CXII. NOTE ON THE EFFECT OF HIGH TEMPERATURES ON THE ACCESSORY FOOD FACTOR CONTENT OF COD-LIVER OIL.

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THE experimental work described in this paper was undertaken because of the result obtained on incidentally testing a sample of heated cod-liver oil for its content of vitamin A. This sample of oil had been used to see whether it contained any volatile constituent up to a temperature of 300° at a pressure of 0.2 mm. The pressure above the oil was reduced to this figure before heat was applied to the surrounding oil bath. Then the temperature of the flask contents was raised slowly to 300° , this occupying a period of 6 hours. The flask contents were immediately cooled, air being excluded. Incidentally this oil was tested on rats, and it was found that even 1 g. added daily to the basal diet failed to show any evidence of the presence of the growth factor. Further, X-ray examination of the bones showed that the calcifying principle had also been destroyed during the process. This experiment indicated that heat alone, apart from any external oxidation, could bring about the destruction of these factors—if distinct. A series of experiments, which will be described later, was therefore carried out to determine the effect of heat alone at a lower level on the vitamin content of cod-liver oil.

The stability of vitamin A to heat and oxidation has been the subject of several investigations. The discrepancies between the results obtained by earlier workers in this field were shown by Hopkins [1920] to be due to the fact that the growth-promoting factor is easily destroyed by the oxygen of the air. He showed, on the other hand, that if butter be heated, air being carefully excluded, its vitamin A content is not much reduced by a temperature of 120° maintained for 12 hours.

More recently the view of E. Mellanby [1918] that the anti-rachitic factor is probably identical with vitamin A, has been attacked on the ground that the two principles are not equally stable when exposed to heat and oxidation, the former factor proving more resistant to these processes [McCollum, Simmonds, Becker, and Shipley, 1922; Goldblatt and Zilva, 1923].

In consequence of these results it was decided to determine the effect of heat alone for varying periods on the vitamin content of cod-liver oil. It has

already been stated that the author found that slowly heating cod-liver oil to 300°, air being rigidly excluded, destroyed the growth-promoting and calcifying factors. A lower temperature, 200°, was therefore decided upon. A sample of unrefined cod-liver oil was obtained and 200 g. of this placed in each of three long-neck round-bottomed flasks fitted with a rubber cork having inlet and outlet tubes and a thermometer. The outlet tubes were connected up with tubing, the other end of which dipped under mercury, thus excluding air, but allowing escape through expansion in the flasks. By means of the inlet tubes the flasks were flushed out with dry carbon dioxide before heating was commenced. The flasks were heated in an oil-bath. The temperature of the flask contents was gradually raised to 200°, this process taking about 1 hour. One flask was thereupon disconnected and cooled, air being excluded. This specimen will be referred to as Heated 1 sample. The temperature of the oil in the other two flasks was maintained at 200° for 2 hours, when one of these was disconnected and cooled. The contents of the remaining flask were maintained at 200° for a further 4 hours. The flask was then disconnected and cooled, air being excluded. The last two lots of oil will be referred to as Heated 2 and Heated 3 samples respectively.

EFFECT ON THE GROWTH CURVE.

A preliminary testing of these three samples for their content of the growth-promoting factor was then carried out on rats, the basal diet of these animals being: starch 50 %, caseinogen 20 %, oxidised heated olive oil 20 %, marmite 5 %, salt mixture 5 % [McCollum, 1917]. It was found that 5 mg. of the unheated sample restored normal growth when this had ceased on the basal diet. About 40 mg. of the Heated 1 sample, and 180 of the Heated 2 sample were required for this purpose, whilst 0.3 g. of the Heated 3 sample gave a very flat growth curve. It is curious to note that while the rats did not grow much on the last sample, yet in no case did they show xerophthalmia.

In Fig. 1 are illustrated the growth curves of a family of ten rats, pairs of which were being tested preventatively with the same amount (namely, 0.1 g. per rat per day) of the unheated and heated specimens of oil, one pair remaining on the basal diet for control. Similar results were obtained with two other sets of rats.

It will be seen that the growth curve given by 0.1 g. of Heated 1 sample is normal, that the growth produced by the same amount of Heated 2 sample is somewhat below normal, whilst the animals fed with 0.1 g. of Heated 3 specimen put on weight extremely slowly.

RELATIVE CALCIFICATION EFFECTS PRODUCED BY THE SAMPLES.

With the diet given, great differences in the calcification of bones were not to be expected. The bones were examined radiographically and their calcium content subsequently estimated. The radiograms of the respective

sets showed that the bones of the rats on the Unheated and Heated 1 specimens were well and equally calcified. Those of the rats on the Heated 2 specimen showed some osteoporosis, which was more marked in the case of those on the Heated 3 specimen. The calcification in this last case showed a slight improvement on that in the bones of rats on the basal diet only.

Fig. 1. Rats 1 and 3. 0.1 g. unheated oil
 „ 2 „ 4. 0.1 g. heated 1 sample
 „ 5 „ 6. 0.1 g. „ 2 „
 „ 7 „ 8. 0.1 g. „ 3 „
 „ 9 „ 10. Basal diet only

The calcium content of the femora of the respective animals worked out as follows:

Specimen of oil	% content of moisture	First set of rats % content of calcium	
		Wet bone	Dry bone
Unheated			
1st rat	25.7	17.0	22.9
2nd rat	23.1	15.9	20.4
Heated 1			
1st rat	22.0	15.1	19.4
2nd rat	23.5	15.0	21.2
Heated 2			
1st rat	28.3	12.6	17.8
2nd rat	29.7	11.9	17.0
Heated 3			
1st rat	31.6	10.3	15.1
2nd rat	31.8	9.0	14.7
Basal diet only			
1st rat	31.2	10.0	15.5
2nd rat	33.6	8.5	12.6

The bones of the other two sets of rats were not analysed for calcium content.

Thus it will be seen that the calcium estimations bear out on the whole the results found radiographically; that is, there is a gradual disappearance of the anti-rachitic vitamin when the oil is heated to, and maintained at, this high temperature.

SUMMARY OF RESULTS.

1. When cod-liver oil is slowly heated out of contact with air to 300° the anti-rachitic and growth-promoting factors contained in it appear to be completely destroyed.

2. If cod-liver oil is slowly heated to 200° and maintained at this temperature for some hours these two factors slowly disappear *pari passu*.

3. Oxidation from external sources is obviously unnecessary for the destruction of these factors at these temperatures.

4. If oxygen be excluded the chemical compound or compounds representing these factors are fairly stable to heat.

The work described above was carried out during the first half of last year, and the main results reported in June, 1924, to the Science Committee of the British Medical Association to whom grateful acknowledgment is made for the grant which defrayed the expenses of the work. Since then Steenbock and his collaborators [1924] have published the results which led them to the conclusion that the presence of the anti-rachitic factor is necessary for the manifestation of growth also.

The possibility of the destruction of the factors in the above experiments being due to the action of the fatty acids liberated by hydrolysis of the glycerides at those high temperatures was one which the author considered and tried to avoid by heating some oil with a weak base out of contact with air. It was found however that as weak a base as magnesium oxide only increased the rate of hydrolysis with much soap formation.

Finally I would express my thanks to Professor Edward Mellanby for his support throughout the work.

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