EFFECT OF HIGH AMBIENT TEMPERATURE IN EARLY AND LATE LACTATION ON LITTER GROWTH AND SURVIVAL IN RATS

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Abstract

The effect of heat stress, applied either during the first half or during the second half of lactation, on total litter production per rat was studied. The survival of the pups, the growth rate of the surviving pups, the liveweight changes, and feed intakes of the rats were also measured. Some observations were also made on maternal and pup behaviour.

Heat stress, whether applied in the first or the second half of lactation, significantly depressed total litter weight at weaning per rat. At weaning, the total weights of pups produced by the groups which had been subjected to heat stress during either the first half or the second half of lactation were not significantly different. Heat in the first half of lactation resulted in a high rate of pup mortality (62% as compared with 2% in the control group). However, those pups which survived in the early heat group grew as well as the pups in the control group. Heat stress in the second half of lactation did not cause a significant increase in pup mortality, but the growth rates of the surviving pups were slower than those of the controls. Heat in early lactation had adverse effects on the maternal behaviour of rats. Large numbers of pups were rejected by their mothers and this probably contributed to the high death rate in this group. Liveweights and feed intakes of mother rats were depressed by heat. Physiological and behavioural mechanisms underlying these results are discussed.

I. INTRODUCTION

There are many reports on the adverse effects of heat stress during gestation and lactation in the rat and mouse (e.g. Hsu 1948; Macfarlane *et al.* 1957, 1959; Biggers *et al.* 1958; Fernandez-Cano 1959; Howard *et al.* 1959; Aldred *et al.* 1961; Skreb and Frank 1963; Pennycuik 1964*a*, 1964*b*, 1964*c*, 1965, 1966*a*, 1966*b*). However, no information is available on the effects of heat stress applied at different times during lactation. Also, where growth rates of pups kept in hot environments during lactation have been examined (e.g. Biggers *et al.* 1958; Pennycuik 1964*a*, 1964*b*, 1966*a*), pups have not been identified individually, whole litters only being measured.

This paper describes a study of the effects of heat stress applied during the first or the second half of lactation on the growth rates and survival of individual pups,

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the total litter weight per rat till weaning, and on the liveweights and feed intakes of the mother rats.

II. Materials and Methods

(a) *Outline of Experiment*

A total of 24 litters of female albino rats were allocated to three equal groups using a table of random numbers (Fisher and Yates 1953). The randomization was stratified within days of littering and according to litter size. The three groups were as follows:

(1) The control group kept during the entire experiment under the environmental conditions of the rat colony at the University of New England at an ambient temperature of approximately 23°C.

(2) The early heat group (EH) kept under control conditions for the first 48–60 hr after parturition and then put into an environment of approximately 33°C and kept there until the pups were 12 days old. From the twelfth day till weaning (at 22 days) they were kept in the control environment. These rats were not put into the hot environment at birth because Pennycuik (1966*a*) found that mice exposed to heat at parturition lost 90% of their pups.

(3) The late heat group (LH) kept in the control conditions for the first 12 days after parturition and then transferred to the hot environment until weaning.

All three groups were kept at a constant photoperiod of 14 hr light, 10 hr darkness.

(b) *Animals and Management*

All rats and pups were weighed 48–60 hr after parturition and the pups were individually marked for future identification. All litters were inspected each morning. Pups which had fallen out or been pushed out of the cage were replaced and food and water replenished if necessary. Behavioural observations were carried out at the time of the daily checks. All rats were kept in individual standard wire cages with wood shavings for nest making. Wood shavings were replenished when necessary. All rats were fed a standard pelleted ration *ad libitum* and had unlimited access to water.

The dry-bulb temperature of the control environment was 22·5–24·5°C. Humidity was uncontrolled and the wet-bulb temperature ranged from approximately 16 to 19°C. When heat-stressed, the rats were kept in a thermostatically controlled chamber with continuous air circulation, a dry-bulb temperature of $33 \pm 0 \cdot 5$°C, and a wet-bulb temperature of 19 ± 1°C.

Every Wednesday and Saturday each rat and each pup was weighed and food intakes were measured. Rats were weighed to the nearest gram, pups to 0·05 g, and food to the nearest gram. All pups were weaned at 22 days.

"Rat" refers throughout this paper to mother rat; "pup" refers to the offspring of "rats"; a "rejected pup" was one which was found out of the cage, having either crawled out or been pushed out of the cage.

III. Results

(a) *Survival and Growth Rate of Pups*

The total productivity of the mother rat may be measured by the total weight of pups produced at weaning. The two components of this are pup survival and the growth rate of the surviving pups.

(i) *Pup Survival*

The average number of pups per litter ($\pm$ S.D.) at the beginning, the end of the first half, and the end of the second half of lactation was as follows:

Group	Beginning	End of first half	End of second half
Control	10·6±2·7	10·4±2·6	9·5±2·4
EH	10·6±2·0	4·0±3·0	3·9±2·9
LH	10·1±2·7	10·1±2·7	8·8±3·0

It is apparent from these values that heat stress in the first part of lactation caused a considerable increase in pup mortality (62% versus 2% in controls). Heat stress applied in late lactation did not significantly decrease survival. A χ^2 test indicated that the ratios of living pups for the three groups at the beginning of the experiment, at the end of the first half, and at the end of the second half of lactation were significantly different. This was mainly due to the χ^2 of the EH group. All rats in the EH group lost some pups; one lost the entire litter within 3 days of the beginning of the heat treatment.

TABLE 1

COMPARISON OF THE TOTAL NUMBER OF PUPS PER GROUP AT BIRTH WITH THE TOTAL NUMBER OF DEATHS AND THE TOTAL NUMBER OF REJECTIONS IN THE FIRST AND IN THE SECOND HALF OF LACTATION

Group	No. of pups at birth	First half		Second half	
		No. dead	No. rejected	No. dead	No. rejected
Control	85	2	1	7	41
EH	85	53	148	1	2
LH	81	0	0	17	32

Table 1 shows that many pups in the EH group were rejected while in the hot environment. Rejections also occurred in the second half of lactation in the control and LH groups. The majority of those in the control group were from one litter towards the end of lactation and may have been "escapes" of pups which were becoming very active rather than rejections.

TABLE 2

PARTITION OF DEATHS IN THE THREE GROUPS DURING THE FIRST AND SECOND HALVES OF LACTATION

Group	First half			Second half		
	Dead	Dead and partly eaten	Missing	Dead	Dead and partly eaten	Missing
Control	1	0	1	1	1	5
EH	36	4	13	0	0	1
LH	0	0	0	9	7	1

It can be seen in Table 2 that 13 pups in the EH group were missing, presumed dead and eaten. All the pups "dead and eaten" or "missing, presumed dead" had previously been rejected.

Dead pups had lighter body weights on the day of death than their live litter mates ($P<0\cdot001$). However, pups that died had not on average been smaller at birth than pups that survived. There was no relationship between pup mortality and feed intake or weight of the rat.

(ii) *Mean Weight of Surviving Pups*

The mean weights $\pm$S.D. (g) of the surviving pups at the beginning, end of the first half, and end of the second half of lactation were as follows:

Group	Beginning	End of first half	End of second half
Control	$6\cdot75\pm1\cdot20$	$18\cdot40\pm2\cdot75$	$38\cdot10\pm6\cdot45$
EH	$8\cdot20\pm2\cdot00$	$16\cdot00\pm3\cdot10$	$38\cdot50\pm6\cdot15$
LH	$8\cdot00\pm1\cdot45$	$18\cdot90\pm3\cdot50$	$29\cdot00\pm5\cdot25$

The weights of the surviving pups in the EH group were not significantly different from those of pups kept under control conditions. At weaning, however, the surviving pups of the LH group were significantly lighter than those in the control and in the EH group.

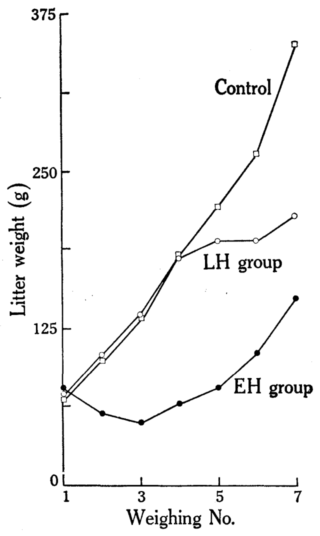

Fig. 1.—Total weight of pups per litter during the experiment; pups were first weighed at 2–3 days after birth then twice weekly till weaning.

(iii) *Total Weight of Pups Produced per Litter*

Figure 1 shows the total weight of pups produced per litter (or per rat) in the three experimental groups during the experiment. Student's *t*-test was carried out on the total weight of pups per rat both at the end of the first half and the end of the second half of lactation. At the end of the first half of lactation the total pup weight per rat of the EH group was significantly less ($P<0\cdot001$) than those of the other two groups (which were under control conditions). At weaning, both the EH and the LH groups had significantly smaller total pup weights per rat than the control group ($P<0\cdot01$). The total pup weight per rat of the LH group was greater than that of the EH group, but the difference was not statistically significant.

(b) *Liveweight and Feed Intakes of Rats*

The initial liveweights and liveweight changes of rats (g), ±S.D., at the end of the first and second half of lactation are given in the following tabulation:

Group	Initial liveweight	Change in liveweight at end of	
		First half	Second half
Control	247±25	+15±11	−10±13
EH	256±39	−24±15	+14±22
LH	271±38	+2±18	−51±21

These values show that heat stress, whether applied during the first or the second half of lactation, caused a significant decrease in the liveweight of the rats. The loss of liveweight was significantly greater in the EH group than in other groups in the first half of lactation ($P<0\cdot001$), while the decrease in liveweight was significantly greater in the LH group than in the other two groups during the second half of lactation ($P<0\cdot001$).

The mean feed intakes (g) per rat per day during the first and second halves of lactation are tabulated below (the number of pups sucking per litter at the beginning and end of each period is shown in parentheses):

Group	During first half	During second half
Control	42·3±9·6 (10·6, 10·4)	67·9±13·6 (10·4, 9·5)
EH	20·6±5·5 (10·6, 4·0)	40·9±21·4 (4·0, 3·9)
LH	40·4±9·9 (10·1, 10·1)	52·1±11·3 (10·1, 8·8)

As lactation advanced the control group ate more ($P<0\cdot001$). Heat stress in the first half of lactation reduced intakes by half when compared with the control group ($P<0\cdot001$) but when the stress was applied in late lactation its depressing effects were much smaller ($P<0\cdot05$). The intakes of the EH group during the second half of lactation were lower than those of the other two groups. However, it should be remembered that, at this time, the EH group had only a few surviving pups per litter and hence probably were producing less milk.

Figure 2 shows that, irrespective of treatment, there was a relationship between feed intake of the rat and weight gain of her litter over that period. As the litter increased in weight so did the feed intake of the rat.

(c) *Behaviour*

As much observation as possible was carried out on the behaviour of the rats and their pups. Whilst these observations were qualitative, it was hoped that they would be of some help in explaining such results as pup survival. It was noticed:

(1) During the first half of lactation pups tended to crawl out of the nest when heat-stressed, whereas the pups kept under control conditions stayed in the nest. Thus, many pups in the EH group were observed falling or crawling through the wires of the cage. In the second half of lactation the pups were too big to fall through the wire of the cage. In general, the pups were more restless under heat-stressed than in control conditions during the first half of lactation. All pups were active during the second part of lactation.

(2) The mother rats were much more restless in the heat, especially during early lactation. They would not remain still, or let the pups suck, and were often seen carrying the pups in their mouths and pushing them out of the cage. Maternal behaviour was much better in the rats heat-stressed in the second half of lactation than in those heat-stressed in the first half.

(3) In the heat, both the rats and their pups had scruffy coats whereas those of the control animals were shiny and sleek.

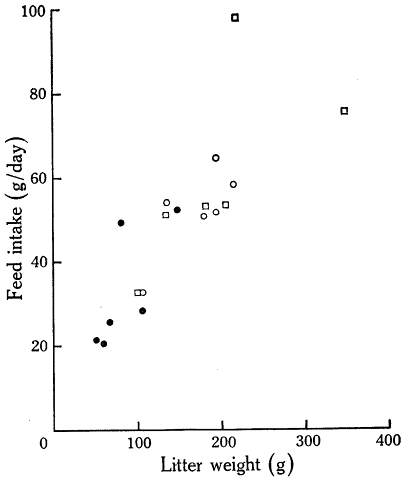

Fig. 2.—Relationship between feed intake per rat during a period and litter weight at the end of that period.
□ Control.
○ LH group.
● EH group.

IV. Discussion

Heat stress, whether applied during the first or the second half of lactation, considerably depressed total productivity per rat. The extent of this reduction in productivity was not significantly different between the EH and LH treatment groups, but the factors contributing to this were different in each group. Heat stress in the early part of lactation resulted in greatly increased mortality rates, but the surviving pups grew as well as those kept under control conditions. Mortality in the LH group was not significantly different from that of the controls, but the growth rates of the surviving pups were significantly lower than those of the controls.

Many factors could have lowered pup survival of the EH group. Firstly there were many rejections in that group and rejection would reduce a pup's chance of survival. Pups were replaced in the cages only once a day and would be without food whilst out of the cage. Many of the rejected pups may have died of starvation whilst out of the cage or because they were too weak to suck when returned to the nest. The mothers contributed to the numbers of rejections by not allowing the pups to suck, by not retrieving pups which were out of the nest, and in many cases by actively pushing the young out of the cage. In fact, as in the experiments of Macfarlane *et al.* (1959) and Pennycuik (1964*c*), cannibalism occurred in the rats heat-stressed in early lactation. It is interesting to note that, in this experiment, all pups which were found partly eaten, or were missing presumed dead, had been rejected previously. Most had low body weights at the time of death. Pennycuik (1964*b*) found that heat-stressed pups had lowered sucking reflexes, which also would tend to lower survival.

Heat stress in late lactation did not significantly increase pup mortality, probably partly because the pups were more robust and could withstand periods of starvation, partly because they were too big to fall out of the cage, and partly because maternal behaviour of the rats was better than when heat stress was applied early in lactation. Maternal behaviour was probably well established at this late stage of lactation and was thus not unduly upset by the heat stress.

The lowered growth rate of pups in the LH group was also probably the result of numerous factors. Pennycuik (1964*a*, 1964*b*) found that heat stress resulted in lowered feed intakes and liveweights of rats, less secretory tissue in the mammary glands, and hence lowered milk production. Since lowered intakes and liveweights followed heat stress in this experiment, it is likely that the heat-stressed animals had lowered milk production. The depressing effect of heat stress on milk production was probably greater earlier in lactation (Pennycuik 1966*b*), but the number of pups competing for this milk in the EH group was so small (38% only survived) that they obtained enough milk to grow well. However, in the LH group mortality was low and the number of pups competing for the limited milk supply was large. This would probably cause the low growth rates of pups in this group.

It would be interesting to find out how the survival and growth rates of pups heat-stressed in early lactation would be affected if the cages were so constructed that it was impossible for the pups to either crawl or be pushed out of the cage. If pup mortality were reduced, would the growth rates of the larger number ofsurviving pups be also depressed because of the reduced milk supply?

Finally, detailed behavioural studies, such as frequency of suckling, the amount of motor activity of the mother and of the pups, etc., might help to explain the contributory causes to pup mortality and growth.

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