II. STUDIES ON THE EFFECT OF HEAT ON MILK.

IV¹. THE IODINE CONTENT.

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EXPERIMENTS have been reported showing that reduction in the soluble calcium of milk by heat [Magee and Harvey, 1926, 1] (a) adversely affects the metabolism of calcium, phosphorus, and nitrogen [Magee and Harvey, 1926, 2], and (b) may eventually give rise to gross signs of rickets [Orr *et al.*, 1926] in young animals fed on heated milk. Symptoms other than those resulting from defective formation of bony tissue have been attributed to the feeding of heated milk to children [Hess, 1920]. Cod-liver oil has been quoted [Maddock, 1927] as having improved the growth-promoting properties of heated milk. Cod-liver oil is comparatively rich in iodine [Kelly, 1925]. These considerations suggested an investigation into the effect of heat on the iodine content of milk. As the investigation proceeded the question of the physico-chemical state of the iodine content was raised. Experiments dealing with this problem are also included in this paper.

Effect of heat on the total iodine.

Separated cow's milk ("fresh," "pasteurised" and "boiled") was prepared as before [Magee and Harvey, 1926, 1]. In addition samples were prepared by bringing separated milk in an open beaker rapidly to boiling point and allowing it to cool on the bench ("boiled in open"). By continuous stirring film formation was avoided. Total iodine was estimated in duplicate by the Leitch-Henderson [1926] modification of Fellenberg's method.

Of the 13 samples studied (Table I) only eight were subjected to all the heating processes. While the changes due to heat in all the samples are comparable with one another, only the findings in regard to these eight samples are averaged for the sake of comparison. It is evident that heat brought about considerable losses in the iodine content. The fact that these were greater in the case of the boiled milk than in the case of pasteurised milk seems to indicate a progressive effect of the heat. Further, the relative evenness of the losses in the case of the boiled milk would suggest that the limit of loss which heat could effect had been nearly reached.

¹ No. III of this series appeared in the *Scottish Journal of Agriculture* [Orr *et al.*, 1926] but by accident was not headed as belonging to the series.

Table I. *Iodine content before and after heating.*

"Fresh" γ per 100 cc.	"Pasteurised" γ per 100 cc.	Loss %	"Boiled" γ per 100 cc.	Loss %	"Boiled in open" γ per 100 cc.	Loss %
6.00	4.00	33	—	—	—	—
6.36	4.31	32	—	—	—	—
4.94	3.78	23	3.68	25	—	—
6.30	5.15	18	4.94	22	4.73	25
4.90	3.90	20	3.60	27	4.40	10
4.73	4.10	13	3.68	22	3.89	18
Av. 5.69	Av. 4.51	Av. 20	Av. 4.23	Av. 26	Av. 4.42	Av. 22
7.46	5.36	15	5.46	27	5.15	31
6.90	5.90	15	5.30	23	5.70	17
5.36	3.89	27	3.47	35	3.89	27
4.94	3.89	21	3.68	25	3.47	30
4.94	3.89	21	3.68	25	4.10	17
4.20	3.68	12	3.26	22	—	—
4.31	3.68	15	3.36	22	—	—

The disappearance of iodine seemed to be due to volatilisation. To confirm this inference approximately 10 g. of iodine-free KOH per 300 cc. were added to several samples of milk which were prepared as before. Obviously, there was then much more alkali present than was necessary to fix, and therefore to prevent volatilisation of, all the iodine present in the milk.

Table II. *Iodine content before and after heating in presence of KOH.*

"Fresh" γ per 100 cc.	"Pasteurised" γ per 100 cc.	Change %	"Boiled" γ per 100 cc.	Change %	"Boiled in open" γ per 100 cc.	Change %
4.20	4.00	-5	4.30	+3	4.00	-5
3.70	3.60	-3	3.80	+3	3.90	+5
4.83	4.52	-7	5.04	+4	4.83	0
4.52	4.20	-7	4.31	-5	4.20	-7
Av. 4.31	4.08	-5.5	4.36	+1.25	4.23	-1.75

The variations found (Table II) in the iodine contents of the heated milks as compared with the raw samples are within the limits of error of the analytical method and are therefore without significance.

The state of iodine in milk.

Harvey [1927] has stated that about 80 % of the total iodine in goat's milk is diffusible, but he did not report the experiments in detail. We have, as being more appropriate, included them in this paper, especially because the iodine estimations were all carried out by one of us (A. E. G.). Fresh separated goat's milk only was used.

Ultrafiltration was carried out by Thiessen's apparatus¹. The membranes used were made by soaking No. 40 Whatman filter papers in a solution containing 2.5 % collodion in glacial acetic acid, allowing them to drip and then washing away all trace of acid in running water. Previous trials had shown that such membranes just held back all the colloids of milk. Pressures of three atmospheres were supplied by means of a simple form of pressure apparatus fitted with a motor tyre valve. Ultrafiltration for 30 hours usually

¹ Supplied by Vereinigung Göttinger Werke, Göttingen.

sufficed to give a perfectly clear ultrafiltrate of about 7 cc. from 10 cc. of milk. Ultrafiltration was always continued until the residue was quite gummy and immobile.

Dialysis was carried out by the method previously described [Magee and Harvey, 1926, 1] but distilled water was used instead of running tap water and all membranes were made from a solution containing 4 % collodion in glacial acetic acid. 20 cc. of milk were put in each sac and three of these at a time were dialysed against 2 litres of distilled water which was changed every 2 hours, the total period of dialysis being 8 hours. The iodine in the contents of each sac was then estimated so that each result is an average of three estimations. The results are shown in Table III.

Table III.

Ultrafiltration:						Average
1. Iodine in separated milk, γ per 100 cc.	17	14	22	20	14	17
2. Iodine in ultrafiltrate, % of total	82	75	75	78	75	77
3. Iodine in residue, % of total	15	15	17	20	22	18
4. Sum (2 + 3)	97	90	92	98	97	95
Dialysis:						Average
Iodine in separated milk, γ per 100 cc.	18	11	13	11	14	13
Iodine in residue, % of total	14	16	15	16	17	16

The results of the ultrafiltration experiments gave an average of 77 % of the total iodine in the ultrafiltrate and 18 % in the residue, *i.e.* 95 % of the iodine was recovered. This result is satisfactory considering the complicated nature of the technique. By dialysis an average of 16 % was obtained in the residue. This figure is in close agreement with the 18 % found by ultrafiltration. The average of these two results, 17 %, therefore, most probably represents the non-diffusible iodine. That is to say, 83 % of the total iodine of separated goat's milk was found to be diffusible.

The iodine content of milk-fat.

The above experiments having been carried out on milk separated by a separator, it seemed of interest to compare the iodine contents of the samples before and after separation. It will be seen (Table III) that the average iodine content of the 11 samples was 15 γ per 100 cc. of separated milk. Before separation the same samples showed an average content of 17 γ per 100 cc. of whole milk, *i.e.* a difference of 2 γ . These findings indicate that the fat contained more iodine than an equal volume of separated milk. Most probably the iodine associated with the fat was chemically bound with the unsaturated fats and presumably therefore non-diffusible. The separated milk used in the experiments unquestionably contained some fat, because the most efficient separator, even when set, as in these experiments, so as to remove the maximum of cream, invariably leaves traces of fat behind. It does not, however, seem possible that the presence of such small amounts of fat in the separated milk could account for all the non-diffusible fraction. It seems

therefore, that this fraction for the most part consists of iodine in close, non-dissociable, chemical union with either organic or inorganic colloids or with both. The average fat content of the above 11 samples (of whole milk) as determined by the Soxhlet method was 6.52 g. per 100 cc.

Effect of heat on the state of iodine.

Separated cow's milk, raw and heated, was used for these experiments. Three lots of 20 cc. from each sample were dialysed as before and the iodine estimated in the residues.

Table IV.

"Fresh" milk		"Pasteurised" milk		"Boiled" milk	
γ per 100 cc. before dialysis	Loss % by dialysis	γ per 100 cc. before dialysis	Loss % by dialysis	γ per 100 cc. before dialysis	Loss % by dialysis
6.0	79	4.0	82	—	—
6.4	77	4.3	78	3.7	74
4.9	87	3.9	88	3.6	88
4.2	88	3.9	86	3.3	87
Av. 5.4	83	4.0	84	3.5	83

The results (Table IV) suffice to show that after heating, the diffusible and non-diffusible fractions of the residual iodine bore the same relation to each other as they did in the original raw milk. Further, the data indicate that the relative amount of diffusible iodine in separated cow's milk is approximately the same as in separated goat's milk (83 %).

SUMMARY.

Heat caused the disappearance by volatilisation of 20 % or more, according to the duration of heating, of the total iodine of separated milk. The ratio of diffusible to non-diffusible iodine was the same in the milk after heating as it was before. Approximately 83 % of the total iodine of separated cow's and goat's milk was found to be in diffusible form.

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