CXLIX. THE INFLUENCE OF FEEDING EITHER FAT AND LIPASE OR LECITHIN ON THE SUGAR EXCRETION OF DEPANCREATISED DOGS.

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An extensive investigation of the metabolism of the phloridzinised dog has led Graham Lusk and co-workers to conclude, by analogy, that the metabolic disturbance in diabetes mellitus and in depancreatised dogs, due to the relative or complete lack of insulin, is a corresponding inability to oxidise carbohydrates. On the basis of this interpretation, these workers have strenuously opposed the view that sugar may be derived from fatty acid in the animal organism. The recent work of Wierzuchowski [1926], confirmed by Deuel, Wilson and Milhorat [1927], which demonstrated the oxidation of glucose by the phloridzinised dog, casts considerable doubt upon the above interpretation of the diabetic state.

The European school, whose views on this subject have been well reviewed by Geelmuyden [1923],—see also Von Noorden and Isaac [1927] and Macleod [1926]—has long advocated the over-production theory of diabetes which, in its simplest terms, attributes the glycosuria and hyperglycaemia of diabetes, not to a defect in oxidation of carbohydrates, but rather to an over-production of glucose from protein and fat, which causes its accumulation in spite of a continued ability to oxidise it. His recent work has led Macleod to advocate gluconeogenesis from fat, and to take that view of the diabetic disturbance which such a conclusion implies.

Macleod [1928] has recently summarised the evidence for the conversion of fat into carbohydrate in the animal organism. Since the publication of his monograph, the work here presented has been completed, and while the results are not of a crucial nature they lend further support to his conclusions. For this reason, and because they illustrate the physiological limitations of this type of experimentation, it seems desirable that these results be placed on record.

Methods.

Totally depancreatised dogs, maintained in an excellent state of health and nutrition by the use of insulin, were used. On the fifth day after withdrawal of all food and insulin, 50 g. of fat mixed with an equal amount of either a simple alkaline, pancreatic lipase extract or a castor bean suspension,

were administered by stomach tube. The lipase extracts, prepared according to Plimmer [1918], were given to facilitate the digestion and absorption of the fat from the intestine. Where lecithin was the substance administered, the addition of lipase was considered unnecessary, in view of the greater ease with which this substance undergoes hydrolysis [Bloor, 1926]. The fats used included olive oil, cotton-seed oil, butter and "intarvin" [Kahn, 1925].

Throughout the experiments, the sugar, nitrogen and total ketone excretion were determined for 24-hour periods. On the day of fat administration, the animal's R.Q. was determined in a carefully controlled respiratory cabinet of the Benedict type. In all cases a careful *post-mortem* examination was made for residual pancreatic tissue and if found the results were discarded for that animal. In some cases, determinations of the liver-glycogen were made by Pflüger's method. Urinary sugar, nitrogen and ketones were determined by the methods of Shaffer and Hartmann [1920–21], Kjeldahl, and Van Slyke [1917] respectively.

Results.

Owing to limitations of space, only one positive, one control and one negative experiment are reported in full (Table I). The remaining experiments are grouped in condensed tabular form (Tables II and III).

Discussion.

Exps. 1, 5 (Table II) and 6 (Table I) clearly show an excess sugar excretion, even after maximal deductions are made for the glucose which might have come from the glycerol portion of the fat, and from extra nitrogen excreted on the day of fat administration. That any of the extra glucose excreted could have come from the muscle-glycogen stores is unlikely. There is at least no evidence that the administration of a food material like a neutral fat can cause a displacement of glucose from the tissue stores and it can be seen from the tables that the control animals in the present experiments showed no such displacement. Since the minuteness of the liver-glycogen stores on the fifth day after withdrawal of food and insulin [Chaikoff, 1927] renders this organ insignificant as a source of the extra sugar excretion, it can be concluded that none of the extra glucose excreted in the positive experiments was derived from the carbohydrate stores of the animal. A comparison of Exps. 1, 5 and 6 with the control Exps. 7, 8 and 9, therefore, indicates that the extra sugar excreted must have been derived from fatty acid.

A large number of experiments yielded negative results (Table III) and there is no reason to doubt that, were the experiments carried on indefinitely, positive results would be obtained in about the same ratio to negative ones as in the present series. A brief consideration, however, of the limitations of this type of experiment, makes it apparent that, with our direct method of approach:

(1) it is quite unlikely that results more pronouncedly positive could ever be obtained;

(2) the number of negative results might reasonably be expected to be relatively great;

(3) to such positive results as have been obtained must be conceded an added significance.

Table I. *Details of typical experiments.*

Day of exp.	Remarks	Total ketone excretion g.	Sugar output g.	N output g.	D : N	
I. *Showing sugar formation from fat. Exp. No. 6.*						
1	Stopped food and insulin.					
2	Weight 7·73 kg.	0	87·08	4·27	20·39	—
3	„ 7·56 „	0·83	49·41	4·95	9·98	—
4	„ 7·22 „	3·87	30·72	4·95	6·21	—
5	25 g. lecithin *per os.* 15 g. lecithin subcutaneously. Food and insulin failed to prevent death on following day. Weight 7·02 kg.	4·86	40·11	5·35	7·50	R.Q. 0·693. Calories 54·6 per kg. *per diem.* *Sugar from glycerol 2·65 g. †Sugar from protein 1·46 g. Sugar from fatty acid 5·28 g.
II. *Control with mineral oil. Exp. 9.*						
1	Stopped food and insulin.					
2	Weight 7·05 kg.	0	43·60	5·01	8·70	—
3	„ 6·70 „	0·24	23·10	4·48	5·16	—
4	„ 6·48 „	1·34	23·43	5·59	4·19	—
5	55 cc. mineral oil +55 cc. pancreatic lipase. Promptly recovered on food and insulin. Weight 6·25 kg.	1·93	21·70	5·66	3·83	R.Q. 0·700. Calories 79·2 per kg. *per diem.* *Sugar from glycerol 3·76 g. †Sugar from protein 0·26 g. Sugar from fatty acid 0 g.
III. *No sugar formation from fat. Exp. 4.*						
1	Stopped food and insulin.					
2	Weight 8·1 kg.	Trace	85·55	5·28	16·21	—
3	„ 7·8 „	1·50	39·05	4·56	8·57	—
4	„ 7·6 „	4·27	21·10	5·27	4·00	—
5	50 g. cottonseed oil +55 cc. pancreatic lipase. Recovered on food and insulin. Weight 7·3 kg.	7·39	33·17	7·38	4·49	R.Q. 0·696. Calories 71·7 per kg. *per diem.* *Sugar from glycerol 3·59 g. †Sugar from protein 7·70 g. Sugar from fatty acid 0·78 g.

* *Calculation for sugar from glycerol.*

Animal required 54·6 calories per kg. *per diem*; animal weighed 7·02 kg.; therefore animal required $7{\cdot}02 \times 54{\cdot}6 = 383{\cdot}3$ calories *per diem.*

Nitrogen output = 5·35 g.; therefore $5{\cdot}35 \times 6{\cdot}25 = 33{\cdot}44$ g. protein were utilised; and therefore $33{\cdot}44 \times 4{\cdot}1 = 137{\cdot}1$ calories were derived from protein.

Assuming that no energy was derived from carbohydrate, $383{\cdot}3 - 137{\cdot}1 = 246{\cdot}2$ calories were derived from fat. Therefore $\frac{246{\cdot}2}{9{\cdot}3} = 26{\cdot}5$ g. fat were utilised for energy; and therefore $\frac{26{\cdot}5}{10} = 2{\cdot}65$ g. glycerol is maximum amount available for conversion into glucose.

† *Calculation for sugar from protein.*

Nitrogen output on 5th day of experiment = 5·35 g.
„ 4th „ = 4·95 g.
Extra nitrogen output on 5th day = 0·40 g.
Glucose available from extra nitrogen = $0{\cdot}40 \times 3{\cdot}65 = 1{\cdot}46$ g.

It will be noted that liver-glycogen was increased in Exps. 10 and 13 but, although this offsets to some extent the lack of extra sugar excretion, there still remain ten experiments which are quite negative in their results. In order that an experiment of the type here reported could yield a positive result, it would be necessary for some of the administered fat to be carried

Table II. *Condensed results of experiments showing sugar formation from fat, and of two control experiments.*

Exp. No. and wt. of dog	Administered *per os* on 5th day of exp.	Total ketone excretion g.	*Extra sugar output g.	*Extra N output g.	D : N	
†1 8·38 kg.	50 g. olive oil + 50 cc. castor bean suspension	0·31	9·20	0	6·76	R.Q. 0·702. Calories 60 per kg. *per diem.* Sugar from glycerol 4·35 g. Sugar from fatty acid 4·85 g.
†5 6·47 kg.	30 g. lecithin	2·33	11·07	0	7·78	R.Q. 0·708. Calories 77 per kg. *per diem.* Sugar from glycerol 4·16 g. Sugar from fatty acid 6·91 g.
†7 4·60 kg.	55 cc. mineral oil + 55 cc. water	0·14	2·17	0·53	3·02	R.Q. 0·696. Calories 72 per kg. *per diem.* Sugar from glycerol 2·30 g. Sugar from protein 1·93 g.
†8 6·19 kg.	55 cc. mineral oil + 55 cc. water	0·59	0	0·39	3·29	R.Q. 0·719. Calories 91 per kg. *per diem.* Sugar from glycerol 4·48 g. Sugar from protein 1·42 g.

* Over the preceding 24 hours. † (1) Died; (5) died; (7) recovered; (8) recovered.

Table III. *Condensed results of experiments not showing sugar formation from fat.*

Exp. No. and wt. of dog	Administered *per os* on 5th day of exp.	Total ketone excretion g.	Extra sugar output g.	Extra N output g.	D : N	
2 6·76 kg.	50 g. olive oil + 55 cc. pancreatic lipase	—	0	—	—	R.Q. 0·704. Calories 86 per kg. *per diem.*
3 6·40 kg.	50 g. cottonseed oil + 55 cc. castor bean suspension	—	2·45	—	—	R.Q. 0·702. Calories 62 per kg. *per diem.*
*10 5·80 kg.	50 g. cottonseed oil + 55 cc. pancreatic lipase (on 6th day of fasting)	1·40	0	0	2·78	R.Q. 0·692. Calories 73 per kg. *per diem.* Sugar from glycerol 3·22 g.
11 8·10 kg.	50 g. cottonseed oil + 55 cc. pancreatic lipase (on 6th day of fasting)	1·00	1·93	0·55	2·68	R.Q. 0·686. Calories 57 per kg. *per diem.* Sugar from glycerol 3·81 g. Sugar from protein 2·00 g.
12 7·61 kg.	50 g. olive oil + 55 cc. pancreatic lipase (on 5th and 6th days of fasting)	0·53	7·40	2·04	2·99	R.Q. 0·701. Calories 54 per kg. *per diem.* Sugar from glycerol 3·2 g. Sugar from protein 7·45 g.
*13 9·50 kg.	Ran parallel to Exp. 12. Male dog. Did not catheterise.					
14 6·48 kg.	50 g. butter-fat + 55 cc. pancreatic lipase	0·19	2·86	0·41	2·74	R.Q. 0·701. Calories 73 per kg. *per diem.* Sugar from glycerol 3·74 g. Sugar from protein 1·50 g.
15	Died on 5th day of exp.					
16 8·40 kg.	50 g. olive oil + 55 cc. pancreatic lipase	4·96	0	0	2·99	R.Q. 0·702. Calories 73 per kg. *per diem.* Sugar from glycerol 4·58 g.
17 5·00 kg.	50 g. olive oil + 55 cc. pancreatic lipase	4·58	0·46	0·61	2·73	R.Q. 0·709. Calories 77 per kg. *per diem.* Sugar from glycerol 2·55 g. Sugar from protein 2·23 g.
18 7·50 kg.	50 g. "intarvin" + 55 cc. pancreatic lipase	4·66	6·62	1·09	4·71	R.Q. 0·654. Calories 79 per kg. *per diem.* Sugar from glycerol 4·37 g. Sugar from protein 3·98 g.

* (10) 3·39 g. extra glycogen in liver. (13) 13·77 g. extra glycogen in liver.

Calculation for extra glycogen in the liver.

Highest average value for liver-glycogen in diabetic dogs on the 5th day after the withdrawal of food and insulin is 0·19 % [Chaikoff, 1927].

Assuming the liver weighs 5 % of total body weight $= \frac{5}{100} \times 5800 = 290$ g.

Estimated glycogen in the liver $= \frac{0{\cdot}2}{100} \times 290 = 0{\cdot}58$ g.

Actual glycogen in the liver $= \frac{1{\cdot}37}{100} \times 290 = 3{\cdot}97$ g.

Extra glycogen in the liver $= 3{\cdot}97 - 0{\cdot}58 = 3{\cdot}39$ g.

to the liver, and there converted into glucose, and this process would have to be superadded on the pre-existing level of gluconeogenesis from tissue protein and fat. But the supply of fat does not necessarily determine its utilisation by the liver. As Macleod has suggested (personal communication):

> "The factor upon which gluconeogenesis from fat will depend is the call of the liver for fat rather than the supply which may be carried to this organ by the blood as a result of fat absorption. If this view be correct, then it might quite well happen in diabetes that in some animals the liver would not yet have developed its demand for fat at the time when the fat-feeding was taking place, in which case, of course, there would be no evidence of gluconeogenesis. In other words, the conditions would be similar to those existing in very fat animals, with plenty of 'depot' fat, some of which, as we know, develop the gluconeogenesis at an earlier stage than others."

This view is borne out by the fact that in most of the above experiments—positive, negative and control—the calculated utilisation of fat is of the same order of magnitude. If the positive results depend on the coincidence of the fat administration with a somewhat increased capacity of the liver for gluconeogenesis, it is hardly surprising that a large number of negative results should be obtained.

The low respiratory quotient obtained in Exp. 18 is very interesting in view of the support it lends to the conversion of fat to carbohydrate. The fact, however, that in most of the experiments the R.Q. does not fall significantly below 0·7, is apparently inconsistent with such a process which, by causing a retention of oxygen, should bring about a lower R.Q. This view rests upon the assumption, not supported by recent work, that the diabetic animal does not burn carbohydrate. If we do not make this assumption, then the more or less constant diabetic R.Q. can be readily explained as being the algebraic sum of two quotients—an R.Q. of 1·0 due to the oxidation of carbohydrates, and an R.Q. of 0·2 resulting from gluconeogenesis from fat [Macleod, 1928, p. 78].

It will be observed that, in the clearly positive experiments, the animal died in every case, despite all efforts to revive it. This further illustrates the physiological limitations which must be overstepped in order to obtain a positive result. In a totally diabetic animal, which has been deprived of all food and insulin for four days, and is already very ill as a result of the high level of gluconeogenesis, we attempt to drive this process to its utmost extreme. When we succeed in any measure, the animal dies. There is, therefore, only a narrow margin within which we can get the animal which does show a positive result to survive long enough to complete the experiment.

Finally, it may be noted in passing that the administration of "intarvin" did not lower the ketone excretion in Exp. 18. On the day of "intarvin" administration the total ketone excretion increased from 2·445 g. of the preceding 24 hours, to 4·659 g. This does not agree with the work of Kahn [1925], but is in accord with the recent work of Moore *et al.* [1928].

SUMMARY.

1. In three totally depancreatised dogs, the administration of fat was followed by an excretion of extra glucose which could not be accounted for by the glycerol portion of the fat, the nitrogen excretion and the carbohydrate stores of the animal.

2. Gluconeogenesis from fatty acid is therefore confirmed.

3. The physiological limitations of this type of experimentation are pointed out, as accounting for the many negative experiments obtained.

4. In one experiment, where "intarvin" was the fat administered, it failed to show its supposed antiketogenic action.

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REFERENCES.

Bloor (1926). *Chem. Rev.* **2**, 243.
Chaikoff (1927). *J. Biol. Chem.* **74**, 203.
Deuel, Wilson and Milhorat (1927). *J. Biol. Chem.* **74**, 265.
Geelmuyden (1923). *Ergeb. Physiol.* **21**, 274; **22**, 51.
Kahn (1925). *Arch. Int. Med.* **36**, 44.
Macleod (1926). Carbohydrate metabolism and insulin. (Longmans Green and Co.)
—— (1928). The fuel of life. (Oxford University Press.)
Moore, Breckenridge and Koser (1928). *J. Lab. Clin. Med.* **14**, 3, 201.
Plimmer (1918). Practical organic and biochemistry (Longmans Green and Co.), 404.
Shaffer and Hartmann (1920–21). *J. Biol. Chem.* **45**, 365.
Van Slyke (1917). *J. Biol. Chem.* **32**, 455.
Von Noorden and Isaac (1927). Die Zuckerkrankheit und ihre Behandlung, 216. (Julius Springer.)
Wierzuchowski (1926). *J. Biol. Chem.* **68**, 385.