The Rat as a Test Animal in evaluating Artificial Human Milk for Infants

MANY attempts have been made to develop artificial milk formulations to replace breast milk in the feeding of young infants. Tests have been carried out on infants themselves, and there are very few reports of attempts to use animals in such studies[1-3].

We believe that animal experimentation is necessary in studies of artificial human milk, and we have tried to find an indicator which would rapidly show the influence of different formularies on the animal. We observed that feeding with human milk or cow's milk, respectively, causes similar changes in the fæces of rats and the fæces of infants. Using the *p*H of the fæces as an indicator, we then tried to modify cow's milk in such a way that its effect on the rat would be similar to that of human milk.

The technique used in our experiments was very simple. Rats previously kept on an ordinary laboratory diet were caged individually and fed the milk or milk mixtures to be studied *ad libitum*. No extra water was given, and since the experimental period was always very short (1–2 weeks) no minerals or vitamins were added to the milks. The fæces were collected during a few hours each day, suspended in distilled water and the *p*H of the suspension determined electrometrically.

In the first experiments, three-weeks old weanling rats were used. In accordance with Scott and Norris[1], it was found that human milk was unsuitable for these young rats, whereas the cow's milk permitted some gain in weight (Table 1). In later experiments, older rats (9–22 weeks) were used. Although neither cow's milk nor human milk produced growth in these rats, the animals maintained their weight, and it was evident that the results obtained during the short experimental periods were not due to inanition.

Table 1. EFFECTS OF COW'S MILK AND HUMAN MILK AS DIET FOR RATS OF DIFFERENT AGES

Age of rats	Diet	Average body-weight (gm.) initially	Average body-weight (gm.) at 2 weeks	Gain in weight in 2 weeks (gm.)	No. of survivors
3 weeks	Cow's milk	34	51	17	5/6
3 weeks	Human milk	32	29	−3	4/6
9 weeks	Cow's milk	145	162	17	7/7
9 weeks	Human milk	143	148	5	7/7
22 weeks	Cow's milk	214	220	6	5/5
22 weeks	Human milk	220	215	−5	5/5

In Fig. 1 are given some typical *p*H curves of the fæces of rats fed either human milk or cow's milk. It can be seen that there is a distinct difference between the rats on these two diets. Feeding with human milk has given *p*H values between 5 and 6 whereas cow's milk, on the other hand, has given *p*H values above 7. This difference was usually apparent as early as two days after the beginning of the experiment.

The purpose of the further experiments was to find out how cow's milk must be modified to get the *p*H of fæces down to the human-milk level. A great number of cow's milk modifications were prepared including changes in the quality and quantity of protein, fats, carbohydrates, minerals and vitamins. In most of these cases the *p*H of the fæces did not change in the desired way. However, decreasing the calcium content of the milk and a simultaneous addition of lactose led to positive results. In all experiments with decreased calcium and added lactose the *p*H of the rat fæces was distinctly acid, the

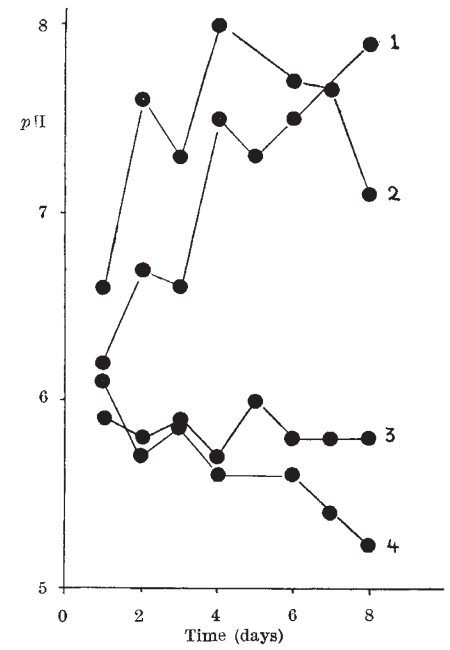

Fig. 1. *p*H of the fæces of rats fed either cow's milk or human milk (averages of 5–7 rats): (1) cow's milk, 9 weeks old rats; (2) cow's milk, 22 weeks old rats; (3) human milk, 9 weeks old rats; (4) human milk, 22 weeks old rats

acidity curve closely resembling that obtained with human milk.

The calcium content of the milk was lowered by treatment with cation exchangers ('Zeokarb 215' proved satisfactory, removing about 30 per cent of the calcium), by precipitation with sodium sulphate, sodium oxalate or sodium carbonate (cf. ref. 4), or by adding sodium hexametaphosphate ('Calgon') to the milk. The last-mentioned substance, which is known to form soluble complex ions with bivalent ions such as calcium and which is generally used for softening water, has earlier been used by Schwartz *et al.*[5] for the preparation of 'soft curd' milk. Of the different substances tried it was found to be the most suitable for reducing the content of the ionizable calcium. Lactose was added to the milk at the level of 5–10 per cent. Other carbohydrates, for example, dextrin, maltose and sucrose, did not lower the *p*H of the fæces when added to the milk in the same quantities.

The effect of the hexametaphosphate–lactose–milk on the *p*H of the fæces is probably due both to changes in the curdling properties of casein in the stomach and its absorption in the intestines, and to alterations in the composition of the intestinal flora. Further investigations into these questions and into the applicability of such milk formulations as food for young infants are in progress.

PAAVO ROINE
HELGE GYLLENBERG
MARJATTA ROSSANDER
RITVA VUOKILA
Department of Nutritional Chemistry,
University of Helsinki. June 9.

[1] Scott, M. L., and Norris, L. C., *J. Nutrition*, **37**, 337 (1949).
[2] Tomarelli, R. M., Linden, E., Durbin, G. T., and Bernhart, F. W., *J. Nutrition*, **51**, 251 (1953).
[3] György, P., Mello, M. J., Torres, F. E., and Barnes, L. A., *Proc. Soc. Exp. Biol. Med.*, **84**, 464 (1953).
[4] Lemke, H., *Monatsschr. Kinderheilk.*, **99**, 409 (1951).
[5] Schwartz, C., Jones, K. K., Mack, T. W., and Vance, R. W., *J. Dairy Sci.*, **23**, 19 (1940).