CXI. STUDIES ON THE EFFECT OF HEAT ON MILK.

II. THE INFLUENCE OF DIETS OF FRESH AND TREATED COW'S MILK ON THE CALCIUM, PHOS- PHORUS AND NITROGEN METABOLISM OF THE YOUNG PIG.

BY HUGH EDWARD MAGEE AND DOUGLAS HARVEY.

From the Rowett Research Institute, Aberdeen.

(Received July 12th, 1926.)

IN the first paper of this series [Magee and Harvey, 1926] we reported, as a result of heating milk, a progressive fall in the diffusible calcium. On the other hand, several workers have demonstrated that the souring of milk causes a rise in the diffusible calcium [Grosser, 1913; Van Slyke and Bosworth, 1916; Wha, 1923] and phosphorus [György, 1923]. It seemed of interest, therefore, to investigate the effect of diets of raw, heated and sour milk on the metabolism of calcium and phosphorus in young animals. Study of the metabolism of nitrogen was also considered desirable; firstly because we observed that heat affected the physical state of the colloids, but not the digestibility of the proteins, and secondly because the lactic acid of sour milk precipitates the caseinogen as a base-free protein [György, 1923].

The most recent review of the literature available [Lane-Claypon, 1916] would lead one to conclude that infants fed on heated cow's milk thrive better than on raw milk. The later experiments of Daniels and Loughlin [1920] and Daniels and Stearns [1924] point to a contrary conclusion. The former attributed the defective nutrition they observed in rats fed on heated milk to loss of calcium by precipitation. The latter found that infants fed on very rapidly boiled milk stored more calcium and phosphorus than on milk pasteurised for half an hour. They contend that the extent to which milk is detrimentally affected by heat depends more on the duration of the heating than on the temperature.

EXPERIMENTAL.

Pigs were used for these experiments. They are more suitable animals for metabolism experiments than calves and, on account of their rapid rate of growth, errors of diet soon become apparent. Cow's milk, however, is not well suited for the requirements of the young pig. It contains on an average

0.16 % CaO and 0.24 % P_2O_5 , *i.e.* $CaO : P_2O_5 = 1 : 1.5$, whereas sow's milk, the natural food of the suckling pig, contains 0.39 % CaO and 0.36 % P_2O_5 , *i.e.* $CaO : P_2O_5 = 1 : 0.92$. It is impossible to supply the food requirements of pigs of the age employed with cow's milk only. Cereals had therefore to be added to their ration. These raised the energy and phosphorus contents, but had little effect on the calcium, so that in regard to the $CaO : P_2O_5$ ratio the diet was rendered still more unsuitable. We decided, nevertheless, as a preliminary to further experiments, to try the effect of feeding a ration of cereals with heated, fresh or sour milk on the calcium, phosphorus and nitrogen metabolism of a young pig. The $CaO : P_2O_5$ ratio of the intake was 1 : 3 and the periods were in the above order. The animal broke down after a month and signs of rickets appeared. The retention of CaO fell steadily until finally only 4 % of the intake was being absorbed as compared with 28 % at the beginning. The rate of retention of N was, however, maintained and indeed increased.

This preliminary experiment showed that the ration of cow's milk and cereals was inadequate for proper growth of a young pig, owing most probably to its low calcium content; for Henderson [1925] has shown that a diet with a $CaO : P_2O_5$ ratio of 1 : 2.5 caused defective bone formation in a young pig in the absence of ultra-violet light. It was, therefore, necessary in the subsequent experiments to supplement the diet of milk and cereals with calcium.

Exp. 1. Animals. Two hog pigs aged 12 weeks, No. 1 weighing 21 kg. and No. 2 16 kg.

Diets per day. Basal rations. Pig 1. Oatmeal, maize and sharps 220 g. each, blood meal 30 g., olive oil 5 cc., $CaCl_2$ solution 100 cc. (5.621 g. CaO) and some saccharin to sweeten. Pig 2. three-fourths, *i.e.* approximately 16/21, of the ration fed to pig 1. The $CaO : P_2O_5$ ratio of the diet when milk was introduced was approximately 1 : 1.2. In regard to the proper ratio for pigs of the age employed, we are still somewhat in the dark, for while a ratio of 1 : 0.92 is proper for a suckling pig, it does not follow that it is so for one 10 or 12 weeks old. The adjustment made must be regarded, therefore, as only approximate.

Periods. (1) Basal ration period—8 days. The above basal ration was fed in order to determine the nature of the balance before milk was introduced.

(2), (3) and (4) Pasteurised, Fresh and Sour Milk periods, each 12 days. 1500 cc. daily of the appropriate milk were given to pig 1 and 1125 to pig 2. For the latter the order was: Fresh, Pasteurised and Sour Periods.

(5) Post-period—6 days. The $CaCl_2$ supplement was reduced by half in order to determine whether big reductions in the intake of soluble CaO would accentuate the effects induced by feeding heated milk. Pig 1 got 1500 cc. fresh milk and pig 2, 1125 cc. per day.

Methods. The methods of collection of excreta and of analyses were similar to those in general use at this Institute [Husband, Godden and Richards, 1923]. Excreta were collected and analysed every 48 hours. At the beginning of each period an interval of 48 hours was allowed to elapse before analyses were begun.

The milk was all from the same cow, one of the Institute herd (the bacterial count of pooled samples of Institute milk at the time of the experiments was found to be between 2000 and 4000 per cc.). The intake of CaO , P_2O_5 and N was determined by previous analyses of the cereals and by drawing off daily suitable quantities of milk from the bulk fed to the pigs and analysing the composite sample at short intervals. "Pasteurised" milk was prepared by keeping a bulk of fresh milk at 70° in a vessel immersed in a water-bath for 30 minutes. The film was removed after cooling rapidly. "Sour" milk was prepared by inoculating the requisite amount of fresh milk for the daily ration with a pure culture of *B. bulgaricus* and incubating 24 hours. The sample for analysis was taken from the bulk of fresh milk, not from the sour milk.

The cereal part of the ration mixed with sufficient milk was all given at the morning feed, the remainder of the milk, if fresh or pasteurised, was kept cool and some formaldehyde added so as to preserve it until the evening feed.

Results. The results are shown in the figure.

Fig. 1.

The 48-hourly balances when plotted gave curves with a great number of small oscillations. In order to smooth these out an average was taken of consecutive pairs of values in each period and *graphed*. In the post-period the three values were taken together and averaged.

In pig 1 (Fig. 1) it is seen that on the addition of milk to the basal ration there is a marked increase in the retention of CaO and P_2O_5 which is continued when fresh milk is substituted for pasteurised. During the latter part of the fresh period the retention falls slightly but increases steadily when sour milk is substituted for fresh. The N curve does not suggest any definite conclusions. In view of the results shown in the N graph of pig 2 (Fig. 2) it appears

probable that pig 1 was on a *luxus* consumption of N throughout the three milk periods. The initial increases in the balance on changing from pasteurised milk to fresh and from fresh to sour seem to support this view.

In pig 2 the retention of CaO and P_2O_5 rises steadily in the fresh period, but falls in the pasteurised and rises again in the sour period. The retention of N, on the whole, follows that of CaO and P_2O_5 . It will be noted, however, that on the introduction of milk the retention of N in both pigs, unlike that of CaO and P_2O_5 , rises rapidly.

In the post-period in both pigs (when the $CaCl_2$ supplement was reduced by half) the retention of CaO, P_2O_5 and N falls markedly. It is interesting to note that the diminished balances of P_2O_5 and N were largely due to increased urinary excretion, as is shown in Table I.

Table I. *Urinary excretion.*

(Average in g. per 2 days.)

Pig 1

Period	Basal	Past.	Fresh	Sour	Post
CaO	1.23	1.09	0.85	0.72	0.20
P_2O_5	0.44	0.68	0.66	1.17	5.33
N	17.36	17.82	19.47	20.31	23.26

Pig 2

Period	Basal	Fresh	Past.	Sour	Post
CaO	1.17	1.12	0.70	0.78	0.29
P_2O_5	0.32	0.62	0.71	0.77	4.04
N	13.65	16.74	18.50	18.50	19.88

The P_2O_5 excretion was increased about fivefold. The next highest value for the urinary P_2O_5 was in the sour period. This supports the finding of Blatherwick and Long [1922] that sour milk ingestion increased the urinary phosphorus.

This experiment seemed to indicate that the retention of CaO, P_2O_5 and N was lower on the ration of pasteurised milk than on that of fresh or sour milk. The next step in the investigation was to try the effect of adding enough soluble calcium to the diet to make good the loss due to pasteurising.

Exp. 2. Animals. Two hog pigs aged 15 weeks and weighing 25 kg. each.

Periods. Pig 1—1st fresh 15 days: pasteurised plus $CaCl_2$ 15: 2nd fresh 12.

Pig. 2—Pasteurised plus $CaCl_2$ 15 days: fresh 15: boiled 12.

Diets per day. Oatmeal, maize and sharps 250 g. each, blood meal 30 g., milk 1500 cc., olive oil 10 cc., $CaCl_2$ solution 75 cc. (4.043 g. CaO) and some saccharin. During the pasteurised plus $CaCl_2$ period 81 cc. $CaCl_2$ solution (4.366 g. CaO) were given. The extra supplement of $CaCl_2$ was equivalent to about double the loss in diffusible CaO after pasteurising as estimated from the data of our earlier experiments [Magee and Harvey, 1926]. The CaO : P_2O_5 ratio of the diet was approximately 1 : 1.6.

The technique was in general the same as in Exp. 1. The boiled milk was prepared by keeping the water in the outside vessel boiling vigorously for an hour: pasteurising was done at 70° for 30 minutes.

Excreta were collected and analysed every 72 hours. An interval of 72

hours was allowed to elapse at the beginning of each period before analyses were begun.

Results. The results are shown in Table II.

Table II.

(Average in g. per 3 days.)

Balance.

Period	Fig 1			Fig 2		
	1st fresh	Past. + CaCl ₂	2nd fresh	Past. + CaCl ₂	Fresh	Boiled
CaO	11.25	13.35	12.57	13.07	11.37	11.94
P ₂ O ₅	14.76	16.21	13.95	15.85	13.71	12.70
N	32.81	33.39	29.64	41.50	32.79	28.91

Urinary excretion.

CaO	1.31	1.36	1.24	0.65	0.63	0.45
P ₂ O ₅	5.52	5.28	6.29	3.91	5.38	5.52
N	43.33	41.44	48.88	34.96	38.84	47.60

As in Exp. 1 the retention of CaO and P₂O₅ did not rise to a steady level until about the 9th day of the first milk period. The first three values in this period were therefore discarded and an average made of the other two. This average represented fairly accurately the equilibrium state. The values in each of the other periods and, in the case of N, in all the periods were obtained by averaging all the results in each. Thus, the results were expressed in tabular form which was to be desired; for, owing to there being five values in each of the first two periods, averaging of consecutive pairs to smooth out the graphs as in Exp. 1 was not possible.

Fig. 2.

It will be seen from the table that the retention of CaO, P₂O₅ and N was highest in the pasteurised plus CaCl₂ period and that the extra supplement of CaCl₂ caused an increase in the retention of CaO equal to more than twice the amount added (0.323 g. CaO). The retention of P₂O₅ and N was lower in the

boiled period than in the fresh, but the retention of CaO was slightly higher in the former than in the latter. This finding is not in conformity with the other results either of this experiment or of the previous one. Very probably the retention was to some extent apparent rather than real, for there was a tendency to constipation during the boiled period. The low urinary CaO in this period would seem to substantiate this view.

As in the post-period of Exp. 1 reduction of the soluble CaO of the intake caused a rise in the urinary P_2O_5 and N. Thus the excretion is lowest in the pasteurised plus $CaCl_2$ period and highest in the boiled period (pig 2).

This experiment suggested that the detrimental effect of heat on milk could be removed, in so far as the retention of CaO, P_2O_5 and N is concerned, by the addition of calcium in solution. Exp. 1 shows, however, that there was practically no difference between the retention of CaO, P_2O_5 and N on the fresh and on the sour milk ration, though the soluble CaO content of the latter was greater than that of the former. There is no apparent reason for this difference.

It would appear from this investigation that the retention of calcium, phosphorus and nitrogen by young animals is lower on a diet containing heated milk than on one containing fresh or sour milk, and further that the addition of soluble calcium to the heated milk ration increases the amount of calcium, phosphorus and nitrogen retained by more than the weight of the calcium added. The changes in the state of the colloids, described in our first paper, had evidently no effect on the assimilation of nitrogen. It is therefore suggested that the reduction in the soluble calcium by heat is an important factor in the lowering of the retention of calcium, phosphorus and nitrogen of young animals fed on heated milk as compared with raw milk.

There are no data in these experiments that would throw any light on the effect on metabolism of alteration by heat in other milk constituents of known or unknown constitution, so that discussion along these lines is not possible.

SUMMARY.

1. A young pig kept under experimental conditions on a diet of cow's milk and cereals showed signs of rickets after 30 days. The addition of a soluble calcium salt to the diet of cereals and milk enabled the animals fed on it to remain in a thriving condition for 60 days.

2. On a diet of cereals and milk the retention of calcium, phosphorus and nitrogen was lower with heated milk than with fresh or sour milk.

3. The addition of soluble calcium to a ration containing heated milk raised the retention of calcium, phosphorus and nitrogen by more than the weight of the calcium added.

4. Reduction of the soluble calcium of the diet increased the urinary phosphorus and nitrogen.

5. It is suggested that heat has a detrimental effect on the nutritive value of milk and that one of the important contributing factors is the reduction in the amount of soluble calcium.

We thankfully express our indebtedness to Dr J. B. Orr, Director of the Institute, for help and advice.

REFERENCES.

- Blatherwick and Long (1922). *J. Biol. Chem.* **53**, 101.
Daniels and Loughlin (1920). *J. Biol. Chem.* **44**, 381.
Daniels and Stearns (1924). *J. Biol. Chem.* **61**, 225.
Grosser (1913). *Biochem. Z.* **48**, 427.
György (1923). *Biochem. Z.* **142**, 1.
Henderson (1925). *Biochem. J.* **19**, 52.
Husband, Godden and Richards (1923). *Biochem. J.* **17**, 707.
Lane-Claypon (1916). Milk and its Hygienic Relations.
Magee and Harvey (1926). *Biochem. J.* **20**, 873.
Van Slyke and Bosworth (1916). *J. Biol. Chem.* **24**, 191.
Wha (1923). *Biochem. Z.* **144**, 278.