or less constant inundation of the intestine with carbon particles. Further, the animals were kept on the described regime from 16 days to 7 months, a much longer time than that reported by other workers.

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Formation of Carbohydrates from Non-Glycerol Fraction of Lipoids in the Rat.

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Although the study of the metabolic disturbance in the depancreatized dog has contributed in a large measure to our present knowledge of metabolism, there is still no universal agreement as to the exact nature of this disturbance. Two main schools of thought exist on this question. The theory which gained early acceptance in this country postulates an inability of the diabetic tissue to utilize carbohydrate. The opposing theory, more widely accepted in Europe, and gaining more credence in this country, states that the diabetic organism can utilize carbohydrate but that the essential disturbance is an overproduction of sugar from non-carbohydrate sources. A necessary corollary to this latter theory is a formation of carbohydrate from the non-glycerol fraction of lipoids.

We have previously reported that carbohydrate is utilized by the completely depancreatized dog not receiving insulin,[1] and many older and more recent reports may be drawn in support of this thesis.[2, 3, 4] The existing evidence for the conversion of fat to carbohydrate in the higher animal species is largely based on the D:N ratio and Respiratory Quotient, and is disputed on theoretical grounds,[5] although it is conceded that this conversion occurs in plants and probably also in insects. The present preliminary report offers direct proof, of a crucial nature, that carbohydrate can be derived from the non-glycerol fraction of lipoids in the rat.

1 Soskin, S., *J. Nutrition*, 1930, **3**, 99.

2 Houssay, B. A., *Endocrinology*, 1931, **15**, 511.

3 Mann, F. C., *Archives Int. Med.*, 1923, **31**, 797.

4 Hedon, L., *Arch. Internat. de Physiol.*, 1926, **26**, 329; 1926, **27**, 254; 1927, **29**, 175.

5 Soskin, S., *Biochem. J.*, 1929, **23**, 1385.

Magne, Mayer and Plantefol[6] observed a marked depletion of the tissue carbohydrates in animals following the administration of dinitrophenol. Later, Dodds and Greville,[7] working with isolated tissues, concluded that the increased metabolism following dinitrophenol administration occurred solely at the expense of carbohydrates. It was difficult to reconcile these results with the work of Hall, Field, *et al.*,[8] who observed a significant decrease in the Respiratory Quotient of animals treated with dinitrophenol, from which they concluded that the fats were the chief source of the liberated energy. In some preliminary experiments in which we attempted to find an explanation for the above discrepancy, we found that while the oxygen intake in the intact dog given dinitrophenol was far in excess of that required to oxidize the carbohydrate which disappeared, the oxygen intake of hepatectomized dogs under the same conditions was less than the amount necessary to oxidize the disappearing carbohydrate. These results enabled us to reconcile the previous work by assuming that while the increased heat production caused by dinitrophenol occurred at the expense of carbohydrate, there also occurred a concomitant production of the necessary carbohydrate from other sources. Since it had been established that even prolonged administration of dinitrophenol does not increase the urinary excretion of nitrogen nor the non-protein nitrogen of the blood,[8] the gluconeogenesis presumably occurred at the expense of fat. The following experiments were therefore performed with a view to the possibility that following the height of the action of dinitrophenol, the rate of formation of new carbohydrate might be sufficiently greater than the rate of its oxidation to permit of the unequivocal demonstration of the former process.

In a number of sets of 2 litter-mate rats of approximately the same weight and fasted 24 hours prior to the experiments, 20 mg. per kilo of 2:4 sodium dinitrophenol was administered subcutaneously in 0.1% solution. In from one to 3 hours after dinitrophenol administration, one rat from each set was killed and passed through a meat-grinder. Aliquots of the thoroughly mixed, minced tissue were taken for the determinations of glycogen, soluble carbohydrates and lactic acid, by the procedure described by Dann and

[6] Magne, H., Mayer, A., and Plantefol, L., *Ann. de Physiol. Physicochim. Biol.*, 1932, **8**, 50.

[7] Dodds, E. C., and Greville, G. D., *Nature*, 1933, **132**, 966.

[8] Hall, V. E., Field, J., Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.*, 1933, **106**, 432.

Chambers.[9] Total lipoid content was determined by a preliminary extraction with an alcohol-ether mixture and the weighing of the residue of a subsequent petroleum-ether extract.[10] Total nitrogen was determined by the Kjeldahl method and total solids by drying the tissues to constant weight at 80°C. Three to 4 hours later, the second rat of each set was killed and treated in a similar manner. All determinations were made on triplicate tissue aliquots, analyzed in duplicate, and calculated on the basis of dry weight.

TABLE I.

	First Rat					Second Rat				
	Carbohydrate*					Carbohydrate*				
Rat No.	Glycogen Mg. %	Soluble CHO Mg. %	Lactic Acid Mg. %	Total Mg. %	Total Lipoids Mg. %	Glycogen Mg. %	Soluble CHO Mg. %	Lactic Acid Mg. %	Total Mg. %	Total Lipoids Mg. %
1-2	378	145	1859	2382	18130	384	181	2602	3167	15694
7-8	300	197	488	985	16630	429	255	624	1308	13610
9-10	413	131	362	906	16000	469	241	465	1175	14250
13-14	399	120	360	879	14230	657	166	405	1228	12200
Total	1490	593	3069	5152	64990	1939	843	4096	6878	55754
% change									+33%	—14%
3-4	585	141	620	1346	17900	334	154	110	598	17300
11-12	250	262	524	1036	15500	255	153	454	862	15600

*Calculated as Glucose All calculations on basis of dry weight of tissue.

Some of our results are given in Table I. It may be seen that in those experiments in which we succeeded in observing the appropriate phase (above heavy line), there was a consistent and significant rise in the total carbohydrates (av. 33%) and a corresponding diminution in the total lipoids (av. 14%) of the second rat as compared to the first. When no increase in carbohydrate occurred (below heavy line), no fall in the total lipoids was found. No significant difference between total nitrogen content of the rats was observed. It is obvious that the glycerol fraction of the fat which disappeared cannot account for the total amount of carbohydrate which appeared since that would necessitate the disappearance of more than four times the amount of lipoid which is actually lost. The carbohydrate must, therefore, be derived from a fraction of the lipoids other than, or in addition to, glycerol.

[9] Dann, M., and Chambers, W. H., *J. Biol. Chem.*, 1932, **95**, 413.

[10] Chen, J. S., *Chinese J. Physiol.*, 1934, **8**, 195.