THE EFFECTS OF ACUTE EXPOSURE TO HIGH TEMPERATURES ON PRENATAL DEVELOPMENT IN THE MOUSE WITH PARTICULAR REFERENCE TO SECONDARY VIBRISSAE

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Summary

(1) Mice of different gestational ages (1–18 days) were exposed to raised temperatures by exposing their mothers to 43°C for 1 hr.

(2) Secondary vibrissa scores were reduced by exposure at 12 days gestation. This reduction was due to the disappearance of vibrissae from all sites other than the supra-orbital.

(3) The correlation between the reduction in vibrissa score and the rise in rectal temperature of the mother was significant only to the 0·1 level.

(4) Measured in terms of means the effect of heat on vibrissa development was greater in mice carrying the tabby gene than in normal sibs. When the results were converted to probits the effects of heat and of the tabby gene were found to be additive.

(5) Foetal losses were highest in the early post-implantation period (7–10 days). A few severe malformations were produced in the same period.

(6) Foot malformations were produced by exposure at 11 and 15 days.

I. Introduction

Morphological characters which are constant for any given species are produced in the face of a variable genetic background. Waddington (1952) used the term canalized characters to distinguish these from the more labile features which separate individuals of the same species. Canalization implies the existence of a balance between developmental processes, a balance which must be maintained over a critical period, imbalance during this period resulting in abnormalities. Imbalance between processes may be due to genetic shortcomings of the individual concerned, or to environmental insults, or to a combination of both. A study of ways in which abnormalities of canalized characters can be produced may reveal something about the mechanism by which canalization is brought about.

A study of this kind was made by Milkman (1960*a*, 1960*b*, 1961, 1962*a*, 1962*b*) in which the posterior crossvein on *Drosophila melanogaster* was used as the constant character. This author worked with one strain of flies in which the wing vein was absent due to selection, a second strain in which the absence of the wing vein was due to a recessive gene, crossveinless *C*, and with normal flies in which the wing vein was missing following heat exposure during pupal development. In the crossveinless stock he was able to show that vein development was under the control of a number of polygenes. In the heat-shock experiments he was able to show that crossvein development was impaired by exposure to raised temperature at 19 and at 24 hr pupal development. Results of these heat experiments were interpreted in terms of the

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denaturation of two of the proteins concerned in the differentiation of the crossvein. It was suggested that these proteins were related in some way to the genes known to be concerned with crossvein development.

Studies of this kind have not been carried out in mammals. It was therefore decided to examine the effect of high environmental temperature and of a mutant gene (tabby) on a constant character in the mouse. The character chosen for study was the secondary vibrissa number, for it was known that development of those structures could be disturbed both by the tabby gene (Dun and Fraser 1959) and by X-irradiation at 11½ days gestational age (Fraser and Hall 1958; Kindred 1964).

Measurements were made on the gestational age at which environmental temperature affected vibrissa development and the relationship between the effect of the tabby gene and raised temperature. In the course of these investigations observations were made also on the number of individuals surviving the experimental treatment, and conversely on the effect of the treatment on foetal or embryonic resorption. Gross external malformations of the young at birth were noted and measured.

II. Materials and Methods

(*a*) *Animals*

The mice used were from an outbred stock designated here as TS. For studies on the effects of genetic background on the influence of temperature, the tabby gene was introduced by mating normal females (++) to males of the same stock carrying the tabby gene (*Ta*·). Pregnant females, therefore, carried female foetuses of the genotype *Ta*+ and normal males (+·).

(*b*) *Aging of Foetuses and Examination of the New Born*

Mating was carried out by placing one male in a cage with several females from Monday evening to Friday morning each week. The mice were examined for the presence of vaginal plugs each morning and the day on which these were found was counted as day 0 of the pregnancy. The embryo at this stage was approximately one-third to one-half a day old (Grüneberg 1943).

The pregnant animals were examined for the presence or absence of a litter by palpation at 16–18 days after vaginal plugs were found. Litter sizes were counted at birth and at 5 and 10 days post-partum.

Two small series of pups were examined before birth; one of these was a group exposed to heat on the twelfth day of gestation, the second was a control series. The embryos were dehydrated, cleared, and examined for abnormalities. One pup from each litter was sectioned and examined.

Pups were examined for gross external malformations at birth. It is possible that a few malformed young were not seen because examination of pups was delayed until after parturition. Some pups may have been still-born, and these could have been eaten by the mother before the litter was found. At 5 and 10 days post-partum the feet of the pups were examined for number of toes and for any malformations of the foot. At weaning the pelage of each mouse was examined. If

abnormalities were observed, a hair sample was cut from the mid-back with a razor-blade and a clump of 300–400 hairs from this sample was scored for the four different basic hair types with the aid of a binocular microscope.

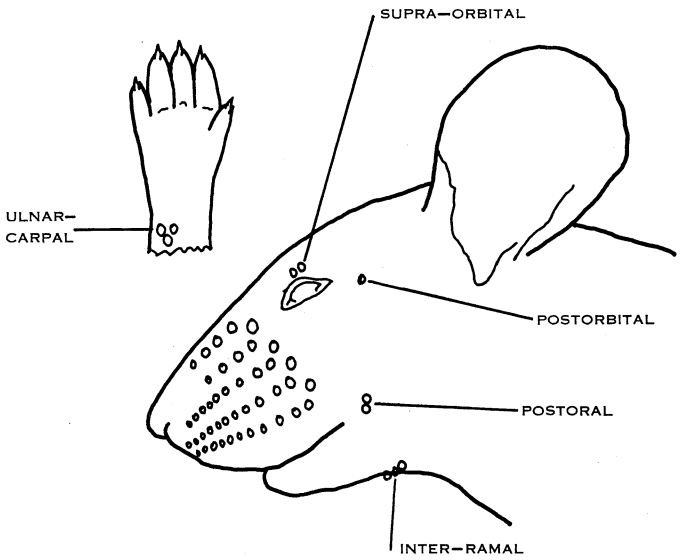

Fig. 1.—Distribution of secondary vibrissae on the face and fore feet of the mouse.

(*c*) *Scoring*

Vibrissa scoring was carried out at 5 and 10 days post-partum. The vibrissae scored are shown in Figure 1. Scores were recorded as follows:

$$\frac{a\ \ b\ \ c}{a_1\ b_1\ c_1}\ \frac{d}{}\ \frac{e}{e_1},$$

where a is the left supra-orbital score, a_1 the score for the right supra-orbital, b and b_1 the postorbital scores, c and c_1 the scores for the postorals, d the score for the inter-ramals, and e and e_1 the scores for the ulnar–carpals. The normal score, written in this form, is:

$$\frac{2\ 1\ 2}{2\ 1\ 2}\ \frac{3}{3}\ \frac{3}{} = 19 \text{ vibrissae (N).}$$

(*d*) *Duration of Heat Exposure*

The body temperature and the duration of heat application required to affect vibrissa number as seen in the 5–10-day-old mouse was determined in a preliminary experiment on animals carrying foetuses of 11, 12, and 13 days gestational age. Exposures of 10 min to 44·4°C reduced the vibrissa scores in foetuses exposed at 12 days gestational age, but many of the mothers died following exposure to this

temperature. Exposures of 45–60 min to body temperatures between 41 and 43°C also reduced vibrissa numbers in infant mice after exposure at 12 days gestational age and a high percentage of adult mice exposed to these temperatures survived to produce viable offspring.

III. Experimental Procedure

(a) *For TS Mice*

Approximately 400 mice were mated as described above. These were allotted at random to the control and experimental groups. Control animals were transferred to individual cages at 16 days gestation where they were allowed to litter. Except for weekends, cages were examined daily for presence of litters. Scoring was carried out as described above.

Experimental animals were treated as follows: the normal rectal temperature of the mouse was measured to within 0·1°C by means of a thermocouple and a Philips automatic compensator. The animals were then transferred, in groups of 2–6, to a cage previously heated in an oven to 42–43°C and mice and cage were placed in the oven.

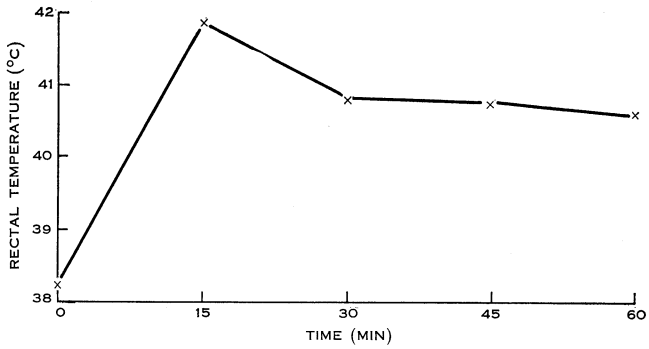

Fig. 2.—Plot of rectal temperatures over a 60-min period for 19 mice exposed to an environmental temperature of 43°C on the twelfth day of gestation. The standard errors were as follows: before exposure 0·07°C, 15 min 0·13°C, 30 min 0·09°C, 45 min 0·14°C, 60 min 0·12°C.

Rectal temperatures were obtained at 15-min intervals by removing the cage and mice from the oven to room temperature. These measurements were completed in 1–2 min. The cumulative exposure to 43°C was 60 min. The rectal temperatures for a group of these animals exposed at 12 days gestational age are shown in Figure 2. The fall in rectal temperature noted after the first heat exposure was due to the fact that the cage and the mice cooled during the course of this second reading. The gestational ages ranged from 1 to 18 days. Treatment of animals in each age group was carried out at random. The experiment was continued until 10 or more mothers in each group had produced offspring which survived until 10 days post-partum. After heat exposure the mice were transferred to individual cages where they were allowed to litter. Scoring was carried out as described for the controls.

(b) *Tabby Mice*

Twenty-eight TS mice were mated to six males of the same strain carrying the tabby gene. During the first pregnancy some of these mice were exposed to 43°C on the twelfth day of gestation. The remainder was allowed to litter without treatment. The females were then re-mated to the same males. Those which were not treated during the first pregnancy were exposed to 43°C on day 12 of gestation. The remainder was allowed to litter without treatment.

IV. Results

(a) *Secondary Vibrissa Scores*

(i) *TS Mice*

The effects of heat exposure on total vibrissa score are shown in Figure 3(*a*). Average total scores are shown in Table 1, together with average scores for individual vibrissa sites. To test the significance of the differences between the results for

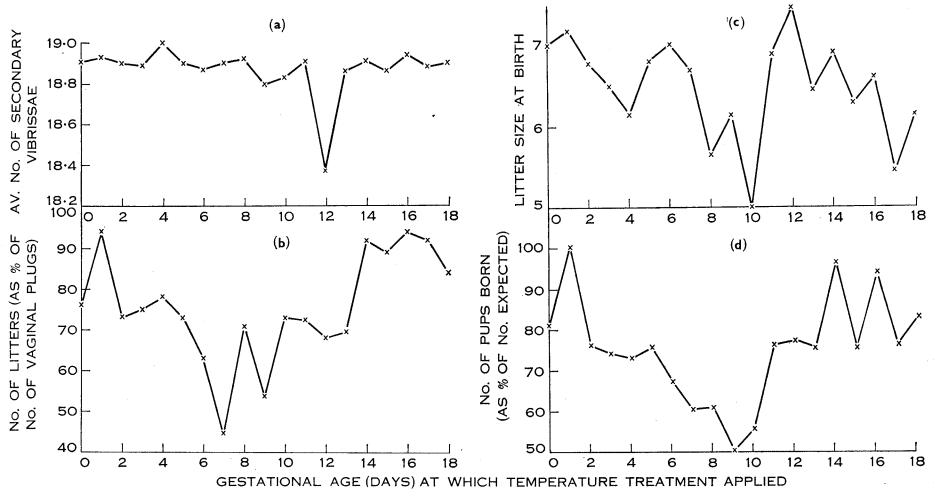

Fig. 3.—(*a*) Average vibrissa scores plotted against the gestational age of the foetus at the time at which temperature treatment was applied. Survivals of litters and pups following this treatment have been expressed in three forms: the number of litters as a percentage of vaginal plugs found (*b*), the average litter size at birth for all animals which produced litters (*c*), and the number of pups born expressed as a percentage of the number of pups expected (*d*). This latter was calculated by expressing the actual number of pups born as a percentage of the number expected if each mouse which mated had produced 6·56 pups, the average litter size for the whole group.

experimental and control groups, χ^2 values were calculated from contingency tables. (To do this, results for all pups born to control animals were taken as a basis for calculating the expected values, and the results for all pups born to experimental animals were grouped into cells for which expected values were available.) The significance of these differences is shown in Table 2, from which it would appear that total vibrissa scores were affected significantly at both 9 and 12 days gestational age. However, the total χ^2 value for all groups, with the exception of those exposed at 12

TABLE 1

TOTAL SCORES AND SCORES FOR EACH SECONDARY VIBRISSA SITE FOR CONTROL MICE AND FOR MICE EXPOSED TO HIGH TEMPERATURES AT AGES RANGING FROM 1 TO 18 DAYS

Age (days)	Total No. of Mothers	Total No. of Pups	Secondary Vibrissa Scores					
			Total	Supra-orbital	Post-orbital	Post-oral	Inter-ramal	Ulnar–Carpal
Controls	76	526	18·91	3·99	2·00	4·00	2·93	6·00
1	16	104	18·92	3·99	2·00	4·00	2·93	6·00
2	14	87	18·90	3·98	2·00	4·00	2·93	5·99
3	18	110	18·89	4·00	1·99	3·98	2·92	6·00
4	14	79	19·01	4·00	2·00	4·04	2·99	5·99
5	16	103	18·90	4·00	1·99	4·00	2·93	5·99
6	17	115	18·87	4·00	2·00	4·00	2·90	5·97
7	16	102	18·90	3·97	2·00	3·99	2·95	5·99
8	15	77	18·92	4·01	2·00	4·00	2·93	5·97
9	15	89	18·79	3·99	2·00	4·00	2·85	5·94
10	11	47	18·83	4·00	1·98	4·00	2·85	6·00
11	15	85	18·92	3·99	1·99	3·99	2·95	6·00
12	19	113	18·39	3·97	1·86	3·92	2·74	5·89
13	18	111	18·86	4·01	2·00	3·99	2·88	5·97
14	11	69	18·91	4·01	2·00	4·00	2·90	6·00
15	15	89	18·87	3·99	1·98	3·99	2·93	5·98
16	15	95	18·94	3·98	2·00	4·00	2·96	6·00
17	11	57	18·88	4·00	2·00	4·00	2·89	5·98
18	15	83	18·90	3·99	2·00	3·99	2·94	5·99

TABLE 2

SIGNIFICANCE OF DIFFERENCES BETWEEN VIBRISSA SCORES FOR UNTREATED CONTROL ANIMALS AND EXPERIMENTAL ANIMALS EXPOSED TO HIGH TEMPERATURES AT AGES RANGING FROM 1 TO 18 DAYS

χ^2 values were calculated from contingency tables. Significant differences are set in italic type

Age (days)	Total	Supra-orbital	Postorbital	Postoral	Inter-ramal	Ulnar–Carpal
1	0·80	0·70	0·50	0·70	0·90	0·70
2	0·80	0·50	0·50	0·70	0·95	*0·05*
3	0·70	0·20	0·30	0·50	0·90	0·70
4	*0·02*	0·30	0·50	*0·001*	0·20	*0·02*
5	0·80	0·70	0·30	0·70	*0·01*	*0·05*
6	0·50	0·90	0·50	0·70	0·50	0·10
7	0·70	0·50	0·50	0·50	0·30	*0·05*
8	0·70	0·30	0·70	0·80	0·95	*0·001*
9	*0·001*	0·95	0·50	0·70	*0·05*	*0·001*
10	0·20	0·50	*0·05*	0·80	0·20	0·80
11	0·80	0·80	0·70	0·30	0·80	0·70
12	*0·001*	0·20	*0·001*	*0·001*	*0·001*	*0·001*
13	0·10	0·20	0·50	0·50	0·20	*0·001*
14	0·70	0·95	0·50	0·80	0·70	0·70
15	0·30	0·50	*0·01*	0·50	0·90	*0·001*
16	0·70	0·50	0·50	0·70	0·70	0·70
17	0·70	0·30	0·70	0·80	0·70	*0·01*
18	0·80	0·90	0·50	0·50	0·90	*0·05*

days, is 70·0615 ($0·50>P>0·30$), suggesting that the significant χ^2 at 9 days was due to sampling error. The significance of the results for the 12-day group is beyond dispute. Examination of the results for individual vibrissa sites shows that this difference is due to a significant reduction in vibrissa numbers at all sites with the exception of the supra-orbital.

The effect of heat exposure, then, was to depress vibrissa development. This is illustrated further in Figure 4 where the numbers of mice in each vibrissa class in

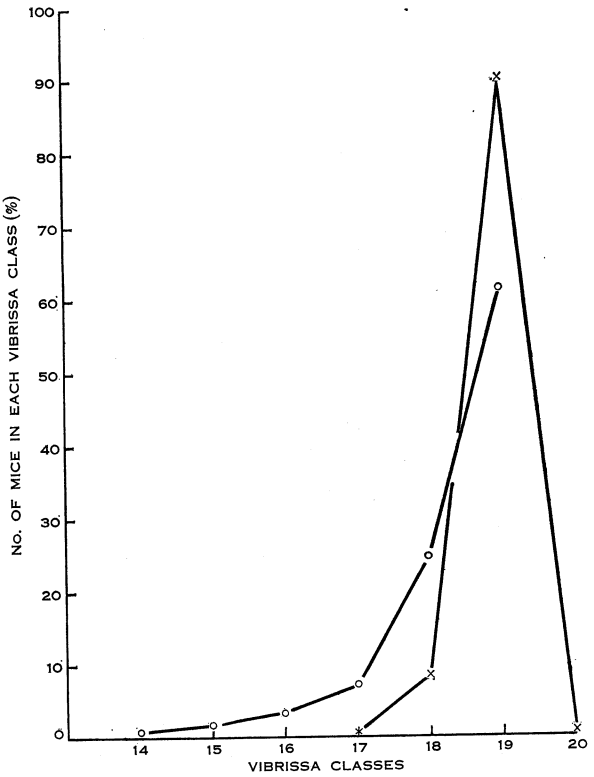

Fig. 4.—Numbers of mice in each vibrissa class plotted against the vibrissa class. ×———× Control TS mice. ○———○ TS mice exposed to raised temperatures at 12 days gestational age.

untreated controls and in mice exposed to raised environmental temperture at 12 days gestation have been plotted. The depression in the average score in the latter group is due, clearly, to an increase in the number of mice falling into the vibrissa classes below 19 (the most usual score for normal untreated mice). This was not accompanied by a spread to classes above 19.

There is a suggestion that the degree to which vibrissa development is depressed by heat exposure at 12 days gestation is related to the degree of hyperthermia induced in the mother. When the correlation coefficient is calculated for the total scores of all pups exposed at 12 days gestational age and the maximum rectal temperature reached

by their mothers during exposure, the value obtained is $-0{\cdot}1609$ which is significant at the 0·1 level.

TABLE 3

SECONDARY VIBRISSA SCORES (TOTAL AND SCORES FOR INDIVIDUAL SITES) FOR OFFSPRING OF $++ \times Ta\cdot$ MATINGS

Sex	Group	Total No. of Mothers	Total No. of Pups	Total	Supra-orbital	Post-orbital	Post-oral	Inter-ramal	Ulnar–Carpal
♀($Ta+$)	Control	28	96	16·67	3·96	1·97	3·00	2·24	5·52
	Treated	28	86	14·36	3·39	1·60	2·43	1·89	5·03
Significance of difference (P):				*>0·001*	*>0·001*	*>0·001*	*>0·001*	*>0·001*	*>0·001*
♂($+\cdot$)	Control	28	100	18·91	4·00	2·00	4·00	2·91	6·00
	Treated	28	107	18·27	4·00	1·82	3·90	2·70	5·85
Significance of difference (P):				*>0·001*	1·00	*>0·001*	*>0·001*	*>0·001*	*>0·001*

Examination of individual vibrissa sites in mice exposed to heat at 12 days gestational age shows that some sites are more sensitive to raised temperatures than others. The supra-orbital sites, which can be detected histologically at this age, were

TABLE 4

NUMBERS OF MICE WITH VAGINAL PLUGS, NUMBERS OF MICE WITH LITTERS, AND LITTER SIZES FOLLOWING EXPOSURE TO RAISED BODY TEMPERATURE AT GESTATIONAL AGES RANGING FROM 1 TO 18 DAYS

Gestational Age (days) at Time of Treatment	No. of Mice with Vaginal Plugs	No. of Mice with Litters	Litter Size at Birth (±S.E.)	Gestational Age (days) at Time of Treatment	No. of Mice with Vaginal Plugs	No. of Mice with Litters	Litter Size at Birth (±S.E.)
Controls	21	16	7·00±0·36				
1	17	16	7·19±0·42	10	15	11	5·00±0·43
2	19	14	6·79±0·38	11	22	16	6·88±0·49
3	24	18	6·50±0·43	12	28	19	7·47±0·39
4	18	14	6·14±0·50	13	25	18	6·89±0·45
5	22	16	6·81±0·50	14	12	11	6·91±0·58
6	27	17	7·00±0·36	15	19	15	6·27±0·48
7	36	16	6·69±0·54	16	16	15	6·60±0·51
8	24	17	5·65±0·68	17	12	11	5·54±0·56
9	28	15	6·13±0·40	18	18	16	6·13±0·69

unaffected, but the postorbital vibrissae (which can also be seen at 12 days) were significantly reduced. So too were vibrissae at the postoral inter-ramal, and ulnar–carpal sites, although no traces of these vibrissae can be detected in the 12-day embryos. There is a suggestion that the ulnar–carpal vibrissae, which are not

detectable histologically until 14 days, are sensitive to hyperthermia at a number of different stages of embryonic development.

The vibrissa sites affected appeared to be independent of the developmental age of the foetus at the time of exposure, because litter mates frequently showed differences in the sites affected and individual mice occasionally lost both early- and late-developing vibrissae. The scores for one such litter are set out below:

Litter TS/264·99

(1) ♀ N

(2) ♀ $\frac{1\ 1\ 2}{2\ 1\ 2}\ 3\ \frac{3}{3}$

(3) ♀ N

(4) ♀ $\frac{2\ 1\ 2}{2\ 1\ 2}\ 3\ \frac{3}{2}$

(5) ♀ $\frac{1\ 1\ 2}{2\ 1\ 2}\ 3\ \frac{3}{2}$

(6) ♂ $\frac{2\ 1\ 2}{2\ 1\ 2}\ 3\ \frac{3}{2}$

(7) ♂ N

(8) ♂ N

(ii) *Tabby Mice*

These mice were exposed to high temperatures at 12 days only. Average values for total scores and for scores at individual vibrissa sites are shown in Table 3, together with probabilities of differences between results for experimental and control groups. It is apparent that heat affected total score significantly in both *Ta*+ and +· offspring. In the case of males this difference was due to a reduction in the number of vibrissae at all sites except the supra-orbital. Results for females show that this site also was affected significantly in mice carrying the tabby gene.

The frequencies of occurrence of different total scores for males and females in both control and experimental runs are shown in Figure 5. The frequency distribution for the males is very similar to that for the TS series (see Fig. 4). Following exposure to high temperatures, the highest proportion of females fell in the 15-vibrissa class. Control females, on the other hand, showed a preponderance of animals in the 19-vibrissa class. In addition, the range of scores was greatly increased following heat exposure.

Judged from its affects on the means of the total scores, heat disrupted vibrissa development more severely in mice carrying the tabby gene than in their normal sibs. However, if the genetic and environmental treatments are reduced to the same scale by using probits (Rendel 1962, 1963) and if the probit value for the 18, 19 cut-off point for each of the four groups (for the sake of argument) is subtracted from the mean value (i.e. 5), it is apparent that the difference between the control *Ta*+ mice and the heated *Ta*+ mice (0·87) is rather less than the distance between the control +· mice and the heated +· mice (1·10). (The control *Ta*+ mean lies 0·64 probit units below the 18, 19 cut-off point while the mean for heated *Ta*+ mice lies 1·51 probits below this point. The +· controls lie 1·34 units above the 18, 19 cut-off, the heated +· mice 0·33 units above this point.) This suggests that the effect of heat on the tabby mice was, if anything, less severe than the effects of heat on their normal sibs.

(b) Survivals of Pups of TS Mice Following Exposure to Increased Body Temperature during the Gestation Period

In Figure 3(*b*) the number of litters born, expressed as a percentage of the number of vaginal plugs, has been plotted against foetal age at the time of heat exposure. This provides a measure of the number of intra-uterine deaths following hyperthermia. The sizes of the groups used are designated in Table 4. The total χ^2 value for all groups is 37·043 (0·01>P>0·001). Except for the group exposed at 7

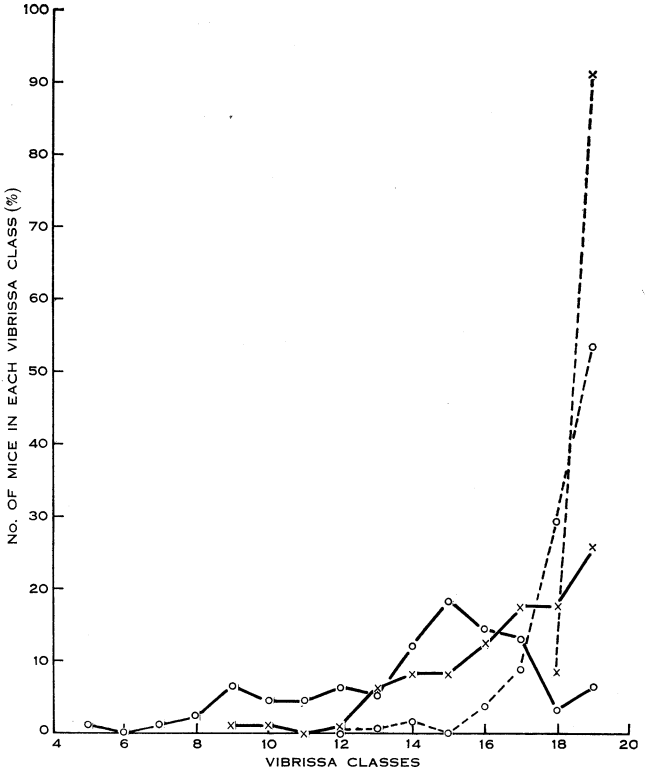

Fig. 5.—Number of mice in each vibrissa class plotted against the vibrissa class. ×———× Control *Ta*+ females. ○———○ *Ta*+ females exposed to heat at 12 days gestational age. ×- - - - -× Control +· males. ○- - - - -○ +· males exposed to heat at 12 days gestational age.

days the χ^2 value is 22·9485 (0·20>P>0·10). Embryos of 7 days of age are apparently particularly sensitive to heat treatment. This is the period at which the implanted embryo has reached the primitive streak stage or a little beyond.

Figure 3(*c*) illustrates the effect of raised body temperature on litter size at birth. Litters were smallest when heat was applied at 10 days of gestational age. When the χ^2 value was calculated for the whole group, the value obtained was 34·29 (0·02>P>0·01); when the group exposed on the tenth day was omitted, the χ^2 value was 20·90 (0·30>P>0·20). The reduction in litter size after heat treatment on the tenth gestational day was therefore significant.

TABLE 5

NATURE AND FREQUENCY OF THE MORPHOLOGICAL ANOMALIES PRODUCED BY HEAT EXPOSURE AT VARIOUS AGES OF GESTATION. DEVELOPMENTAL STAGES REACHED AT EACH AGE HAVE ALSO BEEN INCLUDED

This information was derived partly from observations on the mice used in these experiments and partly from the embryological accounts of Grüneberg (1943), Otis and Brent (1954), Snell (1956), and Dun (1959). The numbers quoted apply to litters and pups alive at 10 days post-partum

Age (days)	Stage of Development	Experimental Results				
		Abnormalities Observed	No. of Mice Exposed	No. of Litters Affected	Total No. of Offspring	No. of Offspring Affected
Untreated controls		Six toes on hind foot on one occasion	76	1	526	1
7	Primitive streak	Mandible missing, maxillary region reduced	16	1	102	1
8	Organogenesis, optic cups not yet invaginated	Microphthalmia; subcutaneous cyst above right eye	15	1	77	1
		Gas in gut		1		1
11	Foot plate present on anterior limb-bud; posterior limb-bud not clearly divided into leg and foot	Foot abnormalities on both limbs (reduction in the number of toes, the lengths of toes, and the formation of the claw)	15	2	85	6
		Innervation of hind limbs affected (mice dragged hind feet at 5 days of age)		2		2
12	Supra-orbital and post-orbital vibrissae begin to develop, other vibrissa sites not visible histologically	Vibrissa number at all sites reduced	19	17	117*	42
		Composition of first hair coat affected		1		2
15	Fingers and toes separate throughout their length, without webbing, and very divergent. End phalanges of fingers begin to appear	Tips of toes rotated usually medially but occasionally laterally	15	2	89	3

* Four additional pups in this value when compared with values for vibrissa scores—this was due to the fact that four pups were missing at second score.

In Figure 3(*d*) the combined results for Figures 3(*b*) and 3(*c*) are shown. The actual number of pups born in any particular group is expressed as a percentage of the number expected if each mouse which mated produced 6·56 pups (the average litter size for the whole group). From this it would appear that the greatest losses were sustained in mice exposed to raised body temperature on days 7, 8, 9, and 10, i.e. during the period of organogenesis.

(*c*) *Abnormalities Observed Following Heat Exposure*

(i) *TS Mice*

In the small series in which prenatal stages were examined, controls showed no detectable abnormalities. Of those exposed to heat on the twelfth day of gestation, 10% (5 out of 50 pups, 2 out of 8 litters) had haemorrhages either beneath the skin or in the subdural space.

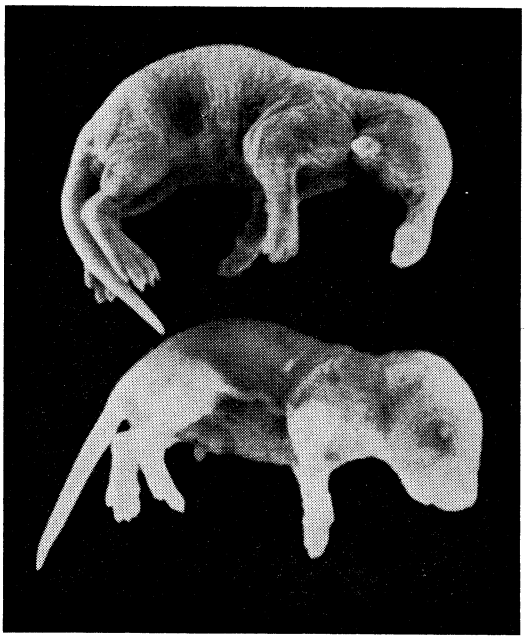

Fig. 6.—New-born pup from mother exposed to raised body temperature at 7 days gestation (top) and normal new-born pup (bottom).

The abnormalities observed at birth and after birth in pups following heat exposure during gestation have been listed in Table 5. The most serious malformations occurred in animals exposed at 7 and 8 days embryonic age, i.e. during early organogenesis (Snell 1956). Following exposure at 7 days, one still-born mouse was found in which the mandible was missing and the maxillary region reduced to a trunk-like projection. Eyes were absent and the form of the head abnormal (Fig. 6). Following exposure at 8 days, one stunted mouse was found in which the eyes were reduced in size and were apparently non-functional, and a cyst was present beneath the skin

above the right eye. This mouse lived to adulthood. A second mouse in this litter had bubbles of gas in its intestine, a situation similar to that described following exposure of mice to X-rays (Russell 1950). The affected animal died during the first few days of extra-uterine life.

Foot malformations of infant mice were observed following maternal exposure at 11 and 15 gestational days. At 11 days of development the foot plate has differentiated in the anterior limb-bud, but not in the posterior limb-bud (Grüneberg 1943). Animals exposed at this stage showed one or more of the following malformations: a reduction in the number of digits on one or more feet; a reduction in the length of one or more digits; malformation of the claws on one or more toes (these usually took the form of claws growing at right angles to the long axis of the toe).

At 15 days the fingers and toes are normally separated throughout their length, no webbing is apparent, and the digits are very divergent. The end phalanges of the fingers begin to appear (Grüneberg 1943). Following the exposure to heat at this stage, there was no change in the number of toes but the direction of the end phalanges was occasionally changed, so that they curved laterally rather than dorsoventrally.

TABLE 6

FOOT ABNORMALITIES IN OFFSPRING OF $++ \times Ta^{\cdot}$ MICE

	No. of Matings	No. of Litters Affected	Total No. of Offspring	No. of Offspring Affected
Controls	28	1	196	1
Exposed at 12 days	28	5	193	18

Following exposure to heat at 11 days gestational age, two young mice were observed to drag their hind feet as they moved about. Normal mice of the same age used their hind feet for locomotion. This peculiarity disappeared when the animals were a few days older and the significance of this defect is not known.

Following exposure to heat at 12 days gestational age, two mice showed abnormal development of the primary hair coat. The coat was less dense than usual and the proportions of the different hair types were changed. Auchenes, which formed 6% of the fibres in the normal coat, were not represented, and the percentage of awls was reduced from 15 to 12%. The proportion of zig-zags in the coat increased in consequence.

(ii) *Tabby Mice*

These were exposed to hyperthermia at 12 days gestational age only. They showed none of the serious malformations observed in the TS series after exposure between 7 and 10 days of age but a number of them showed malformations of the feet. These were similar to those observed in TS mice exposed at 11 days gestation, viz: reduction in the number of digits, usually only on one foot; reduction in the length of one or more digits; fusion of digits; and deformation of the claws. In addition,

some mice showed lateral rotation of the terminal phalanges. The latter deformation was found in the previous series only in mice exposed to heat at 15 days gestation. The incidence of foot malformations is shown in Table 6. Mice with deformed feet were distributed almost equally between the sexes, so it is unlikely that the tabby gene influenced the sensitivity of the feet to heat damage.

V. Discussion

Results for the effects of hyperthermia on vibrissa numbers confirm the observation made with X-rays (Fraser and Hall 1958; Kindred 1964), namely that one of the processes necessary for the production of 19 secondary vibrissae at 5 days post-partum is sensitive to disturbance on the twelfth gestational day. At this stage the different vibrissa sites are at very different stages of differentiation: the supra-orbital and postorbital sites can be detected histologically by the twelfth gestational day, but the remaining vibrissae are not seen until day 13 (postorals and inter-ramals) and day 14 (ulnar–carpals) (Dun 1959). This suggests that the sensitive stage is part of a pathway, so far undetected histologically, which is synchronous at all sites and which becomes linked to vibrissa differentiation at some point in time later than the twelfth day of gestation.

The presence of the tabby gene is also known to reduce the number of secondary vibrissae at 5 days post-partum. However, its time of action is thought to be later than 12 days gestation. Falconer, Fraser, and King (1951) considered that the crinkled gene (tabby is a sex-linked mimic of crinkled) acted from the twelfth to the seventeenth day of gestation and from the twentieth to the twenty-third day after conception. Dun (1959), on the other hand, concluded that the action of the tabby gene was not precisely timed but rather that it had a localized action, the time being determined by the appearance of the first vibrissa in each group. The depression in vibrissa numbers at 12 days gestation by both hyperthermia and X-irradiation raises the possibility that the tabby gene too could act over one short period only, provided its action was not necessary for visible differentiation at that particular time. The fact that the results for hyperthermia and for tabby are additive is not incompatible with this interpretation. However, the gene at the tabby locus is not the only one involved in controlling vibrissa development for there is evidence that certain genes are concerned with the development of all vibrissa sites while others are concerned primarily with vibrissae at one site only (Dun 1959). It is possible that heat and X-irradiation interfere with processes governed by a locus, other than tabby, which is concerned with the development of vibrissae at all sites. Whatever the nature of the gene, there is evidence that it becomes active again during the later life of the affected animal, for in the two mice which showed abnormalities of the primary hair coat the pelage returned to normal in later hair cycles.

The experiments reported here were not designed to show how heat affects vibrissa development. It is possible that damage is due to denaturation by heat of a protein necessary for follicle differentiation or for vibrissa growth. The fact that the affects of the tabby gene and of heat appear to be additive could be interpreted by postulating that the reserves of "vibrissa substance" are lower in tabby animals than in normals, and that heating reduces these reserves below the critical level

necessary for vibrissa growth (cf. Milkman 1961). The fact that X-rays, which do not act by denaturation, produce similar results to those produced by hyperthermia and the lack of a highly significant correlation between maternal body temperature and vibrissa number make such an interpretation doubtful.

The possibility arises then that the effects of heat may be indirect, rather than the result of denaturation of proteins in the differentiating vibrissa cells. At present there is no evidence to indicate the nature of this indirect effect. It could be due to heat damage to the embryo itself, for example tissue necrosis (Škreb and Frank 1963) or foetal haemorrhages (Brinsmade and Rübsaamen 1957) or to the vascular, respiratory, endocrinological, or biochemical adjustments of the maternal organism to heat stress. The possibility that the effects of heat on vibrissa development are indirect receives some support from the fact that the more drastic malformations caused by heat are similar to those produced by other teratogenic agents, and moreover the critical periods involved are similar in the most cases. For example, impaired development of jaws, eyes, and toes has been observed following X-irradiation (Russell 1950), actinomycin-D administration (Tuchmann-Duplessis and Mercier-Parot 1960), and pteroylglutamic acid deficiency (Nelson 1960). Moreover, there is evidence that death of foetuses following heat exposure is due, in some cases at least, to disturbances of maternal physiology. Shah (1956) demonstrated that if rabbits were exposed to 95·9°F for 6 days following copulation most of the foetuses were resorbed. By using a reciprocal transfer technique he was able to show that blastocysts from heated donors developed normally when transferred to untreated does. Fernandez-Cano (1958*a*, 1958*b*) observed a reduction in resorption rate in rats exposed to heat following removal of the maternal adrenals.

The malformations observed in this series of experiments are similar to those observed by other authors following heat exposure. Eye defects were observed by Hsu (1948) in the offspring of rats exposed at $9\frac{1}{2}$ and $10\frac{1}{2}$ gestational days, by Brinsmade and Rübsaamen (1957) in young rabbits after exposure of the doe to artificial fever on days 6–7 and 7–8, and by Škreb and Frank (1963) in rat pups exposed at $8\frac{1}{2}$, $9\frac{1}{2}$, and $10\frac{1}{2}$ days of gestation and killed a day or so before term. Facial defects were observed by Škreb and Frank (1963) although these differed in form from the one instance observed in the present paper. These same authors observed defects of the feet which resembled those noted in this study. However, the brain defects observed in the newborn by Škreb and Frank (1963) were not seen.

Resorptions following short heat exposures have also been reported by other authors. Cameron (1943) found that rabbits exposed to heat 72–80 hr after mating failed to produce litters. Hsu (1948) found that pregnancy was terminated most frequently in rats by heat exposure 0·5–1·5 days after mating. The results of Fernandez-Cano (1958*a*) fell into this same pattern. Only Škreb and Frank (1963) found peak resorptions in the post-implantation period ($11\frac{1}{2}$ days in rats). Since these authors exposed only one horn of the uterus to heat, it seems likely that resorption was due in this case to foetal damage rather than to disturbances of maternal physiology.

Brinsmade and Rübsaamen (1957) speculated on the possibility that fever was responsible for some of the malformations observed in the human embryo. Škreb

and Frank (1963), on the other hand, considered that application of raised environmental temperature to the mothers caused resorption rather than malformations. The present experiments lend support to the possibility that some malformations may result from maternal fever or other hyperthermia due to environmental courses. They also emphasize the fact that the effects vary with the age of the foetus at the time of exposure, for malformations were most common during the period of organogenesis (i.e. in the early post-implantation period) and that the severity of the defects decreased as embryonic development progressed. The disturbance in vibrissa development following exposure at 12 days gestation represents one of the very minor disturbances to canalization resulting from hyperthermia during the later stages of development.

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