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RESORPTION AND LOSS OF FOETUSES IN RATS LIVING AT 35° C

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Various disturbances of reproduction in tropical mammals have been reported. There is a 30% reduction in human conception in the north of Australia during summer relative to the winter conception rate (Macfarlane, unpublished). Castellani & Chalmers (1919) hint at higher abortion and dysmenorrhoea rates for women in hot climates. Pregnancy during summer in the tropics is associated in sheep with the production of small-sized lambs (Moule, 1954; Yeates, 1953) and with small calves in African cattle (Bonsma, 1949). As a result of laboratory studies, Sundstroem (1927) reported that female rats kept at 32° C resorbed their foetuses. Ogle (1934) kept mice at 31–33° C and found that they had small litters of poorly viable offspring.

On subjecting rats to a uniformly hot environment in this laboratory, it was found that some foetal resorption took place. Many other agents causing resorption in rodents have been described. Amongst these are deficiency of food, or specifically, of protein (Barry, 1920) or of vitamins, particularly A, E and B group (Nelson & Evans, 1946). Resorption also follows the removal of the ovaries in rats (Haterius, 1936). An overdose of oestrogen interrupts gestation in rabbits and rats (Huggett & Pritchard, 1945); but, with spaying, interruption is due to uterine compression of the foetus (Frazer, 1954). Hypothyroidism reduces fertility and increases the amount of resorption in rodents (Krohn & White, 1950). An excess of adrenal cortical hormones probably increases foetal resorption rates (Robson & Sharaf, 1952; Leroy & Domm, 1951). Chronic hypoxia can produce a similar condition (Ingalls, Curley & Prindle, 1952). Wilson (1954) induced 30% resorption with injected carbon tetrachloride and with trypan blue. It is likely, also, that some strains of rodent are genetically susceptible to resorption. Frazer (1955*a*) in surveying the fecundity and foetal mortality of colonies of rats in London, showed that high or low implantation numbers, loss of foetuses before the 9th day, and possible

genetic factors were all associated with higher than average resorption rates.

In an attempt to find factors which disturb reproduction in the tropics, rats were exposed during pregnancy to an environment of dry-bulb 95° F (35° C) and wet-bulb about 85° F (29·3° C); 62% r.h. (v.p. 22 mm Hg). Attempts were then made to protect the foetuses in order to gather information on the underlying mechanism of resorption.

METHODS

Rats of 140–180 g from an inbred Wistar strain were used. They were maintained upon a stock diet, supplemented or altered in the experimental groups according to the design of the experiment. The average composition, per cent, of the stock diet (Barastoc dog cubes) was as follows: crude fibre 2·6 together with crude protein 23·1, crude fat 5·6, carbohydrate 52·3, total minerals 6·1, (calcium 1·05, phosphorus 1·0 and iron 0·033) and water 10·3. The protein was derived from wheat and oats (71%), meat meal (18%), liver meal (4%) and milk (7%). Vitamin A was estimated at 9 i.u., riboflavine at 4·7 mg, thiamin 1·7 mg, vitamin D_3 1·0 i.u. and α-tocopherol approximately 2·0 mg/100 g.

Rats were mated, service being confirmed by the presence of vaginal plugs or of sperm in the smear. In a trial series rats were opened at 8, 12 and 16 days: it was found that foetal loss took place in some rats after the 12th day. Since signs of the former presence of a foetus were readily seen at the 16th day, this time was chosen for laparotomy. The number of implantations and the number of damaged or dead foetuses, or of placental sites without foetuses was recorded, and the rats were then allowed to complete pregnancy. The average number of implantations found in cool control and in hot rats, 16 days after service, differed by two in 1953 and by less than one in 1954–5: abortion was not observed.

The experiments fall into two main series, those of 1953 and 1954–5. The 1953 series was divided into six groups, A–F. Group A was a control at room temperature (mean monthly maximum of 27° C in January down to 16° C in July); group B comprised pooled controls at 35° C, the temperature used also for the experimental groups C–F. With the group B rats were some non-pregnant females, used to estimate food and water intakes.

Experimental group C was given 5 g/day/rat of meat as protein supplement, together with extra vitamins, in addition to the stock diet. 5000 i.u. vitamin A, 100 i.u. vitamin D, thiamin 0·25 mg, riboflavine 0·4 mg, nicotinamide 2·5 mg, calcium pantothenate 0·06 mg, pyridoxine hydrochloride 0·025 mg, folic acid 0·07 mg, biotin 0·025 mg, para-aminobenzoic acid 0·25 mg and vitamin E 5 mg were added to the food of each rat each day to reduce the possibility of vitamin deficiency either from the composition of the stock diet or from reduced food intake.

Group D received intramuscular injections of progesterone as steroid, in peanut oil, each second day from the 5th day of gestation; the dose increased from an initial 0·1 to 1·0 mg. The purpose of this was to counteract any excessive activity of oestrogen.

Groups E and F received intramuscular injections of DL-thyroxine from the 5th day of gestation. In group E the dose was 2·5 μg/100 g and in group F it was 3·5 μg/100 g. These two groups were formed because it has been shown by Dempsey & Astwood (1943) that in hot environments there is depression of the thyroid activity of rats.

In the 1953 series the hot chamber in which groups B–F lived was maintained at 34–36° C but the lighting was poor and the humidity varied between 60 and 80% r.h. In all groups mating took place during the night at room temperature. In groups D–F the mated rats were immediately put in the hot chamber and kept there till the 16th day when laparotomy was performed to ascertain the number of implantations and interrupted pregnancies.

It seemed probable after the 1953 series that other factors should be considered, particularly acclimatization and the possible mobilization of adrenal steroids upon sudden change of environ-

mental temperature from 25° to 35° C. The 1954 series, therefore, differed from the 1953 series in two respects. The hot box for the experimental groups was lighted for 9 hr/day, the temperature being closely controlled at 35±0·4° C and the humidity at 62% r.h., with air continuously circulated in this environment at 150 cu.ft./min. In addition, corpora lutea were counted as well as implantations and resorptions. Groups W and X were treated in the same way as groups A and B. Groups Y and Z were, in contrast, acclimatized to 35° C for periods ranging from 14 to 79 days before mating. Rats were mated at room temperature at night and returned to the heat next day.

In the 1955 series the possibility of steroid mobilization was investigated. Two groups, M and N, each received 2 mg/100 g/day of cortisone intramuscularly. Group M was kept at 22° C, while group N was acclimatized to 35° C for 17–29 days, mated and then kept at 35° C, with cortisone injections daily till the 16th day. The hot chamber was that of the 1954 series.

RESULTS

A proportion of conceptuses resorbed (or aborted) at laboratory temperatures in all seasons of the year. Resorptions took place mainly between the 8th and 16th day though occasional abortions or resorptions occurred later.

The results of the 1953 series are shown in Tables 1 and 3. The group B rats lost 7–10% of body weight at the end of 16 days in the heat. Non-pregnant rats lost weight to the same degree. They showed a reduction of food intake of 4·1 g/100 g/day (43% lower intake than rats at room temperature) and water intake increased by 6·9 ml./100 g/day (47% increase).

Proteins and vitamin supplements (group C) did not completely prevent resorption or abortion, for 25% of foetuses were still lost on the fortified diet. Foetal loss was significantly lowered relative to rats at 35° C fed on stock diet, and to rats treated with 3·5 μg/day/100 g thyroxine.

Progesterone injections (group D) significantly reduced the loss from 58 to 32%. The rate of foetal loss was similar to that resulting from thyroxine protection.

In group E the 58% loss in controls was significantly reduced by 2·5μg/100 g/day of thyroxine, to 30%; but interestingly when the dose was 3·5μg/100 g/day the loss rose to 45%. Larger doses than 2·5μg/100 g/day DL-thyroxine were apparently less effective in preventing foetal destruction. The difference between the two thyroxine dose levels was possibly significant ($P > 0{\cdot}05$). This finding is unaffected when the loss of entire litters is ignored and differences are computed for partial losses.

The results of the 1954–5 experiments are shown in Tables 2 and 4. Acclimatization for 14–24 days (group Y) reduced the rate of loss from 31 to 8% at 35° C. The resorption rate of 8% is not significantly different from group W, the controls at room temperature.

Group M in Table 2 is comparable with group W, except that 2 mg/100 g/day of cortisone was administered to group M. This additional hormone significantly trebled the rate of foetal loss, so that it almost equalled the hot control group X.

TABLE 1. 1953 series. Experimental variables, and the associated implantations, foetal losses and the average numbers of foetuses lost by each mother

Group	Temp. (° C)	Treatment	No. of mothers	Total implants	Foetuses lost			Rate of foetal loss	
					Total no. lost	Whole litters lost		Implants lost (%)	Foetuses lost per mother
						Litters	Foetuses		
A	22–28	Stock diet	25	226	15	0	0	7	0·6
B	34–36	Stock diet	23	171	98	6	50	58	4·3
C	34–36	Stock diet + vitamins + protein	9	88	22	1	11	25	2·4
D	34–36	Stock diet + progesterone 0·1–1·0 mg/48 hr	25	209	66	3	23	32	2·6
E	34–36	Stock diet + thyroxine 2·5 μg/100 g/24 hr	11	70	21	1	1	30	1·9
F	34–36	Stock diet + thyroxine 3·5 μg/100 g/24 hr	7	70	32	1	6	46	4·6

Group N, on the other hand, is paired with group Y: in both there was 2–4 weeks' acclimatization before mating. The administration of cortisone, despite the effect of acclimatization, doubled the foetal loss at 35° C. This hot cortisone-treated group N had a resorption rate significantly greater than any other group ($P > 0{\cdot}001$).

Table 5 shows the number of corpora lutea and the number of implantations in the 1954–5 series of groups W–Z. Corpora lutea counted at the 16th day after service were significantly reduced in number by 2–4 weeks' acclimatization

TABLE 2. 1954–5 series. Groups of rats acclimatized before mating or treated with cortisone, compared with control groups, for foetal loss per mother and foetal loss as a percentage of implants

Group	Temp. (° C)	Treatment	No. of mothers	No. implanted	Foetuses resorbed or aborted	No. lost per mother	Foetal loss (%)
W	22	Stock diet	63	561	41	0·63	7
X	35	Stock diet	18	146	45	2·5	31
Y	35	Stock diet, 14–24 days' acclimatization	25	177	15	0·6	8
Z	35	Stock diet, 25–70 days' acclimatization	17	133	16	0·94	12
M	22	Stock diet + cortisone 2 mg/100 g/day	11	100	22	2·0	22
N	35	Stock diet, 17–29 days' acclimatization + cortisone 2 mg/100 g/day	8	55	33	4·1	60

TABLE 3. Comparison by χ^2 test of the various 1953 groups. To the left of the central oblique line are χ^2 values and to the right and above are P values. The values compared are the foetal losses as a fraction of total implantations

Group		Group A	B	C	D	E	F
A	Stock 22° C	—	<0·001	<0·001	<0·001	<0·001	<0·001
B	Stock 35° C	122·7	—	<0·001	<0·001	<0·001	0·10
C	Stock 35° C + protein	20·5	24·4	—	0·25	0·50	0·005
D	Stock 35° C + progesterone	44·6	25·4	1·3	—	0·80	0·03
E	Stock 35° C + thyroxine 2·5 μg	27·3	14·8	0·5	0·06	—	0·05
F	Stock 35° C + thyroxine 3·5 μg	61·1	2·7	7·4	4·6	3·7	—

TABLE 4. Comparison of 1954–55 groups, in which the foetal losses are expressed as a fraction of implantations. The χ^2 values and the probability that any two groups differ, are tabulated

Group		Group W	X	Y	Z	M	N
W	Stock 22° C	—	<0·001	0·60	0·10	<0·001	<0·001
X	Stock 35° C	59·9	—	<0·001	<0·001	0·15	<0·001
Y	Stock 35° C, 14–24 days' acclimatization	0·4	26·4	—	0·30	0·05	<0·001
Z	Stock 35° C, 25–70 days' acclimatization	3·5	14·4	1·1	—	0·03	<0·001
M	Stock 22° C + cortisone	21·2	2·3	10·1	4·1	—	<0·001
N	Stock 35° C + cortisone	131·5	14·3	67·9	46·5	22·38	—

at 35° C. The number of implanted foetuses was also reduced. On longer acclimatization some recovery in implantation and corpus luteum formation rates occurred. The difference between the mean number of corpora lutea and the number of implants was greatest in acutely heated rats (X) and in long acclimatized mothers (Z).

TABLE 5. Reduction in the number of corpora lutea and of implants, following acclimatization for 2–3 weeks, and some recovery with longer (4–11 weeks') acclimatization at 35° C, occurred in this series of observations. *P* values are comparisons of group W with X, Y and Z

Group and temp. (° C)	No. of mothers	*A* Mean corpora lutea per rat	*P*; probability of X, Y or Z differing from W	*B* Mean implants per rat	*P*; probability of X, Y or Z differing from W	*A* − *B*: mean loss before implantation
W 22	63	10·03 ± 1·39	—	8·90 ± 2·19	—	1·13
X 35	14	9·93 ± 1·20	0·8	8·07 ± 2·61	0·6	1·86
*Y 35	25	8·45 ± 1·64	0·001	7·05 ± 2·08	0·01	1·40
**Z 35	17	9·41 ± 1·64	$0·1 < P < 0·2$	7·83 ± 1·67	$0·05 < P < 0·1$	1·58

* Acclimatized at 35° C for 14–24 days. ** Acclimatized 25–79 days.

DISCUSSION

These experiments show that a hot environment brought about high foetal loss in relatively unacclimatized rats. The main part of this mortality took place by foetal resorption. A proportion of the foetuses in any mother died in the hot animals from the 7th or 8th day of pregnancy onward. In group B (heated unacclimatized controls) were six mothers (out of twenty-three) all of whose foetuses resorbed. In the other groups there was almost always some foetus to survive in a litter. This strain of rats lost 12% of litters *in toto* from the various 1953 groups, but in the other 88% of mothers there were some foetal survivors. Heat could have produced this partial loss of litters by disturbance of nutrition following a lowered food intake, but protein and vitamin supplements did not reduce mortality to control levels. No vitamin B_{12} supplement was given, but the meat, vitamin and cereal diet should not have been deficient in this or other necessary food factors.

The possibility that thyroid gland depression (Dempsey & Astwood, 1943) might prejudice foetal survival was tested by thyroxine replacement. Injections of 2·5μg/100 g/day DL-thyroxine (which is approximately the daily requirement of laboratory rats, measured by Griesbach & Purves, 1945) reduced the proportion of foetal losses from 58 to 30% but this rate was still five times greater than that shown by controls, so that this could not be the whole answer. Possibly increased supplements of vitamins and protein, together with thyroxine, would have restored normal function. This has yet to be tested. Additions of 3·5μg of thyroxine *increased* the resorption rate (group F). It is probable that this excess hormone increased the heat load from intrinsic sources and so added to the strain of the environment.

Since Haterius (1936) quoted oestrogen excess as a cause of 'abortion', a progesterone supplement was employed to try to counteract any oestrogen overactivity that might occur in heat (perhaps through deficient inactivation of oestrogen in the liver). Group D suffered only about half the foetal loss of the controls: but the hot environment in its total effect still brought about five times the normal foetal mortality.

It is possible that the common pathway of action of proteins, vitamins, thyroxine and progesterone in saving foetuses runs through the adrenal cortex in its effects upon uterine muscle cells. Robson & Sharaf (1952) have shown that resorption occurred in rabbits and mice injected daily with cortisone. Hoet (1954) described human and rabbit foetal death, associated with excess adrenal steroids which reduce placental glycogen and penetrate to the amniotic fluid. In rats, Alexander, Frazer & Lee (1955) found that deoxycortone acetate protected foetuses from the results of oophorectomy. Our finding that acclimatization was the most effective protective measure against resorption (groups Y and Z) lends support to this theory, since adrenal steroid mobilization almost certainly takes place during heat acclimatization (Conn, 1949). When acclimatized rats (group N) were treated with cortisone, they suffered 60% of resorption, which was twice the rate found in heated unacclimatized rats (group X) and five times the rate of heated acclimatized animals (group Z). Cortisone, therefore, imitated the effect of sudden exposure to 35° C, and overcame the protection that would be expected from acclimatization. This could have been a pharmacological effect from the rather high dose of cortisone given. On the other hand, there is evidence in the difference between groups Y and Z, that acclimatization breaks down with prolonged exposure to 35° C. Additional adrenal hormone in a physiologically strained animal may further alter uterine tension or blood flow, or alter placental metabolism and encourage resorption.

All the factors considered probably act upon the uterus rather than the foetus. Shah (1956) has shown that the heated rabbit resorbs foetuses derived from six-day blastulae transplanted from cool donors, but the converse transplants survive. The uterus of the heated mother appears to be the tissue affected. Whether the tone of the uterus (Frazer, 1955*b*) is the main mechanism causing foetal loss; whether the vascular efficiency of the uterus is impaired in a 35° C environment, or whether adrenal steroids act directly to prevent growth and function at the utero-placental boundary remains to be determined.

Counts of corpora lutea made at the 16th day of pregnancy were undertaken on cool and hot controls and on acclimatized rats. It was not difficult to determine active corpora lutea at this stage, though earlier in pregnancy there may be some confusion with those of previous ovulations. The significant depression of the mean number of corpora lutea in rats undergoing acclimatization

(2–4 weeks at 35° C) was associated with increased loss before implantation. Ovulation or luteinization may have been interfered with by the action of heat through the hypothalamus-pituitary system. Adrenal steroids may act directly on those processes in the ovary and possibly could interfere with implantation. Cortisone has been reported sometimes to produce amenorrhoea in adolescents, but whether by direct action or through the pituitary is unknown (Sprague, Power, Mason, Albert, Mathieson, Hench, Kendall, Slocumb & Polley, 1950).

SUMMARY

1. Rats from the Wistar strain living at 35° C lost up to 58% of foetuses, compared with 7% resorption in controls at 22–28° C.

2. Resorption rates at 35° C were decreased by administration of progesterone, thyroxine or supplements of vitamins and protein.

3. Acclimatization for 2–10 weeks at 35° C before mating produced a highly significant reduction in the rate of foetal loss.

4. Cortisone increased the rate of resorption in unheated rats and the rate of loss reached 60% in heated rats treated with cortisone.

5. The number of corpora lutea formed was significantly reduced in rats acclimatized for 14–28 days at 35° C before being mated.

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REFERENCES

ALEXANDER, D. P., FRAZER, J. D. F. & LEE, J. (1955). The effect of steroids on the maintenance of pregnancy in the spayed rat. *J. Physiol.* **130**, 148–155.

BARRY, L. W. (1920). The effects of inanition in the pregnant albino rat, with a special reference to the change in the relative weights of various parts, systems and organs of the off-spring. *Contr. Embryol. Carneg. Instn*, **11**, 91–136.

BONSMA, J. C. (1949). Breeding of cattle for increased adaptability to tropical and subtropical environments. *J. agric. Sci.* **39**, 204–221.

CASTELLANI, A. & CHALMERS, A. S. (1919). *Manual of Tropical Medicine.* New York: Wm Wood and Co.

CONN, J. W. (1949). The mechanism of acclimatisation to heat. *Advanc. intern. Med.* **3**, 373–393.

DEMPSEY, E. W. & ASTWOOD, E. B. (1943). Determination of the rate of thyroid hormone secretion at various environmental temperatures. *Endocrinology*, **32**, 509–518.

FRAZER, J. F. D. (1954). Causes of foetal loss in the spayed pregnant rat. *J. Physiol.* **126**, 43*P*.

FRAZER, J. F. D. (1955*a*). Foetal death in the rat. *J. Embryol. exp. Morph.* **3**, 13–29.

FRAZER, J. F. D. (1955*b*). The mechanism of foetal loss after pregnant rats are spayed. *J. Physiol.* **130**, 253–256.

GRIESBACH, W. E. & PURVES, H. D. (1945). The significance of the basophil changes in the pituitary accompanying various forms of thyroxine deficiency. *Brit. J. exp. Path.* **26**, 13–17.

HATERIUS, H. O. (1936). Reduction of litter size and maintenance of pregnancy in the oophorectomized rat: evidence concerning the endocrine role of the placenta. *Amer. J. Physiol.* **114**, 399–406.

HOET, J. P. (1954). Adrenocortical function in infants of diabetic mothers. *Cold Spr. Harb. Symp. quant. Biol.* **19**, 182–185.

HUGGETT, A. ST G. & PRITCHARD, J. J. (1945). Placental growth and foetal death in the rat. *J. Physiol.* **104**, 4*P*.

INGALLS, T. H., CURLEY, F. J. & PRINDLE, R. A. (1952). Experimental production of congenital anomalies, timing and degree of anoxia as factors causing foetal death and congenital anomalies in the mouse. *New Engl. J. Med.* **247**, 758–768.

KROHN, P. L. & WHITE, H. C. (1950). The effect of hypothyroidism on reproduction in the female albino rat. *J. Endocrin.* **6**, 375–385.

LEROY, P. & DOMM, L. V. (1951). Observations on cortisone administration in normal and decapitated foetal rats. *Anat. Rec.* **109**, 319.

MOULE, G. R. (1954). Observations on mortality amongst lambs in Queensland. *Aust. vet. J.* **30**, 153–171.

NELSON, M. M. & EVANS, H. M. (1946). Pantothenic acid deficiency and reproduction in the rat. *J. Nutr.* **31**, 497–507.

OGLE, G. (1934). Adaptation of sexual activity to environmental stimulation. *Amer. J. Physiol.* **107**, 628–634.

ROBSON, J. M. & SHARAF, A. A. (1952). Effect of adrenocorticotrophic hormone (ACTH) and cortisone on pregnancy. *J. Physiol.* **116**, 236–243.

SHAH, M. K. (1956). Reciprocal egg transplantation to study the embryo-uterine relationship in heat induced failure of pregnancy in rabbits. *Nature, Lond.*, **177**, 1134–1135.

SPRAGUE, R. G., POWER, M. H., MASON, H. L., ALBERT, A., MATHIESON, D. R., HENCH, P. S., KENDALL, E. C., SLOCUMB, C. H. & POLLEY, H. P. (1950). Observations on the physiologic effects of cortisone and ACTH in man. *Arch. intern. Med.* **85**, 199–258.

SUNDSTROEM, E. G. (1927). Physiological aspects of tropical climates. *Physiol. Rev.* **7**, 320–362.

WILSON, J. G. (1954). Influence on the offspring of altered physiological states during pregnancy in the rat. *Ann. N.Y. acad. Sci.* **57**, 517–525.

YEATES, N. T. M. (1953). The effect of high air temperature on reproduction in the ewe. *J. agric. Sci.* **43**, 199–203.