XXXI. THE DIRECT REPLACEMENT OF GLYCEROL IN FATS BY HIGHER POLYHYDRIC ALCOHOLS. PART II. THE VALUE OF SYNTHETIC MANNITOL OLIVE OIL AS A FOOD.

By

WILLIAM DOBINSON HALLIBURTON, JACK CECIL DRUMMOND
AND ROBERT KEITH CANNAN.

*From the Physiological Laboratory, King's College, London, and the Biochemical
Department of the Research Institute, Cancer Hospital, London.*

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In the preceding communication Lapworth and Pearson [1919] have described the preparation of a synthetic oil by replacing glycerol in olive oil by mannitol. As part of the general enquiry it was also necessary to ascertain what value, if any, this synthetic product possesses as a food-stuff. A sample of the oil was therefore submitted to us for the purpose of making feeding tests. The product supplied was a light greenish brown oil of somewhat dirty appearance, containing a small amount of solid matter in suspension. The amount of this solid matter increased when the oil was kept standing during the cold weather, and from a cursory examination it appeared to consist of the higher melting point esters (stearin). The oil possessed a taste and odour reminiscent of olive oil, but the former was if anything less pleasant than that of the natural oil.

The following data were obtained on analysis, and for comparative purposes the figures obtained on the analysis of a good quality commercial olive oil are also given. The latter was not, however, identical with the olive oil from which the synthetic oil was prepared.

	"Mannitol olive oil"	Olive oil
Free fatty acids, calculated as oleic acid	4.0 %	2.7 %
Saponification value	159	193
Iodine value	82.4	84.7

The investigation of the food value of the mannitol oil was planned to determine first, whether the oil could be assimilated by the mammalian digestive system, and secondly, whether the oil could be administered with safety over considerable periods without any deleterious influence on health. Unfortunately, the amount of material at our disposal was small, and we were obliged to abandon the idea of experiment on man or the higher animals. Such experiments would of necessity have been of very short duration and

quite unsuited to the purpose of the investigation. Accordingly we confined our attention to a study of the absorption of the oil in the digestive tract of the rat, an animal whose suitability for prolonged tests of this type is unquestioned.

As far as we have been able to ascertain, Bloor is the only investigator who has studied the digestibility of mannitol esters of the higher fatty acids in the animal body. He prepared mannide distearate and mannitan distearate and demonstrated that they were hydrolysed by the lipolytic enzymes of the pancreas [1912, 1]. He also carried out feeding experiments of short duration on cats and established the fact that the esters were absorbed from the intestinal tract. One cat received a daily ration of 3.8 g. of mannitan distearate in 5 g. of cotton-seed oil for a period of six days, and showed an absorption of 42.3 per cent. A higher absorption (53.6 per cent.) was observed in an experiment of one day when a much larger amount of the ester was administered. Experiments with isomannide distearate (M.P. 61.5°) showed that approximately 72 per cent. was absorbed, a utilisation comparable with that recorded for tristearin, a natural fat with a melting point of the same order [Arnschink, 1890]. Even better absorbed was isomannide dilaurate [Bloor, 1912, 2], for which coefficients as high as 95—97 per cent. were obtained.

FEEDING TESTS WITH "MANNITOL OLIVE OIL" ON RATS.

To make the tests of the edibility of the mannitol oil as severe as possible it was decided to administer it as the sole source of fat in the diets of the experimental animals. Such a decision introduced a disturbing element into the experiments, for it would necessitate the animals being deprived of the fat-soluble accessory factor, which for several reasons we knew must be absent from the synthetic oil. It was hoped, however, to surmount this difficulty as well as possible by employing animals which were nearly mature. Such animals may live without any apparent disturbance of health upon a diet deficient in fat-soluble A for several weeks [Drummond, 1919]. Two pairs of healthy young adult male rats were selected and confined in suitable metabolism cages. For a preliminary period both pairs were fed upon an artificial diet containing butter fat, after which they received diets containing either olive oil itself or the synthetic mannitol oil for further periods, usually of four days each.

The dietaries were compounded as follows:

	A	B	C
Purified caseinogen	20	20	20 parts
„ starch	50	50	50 „
Yeast extract	5	5	5 „
Salt mixture	5	5	5 „
Orange juice	5	5	5 „
Butter fat	20	—	— „
Olive oil	10	30	— „
Mannitol oil	—	—	30 „

These diets were stiff pastes which were not readily scattered about by the animals during feeding. Every morning fresh water and a ration of diet were placed in the food receptacles in the cages, and the unconsumed diet from the previous twenty-four hours was taken out and weighed. The nitrogen balance was determined in order to follow the general nutritive condition of the animals. Fat was estimated in the food mixtures and faeces by prolonged ether extraction in a Soxhlet apparatus, after dehydration at 110° for twenty-four hours. It was originally proposed to attempt an estimation of the unabsorbed mannitol oil by a polarimetric method, as was done by Bloor [1912, 2], but it was ascertained that the low specific rotation of the oil ($[\alpha]_D^{20} = -1.44^{\circ}$) rendered such a procedure out of the question.

Tables I and II contain the detailed experimental results. The animals all tended to lose weight when receiving the mannitol oil diet, and their food consumption was not good. Rats 3 and 4 both appeared in somewhat poor condition at the termination of their period of 11 days on the mannitol diet. In all cases, however, the absorption of the mannitol oil was good. From the various utilisation values recorded the following approximate averages have been obtained.

	Percentage utilisation
Butter fat and olive oil	97.8
Olive oil	96.6
"Mannitol" olive oil	95.8

From the point of view of the edibility of the mannitol oil it was important to determine whether the slight decline in health which was apparent in the experimental animals on the mannitol oil dietaries was due to some deleterious action of the oil *per se*, or to the fact that they were being maintained on a ration deficient in one of the indispensable accessory factors. To test this point a batch of young rats of uniform age and sex were selected and divided into two sets. One of these received a diet containing mannitol oil together with butter fat as a source of fat-soluble A (Diet E), the other received a diet containing equivalent amounts of natural olive oil and butter fat (Diet F). The composition of the two rations was as given below:

	Diet E	Diet F
Purified caseinogen	20 parts	20 parts
" starch	50 "	50 "
Yeast extract	5 "	5 "
Orange juice	5 "	5 "
Salt mixture	5 "	5 "
Butter fat	10 "	10 "
Olive oil	— "	10 "
Mannitol oil	10 "	— "

Unfortunately the experiments were restricted by the small amount of mannitol oil which was available. The results which were obtained are tabulated in Table III.

Table I.

[illegible]

Table II.

Day	Remarks	Body wt. g.				Rectal temp. °F.				Food eaten g.	% Fat in food	Total fat consumed g.	Fat in faeces g.	Fat absorbed g.	% Utilization	N in food		N in urine		N in faeces		N balance g.
		Rat 3	Rat 4	Rat 3	Rat 4	Rat 3	Rat 4	g.	g.							g.	g.	g.	g.	g.	g.	
1	Butter Period (Diet A)	154	148	101.0	101.1	—	—	15.5 16.7 20.1	32.4	16.95	0.41	16.54	97.6	1.24	0.76	0.07	0.07	+0.41				
2																						
3																						
4	Mannitol Oil Period (Diet C)	171	161	—	—	—	—	25.8 15.6 17.9 12.2	26.0	18.60	0.37	18.23	98.1	2.01	1.04	0.07	0.07	+0.90				
5																						
6																						
7																						
8	Mannitol Oil Period (Diet C)	163	157	100.2	101.8	—	—	13.6 13.0 13.9 12.2	26.0	13.70	0.78	12.92	94.3	1.48	1.22	0.08	0.08	+0.18				
9																						
10																						
11																						
12	Mannitol Oil Period (Diet C)	160	152	99.6	99.2	—	—	9.2 11.3 9.2 6.8	30.1	10.99	0.32	10.67	97.1	0.94	1.06	0.09	0.09	-0.21				
13																						
14																						
15																						

Table III. *Average body weight in grams of two groups of six male rats.*

Days	Diet E Mannitol oil- butter diet	Diet F Olive oil- butter diet
0	64	67
7	72	85
14	94	98
21	106	112
28	121	132
35	136	148
42	153	162

On the whole, the growth of the rats which received the butter-fat-olive-oil mixture was more satisfactory than that of the animals on the mannitol oil diet. The difference in the final weights is not very marked, and it is difficult to express an opinion as to whether it is of any significance, particularly in view of the small number of animals employed.

At the conclusion of the experiment the mannitol-fed animals appeared in quite as good condition as the control lot, so that it appears safe to assume that no toxic factor was present in the sample of synthetic mannitol olive oil. That this oil was less palatable than olive oil appears likely, however, from the fact that the food consumption of the batch on Diet E was appreciably less than of that receiving Diet F.

The importance that was attached to this investigation in the period during the recent war, when urgent demands of fat for glycerol were bringing about a drastic reduction in the amount available for edible purposes, is fortunately now no longer so great. The results are therefore placed on record purely in the light of their scientific interest.

SUMMARY.

1. A synthetic oil prepared by replacing glycerol in olive oil by mannitol is utilised by the animal organism to practically the same extent as olive oil itself.

2. No toxic action was found to follow the prolonged administration of this oil to rats.

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REFERENCES.

- Arnschink (1890). *Zeitsch. Biol.*, **26**, 434.
Bloor (1912, 1). *J. Biol. Chem.*, **11**, 141.
— (1912, 2). *J. Biol. Chem.*, **11**, 421.
Drummond (1919). *Biochem. J.*, **13**, 95.
Lapworth and Pearson (1919). *Biochem. J.*, **13**, 296.