

THE INFLUENCE OF RAW AND STERILIZED MILK ON GROWTH AND REPRODUCTION IN RATS

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By feeding experiments in which young animals received a diet of biscuit made from white flour, together with milk which constituted a considerable part of the caloric intake, Mattick and Golding (1931) studied the comparative values of raw, pasteurized and sterilized¹ milk on the growth and reproduction of the rat. They observed that sterilization¹ of cow's milk markedly decreases its nutritional value for the young rat and that, apart from poor development and growth, this form of heat treatment as applied by them [212° F. (100° C.) for 30 min.] caused a complete loss of reproductive capacity. Growth and reproduction were, however, normal on a diet of raw milk. In contrast, growth with pasteurized milk [145° F. (62.8° C.) for 30 min.] was satisfactory, although reproduction was again less satisfactory than on raw milk. Using similar methods of experiment, Drummond (1933) and Wilson and Cowell (1933) have studied the comparative nutritive values of raw and pasteurized milk on the growth and reproduction of rats and mice respectively. In confirmation of Mattick and Golding (1931) their results agreed in showing that under the conditions of experiment used there was a significant though slight difference between the nutritive value of the raw and pasteurized milks in general development; on the other hand their findings regarding reproduction were in great contrast to the results of those authors, for reproduction either on raw or pasteurized milks was poor and, further, the litters did not survive. Drummond and Wilson and Cowell observed also that the addition of Yeastrel to both the raw and pasteurized groups caused reproduction and the rearing of the young to be achieved, possibly because of the extra vitamin B provided.

The work described in this paper records the results obtained in applying similar experimental methods to a comparison of the relative nutritive values of raw and sterilized milks. It was undertaken because of the results of Mattick and Golding (1931) which showed that sterilization of milk caused some change detrimental to growth. A further examination of this problem is clearly of considerable importance in view of the increasing use of sterilized milk in infant feeding. This has been forcibly brought to the notice of one of us (H. A. C.) in the conduct of an infant welfare clinic in one of the poorer areas

¹ The words "sterilized" and "sterilization" are used in this paper only in the commercial sense in reference to the heat treatment of milk for varying periods of time at temperatures of 100° C. or more. The period of heating and the temperatures are mentioned where they are relevant.

under the control of the Liverpool Corporation. The mothers, warned against the use of raw milk for infant feeding and unable to afford milk from tuberculin-tested cows, use sterilized milk as often as pasteurized milk. Further, from considerations of present knowledge it did not seem likely *a priori* that milk should suffer significantly more damage from the vitamin point of view by sterilization in bottles under modern conditions than from pasteurization. Lastly, while there is much reported work on various types of nutritive study in which sterilized milk has been used, the word "sterilized" has been employed in the widest sense and the conditions used are often ill-defined. Some of the reports suggest that milk which has been so excessively heated as to cause actual browning has been employed, and the impression is left that many of the findings obtained on such milks are of little value and cannot be applied to milk produced by sterilization under modern conditions.

Apart from the general question of a comparison of the nutritive values of the raw and sterilized milks, as measured by growth rates and general appearance, a further object in view in this work was to attempt to demonstrate whether there was any difference in the sterilized milks resulting from two different sterilization processes. In addition, the experiments have been used for a study of reproduction resulting from the use of raw and sterilized milks.

EXPERIMENTAL

The method used has been essentially that of the authors previously mentioned, namely the feeding of young animals on a diet of white flour and raw or sterilized milk.

The milk used. By the kind co-operation of Captain R. W. Lane and of Mr C. E. Keeling, the milks used were obtained from establishments situated at Bletchley and Stockport respectively. The raw and sterilized samples were in each case withdrawn with due precautions from the same bulk supplies. Because of certain differences which were indicated by the results obtained from the experiments, the two processes of sterilization, which differed somewhat, are recorded in detail.

The Bletchley sterilized milk was prepared as follows: The fresh milk was heated to 95° F. (35° C.) and filtered, and after flash pasteurization at a temperature of 145° F. (62·8° C.) it was homogenized at a pressure of 1½ tons per sq. in., cooled and bottled at a temperature of 62° F. (16·7° C.); the bottled milk with the rubber-lined porcelain stoppers lightly in position was then heated to 212° F. (100° C.) (which allowed the escape of the small volume of air between the surface of the milk and the stopper); at this point the spring clips of the stoppers were shut down and the milk maintained at 212° F. (100° C.) for 30 min., after which it was cooled to a temperature of 55° F. (12·8° C.) for delivery.

The Stockport sterilized milk was prepared as follows: The fresh milk, drawn from bulk storage tanks, was preheated to 95–105° F. (35–40·6° C.); it was then filtered, heated to 167° F. (75·0° C.) and homogenized at that

temperature; the bottles were then filled with the milk at 165° F. (73·9° C.), hermetically sealed and passed into a multi-compartment sterilizing machine. The actual sterilization process consisted of the immersion of the bottles in water at 204° F. (95·6° C.), rising gradually to 212° F. (100° C.) in 14 min.; there followed sterilization under steam pressure for 35 min. at 224° F. (106·7° C.); after immersion in water at 212° F. (100° C.), decreasing to 204° F. (95·6° C.) in 14 min., the cooling process continued until the temperature of the bottled milk was reduced to 65° F. (18·3° C.). This process was carried out in a modern automatic apparatus producing six thousand bottles per hour.

The distances of the sterilizing plants from Liverpool presented difficulties as to the transport of the raw milk and, in order to obtain it in as fresh a condition as possible, the samples of raw milk were cooled and then run into vacuum flasks. The tightly stoppered vacuum flasks were enclosed, together with the samples of sterilized milk, in special felt-lined wooden containers which were dispatched by passenger train to Liverpool. These cases were met for immediate delivery to this laboratory; they arrived in Liverpool between the hours of 4.0 and 6.0 p.m. and part of each daily supply was fed to the rats immediately on arrival. The remainder was kept under cool conditions over night and was used for the morning feed on the following day. The raw milk samples used were thus administered to the animals some 10–16 hours after milking. By keeping an adequate reserve of the milks in the ice-chest, it was possible to meet any emergency caused by train delays which, save for Bank Holidays, occurred during winter fog. These delays were relatively few in number and can have played no serious part in the results which were obtained.

Method of experiment. Groups of rats which were litter mates received as a diet biscuit made from white flour, together with raw or sterilized milk *ad lib.* The daily milk consumption was measured for considerable periods for all the animals used and it was found that there was no significant difference in the consumption of raw or sterilized milks. The young animals consumed on an average 15–20 c.c. per day, which increased to 45 c.c. per day as the animals became adult. The dry biscuit was administered to the amount of 5 g. per day to the young animals and was then increased to 8 g. per day for the adult. After the first experiment had been in progress for a month, it was found that the animals of all the groups, both raw and sterilized, showed a marked degree of anaemia, a finding which was to be anticipated in view of the known deficiency of milk in iron and copper. Accordingly, for the remainder of the experiment, suitable supplements of iron, copper and manganese were incorporated in the biscuit. From this point therefore the biscuit was prepared from the dough obtained by kneading 1 kg. of white flour with 475 c.c. of water, in which had been dissolved 0·5 g. of ferric citrate, 1 c.c. of 2 per cent. copper sulphate solution and 1 c.c. of 1 per cent. manganese chloride solution.

The groups of animals used for the first experiment were made up of litter mates from the inbred stock of this laboratory. In the later experiments (Exps. 5 and 6) animals from an outside stock were employed. In Exp. 1,

twelve does were used for each of the groups fed on Stockport raw and sterilized milks; in Exp. 2 similar numbers of does were used for the Bletchley samples. In Exp. 3 groups of four bucks were used for the Stockport samples. Exps. 1-3 began in May and lasted for 48 weeks. In consequence of temporary difficulties in the breeding and consequently in providing 40-50 g. animals, Exp. 4, in which two groups of four bucks each were used for the Bletchley milks, was not started until October and was then carried on for 24 weeks. Exps. 5 and 6, in which each of the groups consisted of three does, began in October and lasted for 24 weeks. The animals were weighed weekly and the experiments varied in time from 24 to 48 weeks. Records of general health, growth and reproduction were made and a careful naked eye examination was made post mortem after killing at the end of the experiments.

RESULTS

(1) *General health.* All the animals were in excellent health throughout the year. They were plump, active and friendly. Their coats were in perfect condition, although the white hair tended to turn cream and the black to become silver. There was no detectable difference between the raw and sterilized groups. There were seven deaths during the year and these bore no apparent relationship to the nature of the milks, there being three deaths of animals receiving the raw milks and four of those receiving sterilized milks. This matter is further discussed later. At autopsy no animal showed the enlargement of the caecum which was encountered by Drummond (1933).

(2) *Blood picture.* Counts were made at intervals during the first nine months of the experiment and, after the iron, copper and manganese mixture was added to the biscuit; the red blood cell count and haemoglobin content remained normal. There was no detectable difference between the raw and sterilized groups.

(3) *Growth.* In Table I are recorded the average weights of the animals in the different groups of Exps. 1-4 at intervals of 12, 24, 36 and 48 weeks,

Table I. *Weights of rats, receiving raw and sterilized milks, at successive stages of experiment*

No. of exp.	Milk	Animals	Average weight (g.)			
			12th week	24th week	36th week	48th week
1	Stockport, raw	♀	137	173	201	211
	Stockport, sterilized	♀	131	160	187	200
	Percentage difference		-4.4	-7.5	-7.0	-5.2
2	Bletchley, raw	♀	134	182	226	226
	Bletchley, sterilized	♀	118	158	193	202
	Percentage difference		-11.9	-13.2	-10.7	-10.6
3	Stockport, raw	♂	159	232	275	292
	Stockport, sterilized	♂	153	227	258	270
	Percentage difference		-3.8	-2.1	-6.2	-7.5
4	Bletchley, raw	♂	215	261		
	Bletchley, sterilized	♂	199	234		
	Percentage difference		-7.4	-10.3		

together with the percentage difference between those of the sterilized and raw groups, calculated as a percentage of the raw group value.

The results of Exps. 1-3 in Table I show that at the end of 48 weeks the average weights of the different groups of animals receiving sterilized milk are somewhat less than those of the corresponding groups receiving raw milk. For comparison with our results, reference may be made to the very similar experiments on raw and pasteurized milk reported by Drummond (1933). He recorded the weights of his animals at 38 and 50 weeks and these may be taken as comparable with those of our animals at 36 and 48 weeks respectively. Calculation from his figures shows that the percentage decreases in weights of the animals receiving pasteurized milk were as recorded in Table II. While

Table II. *Decreases in the weights of rats receiving raw and heated milks, calculated as a percentage of the weights of the former*

Milk	Animals	Weeks			
		36	38	48	50
Pasteurized milk (Drummond)	♂	—	6.3	—	11.8
	♀	—	7.8	—	14.4
	Mean	—	7.0	—	13.1
Sterilized milk (present authors):					
Stockport	♂	6.2	—	7.5	—
	♀	7.0	—	5.2	—
Bletchley	♂	10.7	—	10.6	—
	Mean	8.0	—	8.0	—

strict comparison with results of another author cannot be made with certainty in feeding experiments of this type, the figures recorded in Table II appear definitely to show that the nutritive value of sterilized milk for the rat does not differ from that of pasteurized milk in the conditions of these experiments.

These results are however in considerable contrast with an experiment recorded by Mattick and Golding (1931). In comparing the growth rates of rats receiving raw and sterilized milks, these authors found little difference in one experiment, but in a second growth on sterilized milk was greatly retarded. Thus 118 days after the beginning of the experiment the average weight of the bucks receiving raw milk was 256 g., whereas that of those receiving sterilized milk was 127 g., no more than half as great; the corresponding figures for the does were 184 and 150 g. respectively. Apart from this apparently profound effect of sterilized milk in retarding growth, the authors reported that at a later stage of the experiment three of the six bucks receiving sterilized milk died, one of them showing typical signs of vitamin B₁ deficiency. In addition they state that, besides the very poor general condition of all the animals receiving sterilized milk, their coats were very staring. The great contrast between these results of Mattick and Golding and those reported in this paper is perplexing. As has already been pointed out, excellent growth was obtained by us from the use of sterilized milk and from the general appearance of the animals it was quite impossible to tell which were receiving raw and which sterilized milk. During the whole year's work only four animals

receiving sterilized milk and three receiving raw milk died or were killed. Details of these seven animals were as follows:

Raw milks:

Stockport: ♀. Normal growth for 105 days; litter of nine at 112 days; growth ceased, without weight loss for next 35 days, when animal was killed. ("Spinner"; ? middle ear disease.) ♀. Normal growth for 132 days; killed with hind limb paralysis. Post-mortem finding of extensive haemorrhage into intestine.

Bletchley: ♀. Normal growth for 92 days, followed by steady moderate weight loss for 57 days; "Spinner"; killed.

Sterilized milks:

Stockport: ♂. Killed after accidental fracture of hind leg after normal growth for 145 days.

Bletchley: ♂. Died after 28 days; pneumonia. ♀. Subnormal growth for 92 days, followed by rapid weight loss and death. No explanation on post-mortem examination. ♀. Normal growth for 132 days, followed by rapid weight loss and death after 14 days. No explanation on post-mortem examination.

Of these seven deaths, those from middle ear disease may be deleted, since a number of such cases occurred in our normal animals at this particular time; similarly the death from pneumonia and the killing after the accidental fracture may be also left out of consideration. On these premises, one death occurred among the raw milk animals and two among the sterilized milk animals from causes which may have been associated with the diet. Thus the mortality figures do not seem related to the question as to whether the animals received raw or sterilized milk. The remarks concerning general health and growth apply to all the groups of animals throughout the year's work and there appears no obvious explanation of the conflict of our results with those of Mattick and Golding.

In one aspect our results with sterilized milks differ somewhat from those of Drummond with pasteurized milk, in that he found that there was no detectable difference in the rates of growth in the animals receiving raw and pasteurized milk until the experiments had been in progress for 34 weeks. In our results, however, the animals receiving sterilized milk showed consistently lower weights throughout the experiments (Table I).

From the point of view of comparing the nutritive value of the two sterilized milks with each other, the figures in Table I suggest that the Stockport samples had given rather better results than those from Bletchley. Since the method of sterilization might be of considerable importance in the effect of heat treatment of milk, Exps. 5 and 6 were carried out, in order to obtain further evidence on these possible differences. The results are recorded in Table III.

Table III. *Average weights of rats receiving raw and sterilized milks*

	12th week	24th week
Stockport, raw (g.)	176	203
Stockport, sterilized (g.)	166	187
Decrease (%)	5.6	8.0
Bletchley, raw (g.)	170	195
Bletchley, sterilized (g.)	155	181
Decrease (%)	9.0	7.0

These experiments, which were begun in October, were carried on for 24 weeks only and the results suggest that there is no difference in the nutritive value of the two samples. On the other hand, it must not be overlooked that, if the Bletchley process of sterilization caused more damage to some milk constituent than did the Stockport one, this result might be equally well explained if the amount of that constituent was greater in the Bletchley raw milk than it was in the Stockport raw milk at this period of the year. Clearly with so many variables no conclusion can be reached on this point and, in view of the findings in Exps. 1-4, together with the fact that small groups of animals only were employed in Exps. 5 and 6, this question needs further investigation.

(4) *Reproduction.* Studies were also made on reproduction by following the oestrous cycle by use of the vaginal smear technique and by mating with stock bucks of known fertility. Further, the bucks which had themselves been fed on the milk diets were tested for their fertility against stock does of proven reproductive powers.

For 11 weeks each doe was examined by the vaginal smear technique and the stages of the oestrous cycle carefully followed. Stock bucks of known fertility were caged with the does for 24 hours, beginning with the early oestrous stage. The findings are given in Table IV.

Table IV. *Production of litters by does, receiving a milk diet, after mating with stock bucks*

	No. of does tested	No. of matings	No. of litters	No. of young	No. reared up to 4 days	Resorption or infertile matings
Stockport, raw	8	34 (9 pos.)	9	72	0	0
Stockport, sterilized	9	47 (6 pos.)	5	34	0	1
Bletchley, raw	10	52 (9 pos.)	7	56	8	2
Bletchley, sterilized	10	57 (8 pos.)	3	13	0	5

The results of Table IV show that reproduction in animals receiving raw milk was satisfactory, in that of eighteen positive matings sixteen resulted in litters, the average number of animals in each being eight. With both sterilized milks, however, reproduction was far less satisfactory, for following fourteen positive matings only eight litters resulted and the average number of animals in each litter was six. As in the growth curves in Exp. 1, the Bletchley sterilized milk appeared to give a less satisfactory result than the corresponding Stockport milk.

Although the ability to reproduce was satisfactory on the raw milk diet, in no case did any litter survive. Of the twenty-four litters from does receiving

either raw or sterilized milk, only one was reared beyond the fourth day. The parent of this particular litter, a doe receiving Bletchley raw milk, had a litter of eight, reared five for 2 weeks, one for 3 weeks and two for 4 weeks, but these two finally died also. She had a second litter which all died within 4 days. The results in mating milk-fed does with stock bucks therefore show that reproductive capacity, which appears normal when raw milk is used, is lessened when sterilized milk is used, and that the rearing of the young is not achieved, whether the does receive raw or sterilized milk.

In contrast, Mattick and Golding (1931) reported that a diet of raw milk and biscuit was adequate for the rearing of rats through several generations. Both Drummond (1933) and Wilson and Cowell (1933) were unable to confirm this finding and our results are thus in agreement with those of these latter

Table V. *Matings with stock does of bucks receiving raw and sterilized milks*

	Rat No.	No. of matings	No. positive
Stockport, raw	43	2	1
	44	3	2
	45	3	1
	46	8	0
Stockport, sterilized	47	3	1
	48	2	1
	50	4	1
	49	9	0
Bletchley, raw	69	3	1
	73	3	1
	68	6	0
	70	6	0
Bletchley, sterilized	60	2	2
	61	1	1
	62	1	1
	63	6	0

authors. In another respect however they differ somewhat from those of Drummond, in that he reported that reproduction on the raw milk produced very few litters of animals of apparently subnormal weight, while in our experiments eighteen positive matings resulted in sixteen litters in our raw milk groups, as has already been pointed out. Further, in all cases these litters consisted of good-sized healthy animals. While reproduction on sterilized milk seemed less satisfactory, the animals in these litters also appeared to differ in no way from those of stock litters.

As to the explanation of unsuccessful rearing on either milk, we have no information. The suggestion of Drummond that a lack of manganese might be involved is not applicable in our experiments, for manganese was added in the preparation of the biscuit. Nor is it definitely established that it is a failure in lactation which prevents the rearing of the young. Observations showed that the young made normally strong efforts to suck during the first 24-48 hours of life, but that they became blue, cold and flaccid in 2-4 days. This suggests that lactation had failed and an attempt was made to obtain evidence on this question. To this end part of the litter of a Bletchley raw milk doe and part of one of a Stockport raw milk doe were removed on the second day and

added to two stock lactating does with litters of the same age; they died before the fourth day. This use of a foster mother has however been successfully accomplished with the young of litters from mothers receiving a normal diet.

Bucks from the four milk groups were also tested for fertility by mating them with does receiving the normal stock diet. The results are given in full in Table V, as a summary would mask the picture. According to these limited figures, therefore, the bucks fed on raw milk do not differ in fertility from those fed on sterilized milk.

SUMMARY

1. Groups of young rats have been fed for periods up to one year on a diet consisting of raw or sterilized milk, with a biscuit made from white flour, to which were added supplements of iron, copper and manganese salts. By this method two samples of commercially sterilized milks, prepared by two different processes, have been compared with the corresponding raw milks drawn from the same bulk supplies.

2. At the end of one year the weights of the groups receiving sterilized milks were about 10 per cent. lower than those of the animals receiving raw milks from the same bulk supplies. This finding is similar to that of Drummond (1933) in a study of the relative values of raw and pasteurized milks by the same methods. It is however in considerable contrast to that of Mattick and Golding (1931) who in comparable experiments found that sterilized milk did not adequately support growth.

3. Animals receiving sterilized milks remained in excellent condition throughout the experiment and they could not be differentiated as regards general health from those which received the raw milks.

4. Adequate reproduction occurred on the raw milks, but no litters were reared. Reproduction was less good on the sterilized milks and, as with the raw milks, no litters were reared.

5. The results with the milk prepared by one sterilization process seemed on the whole better than those from the other; but the evidence was not sufficiently conclusive to make this deduction certain.

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