- tion	CO <sub>2</sub> elimina- tion	R.Q.	O <sub>2</sub> per kgm. hour	Remarks
November 1, 1926. Dog A. White wire-haired terrier. Weight 6120 grams. Last previous feeding October 30, 9:00 a.m.					
8:55- 9:55 a.m.	cc. 4,566	cc. 3,485	0.76	cc. 746	Quiet Very quiet
9:55-10:55 a.m.	4,192	3,073	0.733	685	Slight movements Very quiet
11:05 a.m. 11:50 a.m.					Electric power off 25 grams glucose in 150 cc. water
12:15- 1:15 p.m.	4,572	3,719	0.814	747	Very quiet Sitting up. Quiet 3 movements
1:15- 2:15 p.m.	4,150	3,595	0.866	678	Occasional movement
2:15- 3:15 p.m.	3,644	3,078	0.845	595	Quiet Dog fed 4:00 p.m.
November 3, 1926. Same dog. Weight 6000 grams. Last previous feeding November 1, 4:00 p.m.					
8:35- 9:35 a.m.	4,563	3,340	0.732	760	Quiet Very quiet
9:35-10:35 a.m.	4,074	3,144	0.772	679	Very quiet
10:45 a.m.					25 grams dihydroxyace- tone
11:10-12:10 p.m.	4,032	3,969	0.984	672	Very quiet
12:10- 1:10 p.m.	3,796	3,001	0.896	633	Very quiet
1:10- 2:10 p.m.	3,745	3,139	0.838	624	Very quiet
2:10- 3:10 p.m.	3,707	2,943	0.794	618	

movement recorder, as well as visual observation of the animal, permits us to state that in no case was it responsible for any significant oxygen utilization after the preliminary control period (not shown) was com-

plete. Tables 1 to 4 show the results obtained. As might be expected, there was a continuous fall in the weights of the animals and with it a fall in the basal oxygen consumption per kilogram hour. It appears more satisfactory, therefore, to view the alterations in oxygen con-

TABLE 2

Period	O <sub>2</sub> absorp- tion	CO <sub>2</sub> elimina- tion	R.Q.	O <sub>2</sub> per kgm. hour	Remarks
November 9, 1926. Dog A. Weight 5550 grams. Last previous feeding November 7, 1926					
9:10-10:10 a.m.	cc. 3,491	cc. 2,697	0.772	cc. 629	Very quiet
10:10-11:10 a.m.	3,315	2,412	0.728	597	Very quiet
11:42 a.m.					25 grams dihydroxyace- tone
11:55-12:25 p.m.	1,719	1,638	0.953	620	1 movement
12:25-12:55 p.m.	1,904	1,628	0.855	686	Very quiet
12:55- 1:25 p.m.	1,886	1,730	0.917	580	3 movements
1:25- 1:55 p.m.	1,938	1,628	0.840	698	Quiet
1:55- 2:55 p.m.	3,238	2,508	0.775	583	Very quiet
2:55- 3:55 p.m.	3,349	2,371	0.708	603	1 movement
3:55- 4:55 p.m.	3,117	2,351	0.754	562	Quiet
November 16, 1926. Same dog. Weight 5630 grams. Last previous feeding, November 14, 1926					
9:45-10:45 a.m.	2,846	2,061	0.724	506	Occasional movements
10:45-11:45 a.m.	2,903	2,160	0.744	516	Quiet
11:48 a.m.					25 grams glucose given
12:00-12:30 p.m.	1,404	1,074	0.765	498	Quiet
12:30- 1:00 p.m.	1,379	1,221	0.886	490	Very quiet
1:00- 1:30 p.m.	1,458	1,338	0.918	518	Quiet
1:30- 2:00 p.m.	1,471	1,272	0.865	522	1 movement
2:00- 3:00 p.m.	3,054	2,524	0.826	542	Moving a little
3:00- 4:00 p.m.	2,557	1,923	0.752	454	

sumption in terms of the average consumption per kilogram hour. Table 5 summarizes the data.

For comparison with these we have the unpublished data of Macleod (13) of six experiments on a normal dog fasting continuously except

that it was receiving 20 grams of glucose in each experiment. In this animal seventeen control hours show an average oxygen consumption per kilogram hour of 573 cc. while the average oxygen consumption per kilogram hour for the three hours following administration of glucose was 554 cc., a negligible difference. The animal, on con-

TABLE 3

Period	O <sub>2</sub> absorption	CO <sub>2</sub> elimination	R.Q.	O <sub>2</sub> per kgm. hour	Remarks
November 4, 1926. Dog B. Wire haired terrier. Weight 6,200 grams. Last previous feeding November 2, 4:00 p.m.					
	cc.	cc.		cc.	
8:30- 9:30 a.m.	4,159	2,982	0.717	591	Moving occasionally
9:30-10:30 a.m.	3,458	2,676	0.774	495	Quiet
10:32 a.m.					25 grams glucose
10:45-11:15 a.m.	2,373	1,872	0.789	746	Fairly quiet
11:15-11:45 a.m.	2,091	1,827	0.874	674	Quiet
11:45-12:15 p.m.	1,889	1,740	0.921	610	Very quiet
12:15-12:45 p.m.	1,940	1,715	0.884	626	Very quiet
12:45- 1:45 p.m.	3,847	3,063	0.796	620	Quiet
1:45- 2:45 p.m.	3,457	2,679	0.775	558	Movements slight
2:45- 3:45 p.m.	3,503	2,554	0.729	565	Quiet
November 10, 1926. Same dog. Weight 5960 grams. Last previous feeding November 8, a.m.					
8:25- 9:25 a.m.	3,276	2,768	0.753	550	6 movements; then quiet
9:25-10:25 a.m.	2,910	2,267	0.779	488	Fairly quiet
10:32 a.m.					25 grams dihydroxyacetone
10:45-11:15 a.m.	1,485	1,628	1.10	498	Quiet
11:15-11:45 a.m.	1,833	1,593	0.869	616	Moving
11:45-12:15 p.m.	1,903	1,796	0.944	638	Moving considerably
12:15-12:45 p.m.	1,800	1,412	0.784	604	Quiet
12:45- 1:45 p.m.	2,698	2,137	0.792	453	1 slight movement
1:45- 2:45 p.m.	3,122	2,312	0.741	524	Moving

tinued fasting, it may be noted, shows the same tendency to reduction in oxygen consumption per kilogram hour with fall in body weight as is noted in our two animals on discontinuous fasting. It may also be remarked that in none of these results is there any evidence of specific dynamic action of glucose in the sense that the term is used in reference to proteins. It is true that there is a temporary rise in the oxygen

consumption following administration of glucose but this is not marked and is equalized within three to four hours by a reduced oxygen consumption. As has been previously noted (1, 3), the respiratory quotient in normals after dihydroxyacetone administration rises more

TABLE 4

Period	O <sub>2</sub> absorption	CO <sub>2</sub> elimination	R.Q.	O <sub>2</sub> per kgm. hour	Remarks
November 12, 1926. Dog B. Weight 5750 grams. Last previous feeding November 10, 4:00 p.m.					
10:20-11:20 a.m.	3,033	2,251	0.742	527	Occasional movements
11:20-12:20 p.m.	2,959	2,239	0.757	515	Very quiet
12:25 p.m.					25 grams dihydroxyacetone
12:40- 1:10 p.m.	1,486	1,580	1.06	516	Quiet
1:10- 1:40 p.m.	1,808	1,598	0.884	628	Moving slightly
1:40- 2:10 p.m.	1,773	1,643	0.927	616	Moving slightly
2:10- 2:40 p.m.	1,646	1,476	0.896	572	Quiet
2:40- 3:40 p.m.	2,911	2,445	0.840	506	Very quiet
3:40- 4:40 p.m.	2,790	2,132	0.764	485	Quiet
November 15, 1926. Same dog. Weight 5570 grams. Last previous feeding November 13, a.m.					
9:45-10:45 a.m.	2,756	1,974	0.716	495	Restless at first
10:45-11:45 a.m.	2,674	1,974	0.738	480	Quiet
11:57 a.m.					25 grams glucose
12:06-12:36 p.m.	1,384	936	0.676	496	Very quiet
12:36- 1:06 p.m.	1,305	1,119	0.858	468	Very quiet
1:06- 1:36 p.m.	1,371	1,257	0.917	492	Very quiet
1:36- 2:06 p.m.	1,507	1,359	0.900	542	Very quiet
2:06- 3:06 p.m.	3,083	2,793	0.906	553	3 movements
3:06- 4:06 p.m.	2,741	2,300	0.839	492	Quiet

sharply than when glucose is administered and, also, reaches values higher than were attained when glucose in equal quantities is administered. In tables 1 to 4 these results are confirmed by continued observation of the respiratory exchange of normal dogs previously

fasted throughout a test period of three to five hours. In the case of dihydroxyacetone the rise in oxygen consumption is sharper, sometimes being apparent in the second half-hour p. c., is distinct in the second hour, but falls in the later periods so that the average oxygen consumption per kilogram hour is 578 cc., approximately the same value as for glucose administration. Mason's results on normals, though obtained by discontinuous determinations of ten minutes per

TABLE 5  
*O<sub>2</sub> consumption, cubic centimeters per kilogram hour*

Animal	Date	First control hour	Second control hour	First hour	Second hour	Third hour	Fourth hour
1. Glucose tests							
A	November 1	746	695	747	678	595	
A	November 16	506	516	494	520	542	454
B	November 4	591	495	720	618	620	558
B	November 15	495	480	482	517	553	492
Average.....		584	546	611	608	602	501
2. Dihydroxyacetone tests							
A	November 3	760	679	672	633	624	618
A	November 9	629	597	653	689	583	603
B	November 10	550	488	557	621	453	524
B	November 12	527	515	572	594	506	485
Average.....		616	570	588	627	541	557

*cc. per kgm.  
hour*

Average of 16 control periods.....	579
Average of 15 post glucose periods.....	580
Average of 16 post dihydroxyacetone periods.....	578

hour and, therefore, not entirely suitable for this calculation, when averaged show approximately 1 per cent increased oxygen consumption over basal values. The amount of carbohydrate he calculates as being burned bears no more relation to dihydroxyacetone administration than to the glucose, and the marked variations in nitrogen excretion in the different periods ably demonstrate the essential inaccuracy of the so-called non-protein respiratory quotient in this type of



experiment. All these results agree in all essential particulars with the added advantage that our results on normal animals are available for comparison with the results on depancreatized dogs previously published.

As has been previously shown (7), an increase in oxygen consumption follows the administration of either glucose or dihydroxyacetone

TABLE 6  
*CO<sub>2</sub> elimination per kilogram hour*

Animal	Date	First control hour	Second control hour	First hour	Second hour	Third hour	Fourth hour
1. Glucose tests							
A	November 1	596	526	607	587	503	
A	November 16	366	366	407	463	448	341
B	November 4	481	431	596	557	494	432
B	November 15	354	354	369	469	500	413
Average.....		449	419	495	519	486	395
2. Dihydroxyacetone tests							
A	November 3	558	524	661	500	523	490
A	November 9	509	434	588	605	452	427
B	November 10	464	380	540	538	358	388
B	November 12	391	389	553	542	390	371
Average .....		480	432	585	546	431	419

TABLE 7  
*Average CO<sub>2</sub>/O<sub>2</sub> ratios*

Periods	First control hour	Second control hour	First hour	Second hour	Third hour	Fourth hour
Glucose.....	0.77	0.77	0.81	0.85	0.81	0.79
Dihydroxyacetone.....	0.78	0.76	1.0	0.87	0.80	0.75

to a fasting completely depancreatized dog, but the respiratory quotient in these animals does not rise and equivalent amounts of glucose are excreted in the urine. This demonstrates the independence of oxygen consumption and combustion of the triose or glucose and illustrates the fact that other intermediary processes between ingestion and excretion may influence the level of oxygen absorption

independently of actual combustion of a food administered and we must, therefore, conclude that such temporary changes in oxygen consumption as may occur are probably associated with such intermediary processes, e.g., formation of fat, work expended by the liver in transforming the triose into glucose, kidney work, etc., or to another very important mechanism—the additional muscular work required in carrying on hyperventilation.

While, as has been shown above, the total respiratory exchange is practically unchanged from the basal during the whole period of observation there are changes in oxygen consumption during the individual test periods (table 5). These, however, do not necessarily correspond with the alterations in the so-called respiratory quotient. In fact, as table 6 shows, the highest  $\text{CO}_2$  output occurs in the first half-hour or hour after triose administration while the oxygen consumption (table 5) is greatest in the second hour. The ratio of  $\text{CO}_2$  elimination to oxygen uptake (table 7) is consequently decidedly different from that occurring after glucose administration, in which case the rise in respiratory quotient is less abrupt and more prolonged. Considering the data in tables 1 to 4 together with that of other workers, one is struck by the frequency with which the so-called respiratory quotient exceeds 1.0. Even holding the view that the respiratory quotient is an expression of dynamic equilibrium in food stuffs transformed, burned or stored, such ratios cannot be explained as due to combustion of carbohydrate alone but must include the formation of fat, and calculations of carbohydrate consumption based thereon must be in error. Since, however, the oxygen intake is not decreased but the initial rise in the  $\text{CO}_2/\text{O}_2$  ratio is due to additional  $\text{CO}_2$  elimination, fat production does not appear to furnish a probable explanation for the sequence of events. While it appears inherently improbable that combustion would occur in isolated stages, it may be pointed out that the change from sugar to lactic acid is anaerobic and requires no oxygen and produces no  $\text{CO}_2$ . It would appear then that any additional energy expenditure is required for some other purpose and that such expenditure is accompanied by  $\text{CO}_2$  production in the absence of oxygen or that hyperventilation is the cause of the excess  $\text{CO}_2$  production, or that both these processes take place in differing proportions. It is again significant that the total oxygen intake after

triose within a relatively short time approximates that of the basal period as well as that of the post glucose period, showing how little the metabolism of the individual is disturbed by the administration of the triose. Possibly such disturbance as exists might be accounted for by additional muscular work required for elimination of the additional quantities of  $\text{CO}_2$ . The subject will be further discussed in the succeeding paper.

#### SUMMARY AND CONCLUSIONS

Protocols are presented showing, in confirmation of previous work, that differences in respiratory quotient, oxygen intake, and  $\text{CO}_2$  elimination occur when a normal animal is fed with dihydroxyacetone, as compared to the same animal given glucose. It is pointed out that while depancreatized animals also show a rise in oxygen intake and  $\text{CO}_2$  elimination the respiratory quotient remains down, and the animal excretes the triose as glucose. Further analysis of the protocols indicates that these changes are temporary and are inconsistent with the explanation that fat is being produced or large quantities of triose are being burned. Hyperventilation is suggested as a cause for the experimental results obtained.

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#### BIBLIOGRAPHY

1. Mason, E. H., *J. Clin. Invest.*, 1926, ii, 521. Dihydroxyacetone Studies. I. Its Respiratory and Carbohydrate Metabolism in Normal Men.
2. Mason, E. H., *J. Clin. Invest.*, 1926, ii, 533. Dihydroxyacetone Studies. II. Its Respiratory and Carbohydrate Metabolism in Diabetes Mellitus.
3. Lambie, C. G., and Redhead, F. A., *Biochem. J.*, 1927, xxi, 549. Studies in Carbohydrate Metabolism. III. The Influence of Dihydroxyacetone upon the Respiratory Metabolism and upon the Inorganic Phosphate of the Blood.
4. Rabinowitch, I. M., *Can. Med. Assn. J.*, 1925, xv, 374. Observations on the Use of Dihydroxyacetone in the Treatment of Diabetes Mellitus (Preliminary Report).
5. Campbell, W. R., *J. Biol. Chem.*, 1926, lxxvii, 59. The Quantitative Determination of Dihydroxyacetone.
6. Campbell, W. R., and Hepburn, J., *J. Biol. Chem.*, 1926, lxxviii, 575. The Effect of Dihydroxyacetone on Insulin Hypoglycemia.

7. Campbell, W. R., and Markowitz, J., *Am. J. Physiol.*, 1927, lxxx, 561. On the Metabolism of Dihydroxyacetone in Pancreatic Diabetes.
8. Campbell, W. R. and Markowitz, J., *J. Clin. Invest.*, 1927, iv, 37. Preferential Utilization of Carbohydrate in Diabetes.
9. Markowitz, J., and Campbell, W. R., *Am. J. Physiol.*, 1927, lxxx, 548. The Fate of Dihydroxyacetone in the Animal Body.
10. Himwich, A. E., Rose, M. I., and Malev, M. R., *Proc. Soc. Exp. Biol. and Med.*, 1926-7, xxiv, 238. Changes in the Respiratory Quotient Produced by Subcutaneous Injection of Dioxycetone and Glucose.
11. Cathcart, E. P., and Markowitz, J., *J. Physiol.*, 1927, lxxiii, 309. The Influence of Various Sugars on the Respiratory Quotient.
12. Macleod, J. J. R., *Carbohydrate Metabolism and Insulin*. 1926. Longmans, Green & Co., London and New York.
13. Macleod, J. J. R., Personal communication.