

PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF MICE MOVED TO 21°C AFTER 10 GENERATIONS AT 34°C

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Summary

The effects of maintaining mice for 10 generations at 34°C were measured by comparing the reproductive productivity, growth rate, oxygen consumption, hair growth, density of the subepidermal capillary net, and frequency of the pink-eye gene in mice selected for productivity at 34°C (R95), the same mice moved to 21°C (L95), controls at 21°C (R70), and offspring of crosses between R95 and R70 mice and an unrelated stock (Wild) kept at 21°C (W95 and W70).

Reproductive productivity and growth were depressed in R95 mice at 34°C. When these mice were returned to 21°C (L95), productivity and growth returned to control levels. However, W95 pups grew more slowly than W70 pups suggesting that exposure to 34°C for a number of generations had affected growth potential. Oxygen consumptions of L95 mice differed significantly from those of R70 and R95 animals at 32 and 34°C. Oxygen consumption curves of W70 mice resembled those of the Wild stock; those of W95 animals resembled those of L95 animals. Both results suggest that exposure to 34°C for 12 generations altered the pattern of oxygen consumption. Exposure to 34°C reduced hair growth equally in R70 and R95 mice; transfer of R95 mice to 21°C caused hair growth to return to control levels. The density of the subepidermal capillary net and the frequency of the pink-eye gene were unaffected by temperature or by several generations at 34°C.

I. INTRODUCTION

High environmental temperature reduces fecundity of invertebrates and vertebrates to dangerously low levels, but there is evidence that animals can become adapted to new temperature conditions and that their reproductive performances improve with increasing generation number (Ogaki 1962; Brun 1965). Before this improvement can take place, changes must occur in the physiological and morphological characteristics of the strain. These can only be distinguished from differences due to the direct effects of the environment by measuring the relative performances, in an identical environment, of stocks reared at different environmental temperatures.

Most studies of this kind have been confined to an examination of geographical races, for example cattle, sheep, etc., or to examination of differences between laboratory stocks kept under different climatic conditions (Howard 1963). No-one appears to have investigated the differences between strains deliberately bred at high and more moderate temperatures.

In this laboratory random-breeding mice have now been maintained for 12 generations at 34°C (Pennycuik 1969). When animals of the tenth generation became available it seemed profitable to compare the performance of this heat-selected stock

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with that of the control stock maintained at 21°C when both were exposed to identical temperatures. The characteristics chosen for study were reproductive productivity, growth rate, oxygen consumption, hair growth, and the density of the subepidermal capillary net. In addition, the frequency of the pink-eye gene was measured in the base population and in the tenth generation of both the 34°C stock and the control strain. Similar measurements were also made on stocks formed by crossing the 34°C and the control stocks with an unrelated stock with different patterns of productivity, growth rate, and oxygen consumption.

II. MATERIALS AND METHODS

(a) Temperature Control

The incubator and the animal room are described in the preceding paper (Pennycuik 1969).

(b) Animals

The heat-reared stock (R95) and the control stock from which it was derived (R70) are also described in the preceding paper (Pennycuik 1969). When the R95 strain reached the tenth generation, some animals were moved to 21°C at about 3 weeks of age. When these reached approximately 10 weeks of age the females were divided into two groups: some were mated to R95 males acclimatized to 21°C and some to Wild males. This latter stock is described by Pennycuik (1967). A corresponding number of R70 females was mated to the same Wild males as those used in the R95 matings. The offspring of the R95 matings at 21°C became generation 1 of the L95 stock; those of the R95 × Wild matings, generation 1 of the W95 stock; and those of the R70 × Wild matings, generation 1 of the W70 stock. One further generation of each of these stocks was obtained by mating these F₁ animals. Sib matings were avoided. The interrelationships of these various stocks and the temperatures to which they were exposed are illustrated in Figure 1.

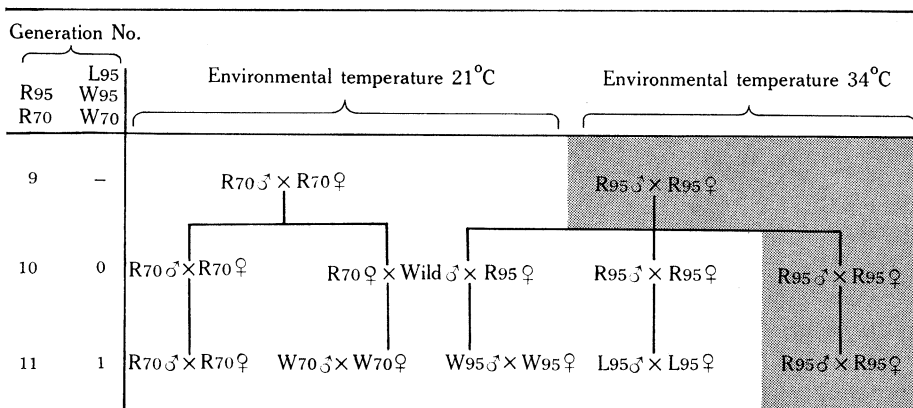


Fig. 1.—Interrelationships of the R70, R95, W70, W95, and L95 stocks and the temperature to which each generation was exposed. Both parents of generation 0 of the L95 stock and the female parent of generation 0 of the W95 stock were exposed to 34°C until weaning when they were transferred to 21°C.

Two further groups were used to compare the effects of exposure of individuals to high temperatures with the effects of maintenance for a number of generations at 34°C; namely, R70 males introduced into the incubator (34°C) at 3 weeks of age and R70 males born to mothers gestating at 36°C and kept at this temperature throughout their lives. These groups were used for measurements of the hair coat and of capillary densities.

(c) Measurement of Reproductive Productivity and Growth Rate

Reproductive productivity and growth rate of the R70, R95 and L95 stocks were measured as described previously (Pennycuik 1969). Only litter size, pup survival, and growth rate were measured for the W70 and W95 stocks.

(d) Metabolic Rate Measurements

The apparatus and the procedure followed are described by Pennycuik (1967). The groups examined included: R70, R95 (generation 11), L95 (generation 1), Wild, W70 (generation 1), and W95 (generation 1). All mice, except those of the R95 stock, were acclimatized to 21°C throughout life. The R95 animals were acclimatized to 34°C.

(e) Age at Time of Emergence of the Second Hair Coat

This was measured for two generations (3 and 11) of R70 and R95 animals and for one generation (1) of L95 animals. The hair of the lower back was removed with clippers when the animals were between 21 and 25 days of age. They were then examined daily until the skin thickened and the new hair coat began to emerge through the skin of the clipped area. Records were kept of the age and body weight at time of emergence of the second hair coat.

(f) Quantity of Hair, Number of Hairs per Follicle, and Skin Capillary Supply

These were measured in the following groups: R70 at 21°C, R70 at 34°C, R70 at 36°C, R95 (generation 12) acclimatized to 34°C, and L95 (generation 2) at 21°C. Males of 12 weeks of age were killed and weighed. The hair was removed with clippers, weighed, and the quantity present expressed as milligrams per square centimetre of surface area [surface area = $13.2 \text{ weight}^{0.667}$ (Brody 1964)]. India ink was injected through the dorsal aorta into the posterior part of the body. The skin of the lower back was removed, pinned to a cork frame with minimal stretching, and fixed in neutral formalin. Approximately 1 sq cm of skin, which appeared to be injected satisfactorily, was stained with oil blue N (which stained the sebaceous glands making the hair follicles easy to identify) and cleared in glycerol. The numbers of hairs per follicle were counted for each of 100–130 follicles. The specimen was then examined on a projection microscope, the subepidermal plexus (Durwood and Rudall 1958) was identified, and an area which was well injected was traced on clear celluloid. Only the skins of the R70 mice at 36°C were unsuitable for measuring the subepidermal plexus.

(g) Frequency of the Pink-eye Gene

The frequency of this gene was measured in generations 0 and 10 of the R70 stock and in generation 10 of the R95 stock.

III. RESULTS

(a) Reproductive Productivity

The total number of pups reared to 6 weeks of age by R70, R95, and L95 mice is shown in Table 1, and the contributions of the four major components of productivity (i.e. percentage of pairs which produced one or more litters, number of litters born to each pair, litter size at birth, and pup mortality) are shown in Tables 1 and 2. The results of the R95 mice differed significantly from those of the R70 and L95 animals, but the L95 results were not significantly different from those of the R70 animals. However, there was a suggestion that L95 mothers born at 21°C (generation 1) produced more litters per pair than mice in the other groups. This was due to the high proportion of post-partum pregnancies (Table 1) and to a shortening of the between-litter interval (Pennycuik, unpublished data).

TABLE 1
REPRODUCTIVE PRODUCTIVITY OF R70, R95, L95, W70, AND W95 MICE

Reproductive productivity was measured in terms of the number of pairs which produced one or more litters, the number of litters born to each pair which produced one or more litters, the number of 6-week-old pups reared by all pairs, and the number of post-partum pregnancies as a percentage of all second and subsequent pregnancies. The significance of differences between groups were calculated using the *F*-test; values in italics reached the 5% level or higher

Stock	Temp. (°C)	Gener- ation	Dates when Matings were Set Up	Time for which Males and Females were Together (weeks)	No. of Matings	No. of Fertile Matings (%)	No. of Litters Born per Pair (mean \pm S.E.)	No. of 6-week-old Pups per Pair (mean \pm S.E.)	No. of Post-partum Pregnancies (%)
R70	21	10	30.iii.66	12	13	100	3.3 \pm 0.20	23.4 \pm 1.58	86.6
		11	18.viii.66	12	13	100	3.3 \pm 0.20	25.6 \pm 1.59	96.7
R95	34	10	30.iii.66	12	13	100	2.3 \pm 0.25	7.5 \pm 0.65	64.7
		11	17.viii.66	12	13	76.9	2.3 \pm 0.25	4.9 \pm 0.95	42.2
L95	21	0	27.iv.66	12	11	90.9	3.3 \pm 0.32	19.6 \pm 2.85	91.3
		1	22.ix.66	12	10	100	3.7 \pm 0.15	24.3 \pm 2.25	92.6
W70	21	0	27.iv.66	3	9	—	—	—	—
		1	9.ix.66	3	16	—	—	—	—
W95	21	0	27.iv.66	3	9	—	—	—	—
		1	9.ix.66	3	16	—	—	—	—
<i>F</i> values*							6.317	29.796	
<i>F</i> values†							0.729	1.577	

* For generations 10 and 11 of stocks R70 and R95 and for generations 0 and 1 of stock L95.

† For generations 10 and 11 of stock R70 and for generations 0 and 1 of stock L95.

Crossing R95 and R70 mice to an unrelated stock (Wild) revealed no significant differences between those maintained for a number of generations at 34°C and those maintained at 21°C (Table 2).

TABLE 2
LITTER SIZES AT BIRTH, 3, AND 6 WEEKS OF AGE OF THE R70, R95, L95, W70, AND W95 STOCKS
The significance of differences between groups were calculated using the *F*-test; values in italics reached the 5% level or higher

Stock	Gener- ation	Litter Size (mean \pm S.E.)			
		Total No. Born	No. Alive at Birth	No. Alive at 3 Weeks	No. Alive at 6 Weeks
R70	10	7.95 \pm 0.40	7.65 \pm 0.45	7.21 \pm 0.49	7.07 \pm 0.48
	11	8.65 \pm 0.35	8.35 \pm 0.37	7.86 \pm 0.40	7.74 \pm 0.38
<i>F</i> values		1.777	1.421	1.062	1.206
R95	10	4.67 \pm 0.36	4.23 \pm 0.35	3.67 \pm 0.36	3.23 \pm 0.31
	11	4.48 \pm 0.39	4.30 \pm 0.40	3.22 \pm 0.36	2.74 \pm 0.30
<i>F</i> values		0.127	0.018	0.763	1.207
L95	0	7.03 \pm 0.41	7.03 \pm 0.41	6.67 \pm 0.46	6.52 \pm 0.45
	1	7.46 \pm 0.46	7.43 \pm 0.46	6.81 \pm 0.50	6.57 \pm 0.50
<i>F</i> values		2.924	1.657	1.334	1.594
W70	0	8.22 \pm 1.00	8.22 \pm 1.00	6.89 \pm 0.93	6.78 \pm 0.89
	1	9.00 \pm 0.74	8.76 \pm 0.78	7.59 \pm 0.84	7.35 \pm 0.84
<i>F</i> values		0.391	0.179	0.274	0.182
W95	0	8.33 \pm 0.47	8.33 \pm 0.47	8.00 \pm 0.62	7.67 \pm 0.69
	1	7.29 \pm 0.62	7.12 \pm 0.66	7.00 \pm 0.62	6.59 \pm 0.59
<i>F</i> values		1.282	1.550	1.056	1.268
Other <i>F</i> values*		10.367	9.884	9.697	11.803
Other <i>F</i> values†		1.881	1.298	0.747	0.850

* For generations 10 and 11 of stocks R70 and R95 and for generations 0 and 1 of stocks L95, W70, W95.

† For generations 10 and 11 of stock R70 and for generations 0 and 1 of stocks L95, W70, and W95.

(b) Body Weights at 3 and 6 Weeks of Age

Exposure to 34°C caused a significant reduction in the body weights of pups at 3 and 6 weeks of age (Table 3). Mothers of the initial generation of the L95 stock were reared at 34°C until they were 3 weeks old before transfer to 21°C. Pups born to these mothers were heavier than those born to mothers kept continuously at 34°C, but lighter at 3 weeks of age than those born to mothers kept continuously at 21°C (cf. L95 and W95 generations 0 with R95 generations 10 and 11 and R70 generations 10 and 11). On the other hand mothers of the L95 stock kept throughout life at 21°C were able to rear pups equal in weight at both 3 and 6 weeks to those reared by R70 mothers at 21°C at the same season of the year (cf. L95 generation 1 and R70 generation 11); i.e. although body weight declined during 10 generations at 34°C (Pennycuik 1969), growth at 21°C was apparently unaffected.

TABLE 3
BODY WEIGHTS (MEAN \pm S.E.) AT 3 AND 6 WEEKS OF AGE OF PUPS OF R70, R95, L95, W70, AND W95 STOCKS
The significance of differences between groups were calculated using the *F*-test; values in italics reached the 5% level or higher

Stock	Temp. (°C)	Gener- ation	3-week-old Females		3-week-old Males		6-week-old Females		6-week-old Males	
			No.	Weight (g)	No.	Weight (g)	No.	Weight (g)	No.	Weight (g)
R70	21	10	53	8.0 \pm 0.18	60	8.3 \pm 0.17	53	16.1 \pm 0.32	60	18.8 \pm 0.28
		11	165	8.6 \pm 0.12	173	8.9 \pm 0.13	164	17.2 \pm 0.17	169	19.6 \pm 0.20
<i>F</i> values				<i>5.014</i>		<i>5.814</i>		<i>11.031</i>		<i>5.086</i>
R95	34	10	53	6.0 \pm 0.19	56	6.4 \pm 0.23	47	12.9 \pm 0.37	50	14.5 \pm 0.44
		11	40	6.2 \pm 0.26	31	6.6 \pm 0.32	34	12.3 \pm 0.40	29	14.0 \pm 0.39
<i>F</i> values				0.641		0.113		1.131		0.561
L95	21	0	76	7.8 \pm 0.16	89	8.0 \pm 0.16	74	16.5 \pm 0.26	82	18.8 \pm 0.33
		1	120	8.9 \pm 0.13	132	8.9 \pm 0.13	113	17.2 \pm 0.16	130	19.3 \pm 0.20
<i>F</i> values				<i>29.843</i>		<i>19.938</i>		<i>4.914</i>		<i>2.489</i>
W70	21	0	26	9.0 \pm 0.34	36	9.1 \pm 0.33	25	17.9 \pm 0.50	36	20.7 \pm 0.39
		1	67	9.9 \pm 0.16	62	10.0 \pm 0.18	64	17.5 \pm 0.23	61	20.6 \pm 0.27
<i>F</i> values				<i>7.316</i>		<i>6.048</i>		0.577		0.077
W95	21	0	40	6.8 \pm 0.13	32	6.9 \pm 0.14	39	13.2 \pm 0.34	30	16.5 \pm 0.44
		1	68	8.9 \pm 0.14	51	9.4 \pm 0.23	67	15.2 \pm 0.26	45	17.4 \pm 0.43
<i>F</i> values				<i>104.991</i>		<i>67.813</i>		<i>23.054</i>		<i>2.201</i>
Other <i>F</i> values:*										
R70(10), W70(0)				7.509		6.867		9.942		17.140
R70(11), W70(1)				36.489		22.134		0.810		6.299
R95(10), R95(11), L95(0), W95(0)				21.398		15.418		40.577		32.436
R95(10), R95(11), W95(0)				3.997		0.733		1.339		7.049
L95(1), W95(1)				0.005		4.061		46.711		19.927
L95(1), R70(11)				3.610		0.072		0.059		1.046
W70(1), W95(1)				19.975		3.835		44.158		41.707

* Generation number given in parenthesis.

However, the results of crossing the heat-reared and the control stocks to an unrelated stock with different growth characteristics suggest that life at 34°C did have some effect on growth potential at 21°C. The weights at 3 and 6 weeks of the unrelated stock used (Wild) were as follows: females at 3 weeks 8.9 g, males at 3 weeks 9.2 g, females at 6 weeks 15.0 g, males at 6 weeks 16.0 g. When comparisons are made between the weights of mice of W95 (generation 1), L95 (generation 1), R70 (generation 11), and W70 (generation 1), it is clear that W95

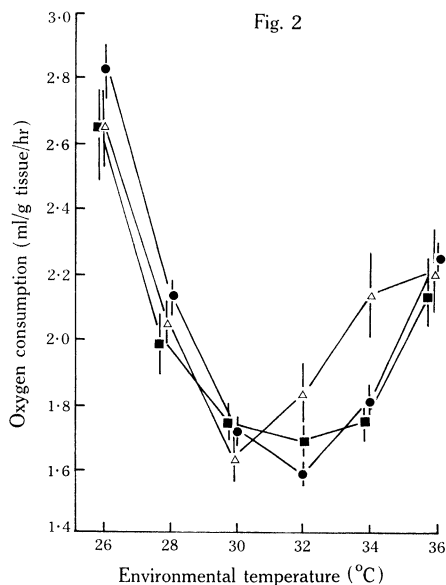


Fig. 2.—Oxygen consumptions measured at six different temperatures of 47 R70 (●) and 11 L95 (△) mice reared at 21°C and of 8 R95 (■) mice reared at 34°C. The vertical bars represent standard errors.

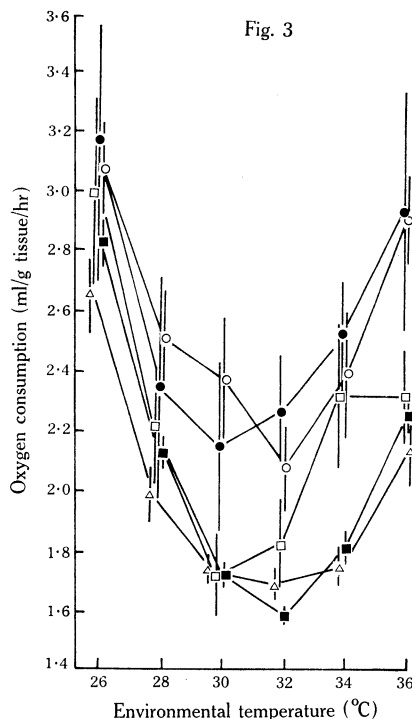


Fig. 3.—Oxygen consumptions, measured at six different temperatures, of 47 R70 (■), 12 Wild (○), 15 W70 (●), and 5 W95 (□) mice reared at 21°C and of 11 R95 (△) mice reared at 34°C. The vertical bars represent standard errors.

animals were significantly lighter at 6 weeks than all other stocks, that W70 animals were significantly heavier at 3 weeks than all other stocks, and that W95 and W70 animals differed significantly in weight at both ages. These differences were not seasonal in origin for the animals were born at approximately the same time of year (Table 1).

(c) Oxygen Consumptions

These are shown in Figures 2 and 3. No significant difference could be detected between R95 mice (generation 12) acclimatized to 34°C and R70 mice acclimatized to 21°C (Fig. 2). Unexpectedly, return of the R95 mice to 21°C caused a significant

change in the pattern of oxygen consumption: the thermoneutral point was depressed (30°C instead of 32°C) and at temperatures above this point, oxygen consumption rose steeply. The values at 32 and 34°C were significantly different from those of the R70 and R95 animals (32°C, $F = 5.88$, $1.0 > P > 0.1$; 34°C, $F = 4.30$, $5.0 > P > 1.0$). The value at 36°C was not significantly different from that of the other two stocks, but this resulted from the fact that the L95 mice could not be kept at this temperature for long enough to obtain reliable readings.

The difference between the pattern of oxygen consumption of the L95 mice and R70 mice was also apparent when the mice were crossed to an unrelated stock (Wild) with a different pattern of consumption. This latter stock had a significantly higher rate of consumption than the R70 and L95 stocks at all temperatures other than 26°C (Fig. 3). Mice of the W95 stock had a pattern of consumption similar to that of

TABLE 4

AGES AND WEIGHTS (MEAN \pm S.E.) OF PUPS OF THE R70, R95, AND L95 STOCKS AT THE TIME WHEN THE SECOND HAIR CYCLE EMERGED FROM THE SKIN SURFACE

The significance of differences between groups were calculated using the F -test; values in *italics* reached the 5% level or higher

Stock	Temp. (°C)	Gener- ation	Females			Males		
			No.	Age (days)*	Weight (g)*	No.	Age (days)*	Weight (g)*
R70	21	3	48	42.77 \pm 0.65	—	64	37.97 \pm 0.60	—
		11	71	40.58 \pm 0.54	16.3 \pm 0.22	78	38.12 \pm 0.41	17.4 \pm 0.22
R95	34	3	21	45.14 \pm 1.65	—	20	44.45 \pm 1.92	—
		11	29	43.62 \pm 0.83	12.7 \pm 0.39	34	43.09 \pm 1.23	14.6 \pm 0.45
L95	21	1	32	39.16 \pm 0.62	15.4 \pm 0.22	34	37.91 \pm 0.57	16.1 \pm 0.26
F values:†								
R70(3,11), R95(3,11), L95(1)			7.698			12.434		
R70(3,11), L95(1)			7.204			0.042		
R70(11), L95(1)			2.439			0.084		
R95(3,11)			0.797			0.392		

* At emergence of second hair cycle.

† Generation number given in parenthesis.

their R95 relatives acclimatized to 21°C (L95). The W70 mice, on the other hand, had a pattern similar to that of their Wild ancestors.

These results suggest that exposure to 34°C for a number of generations favoured a pattern of oxygen consumption different from that at 21°C and that this, unlike the pattern of the base stock, tended to dominate over that of the unrelated stock when the two lines were crossed.

(d) Age at Time of Emergence of the Second Hair Cycle

The ages of the pups at the time of emergence of the second hair cycle are shown in Table 4. Exposure to 34°C increased the interval between birth and

emergence of the hair and slowed growth rate. There was a slight decrease in the age at which the second hair coat emerged after the mice had been at 34°C for a number of generations, but the difference between the values for generation 3 and generation 11 were not significant. Pups born to R95 mothers transferred to 21°C (L95, generation 1) grew hair at approximately the same age as R70 controls. Apparently the delay in the emergence of the second hair coat at 34°C was an environmental effect associated with a slowing of the growth rate.

(e) *Quantity of Hair*

The amount of hair carried by R70 mice at 21, 34, and 36°C, R95 mice at 34°C, and L95 at 21°C is shown in Table 5. Exposure of R70 mice to 34 or 36°C caused a decrease in the amount of hair carried per unit area. The differences between these

TABLE 5

AMOUNT OF HAIR CARRIED BY 12-WEEK-OLD MICE FOLLOWING DIFFERENT PERIODS OF ACCLIMATIZATION TO 34 AND 21°C

The significance of difference between groups were calculated using the *F*-test; values in italics reached the 5% level or higher

Stock	Temp. (°C)	Gener- ation	No. of Mice	Body Weight (g)	Amount of Hair (mg/sq cm) \pm S.E.
R70	21	10	12	29.9 \pm 0.74	2.86 \pm 0.12
	34	12	13	25.0 \pm 0.57	2.66 \pm 0.10
	36	10	10	19.0 \pm 0.92	2.37 \pm 0.17
R95	34	12	9	23.4 \pm 0.54	2.30 \pm 0.07
L95	21	2	11	26.0 \pm 0.81	2.82 \pm 0.07
<i>F</i> values:*					
R70(21,34,36), R95(34), L95(21)					4.821
R70(21), L95(21)					0.182
R70(34,36), R95(34)					2.767

* Temperature (°C) given in parenthesis.

groups and R95 mice at 34°C were not significant. When R95 mice were returned to 21°C their offspring were found to grow quantities of hair similar to those grown by R70 mice at 21°C. Examination of skin specimens from the five different groups suggests that the difference in the weight of hair at 21 and 34°C was due to differences in the number of hairs held in each follicle. Whether the low number at 34°C was due to increased shedding or to reduced growth is uncertain. Like time of emergence of the second hair cycle, the changes observed in the amount of hair grown by the R95 stock appeared to be environmental in origin.

(f) *Density of the Subepidermal Capillary Net*

Examination of injected skin specimens from mice at 21 and 34°C revealed no differences in the density of the subepidermal capillary net.

(g) *Frequency of the Pink-eye Gene*

Approximately half the mice in the base population were homozygous for pink-eye. By generation 10 the frequency of this gene had increased to 66% in the R70 stock and 55% in the R95 stock, but the differences between the three groups did not reach significance.

IV. DISCUSSION

Geographical races of domestic animals usually show distinct morphological and physiological differences when exposed to identical environmental conditions. British and Indian breeds of cattle differ in the amount of hair cover and in the pattern of shedding of the coat (Turner and Schleger 1960). They also differ in growth rate, heat production, and the position of the zone of thermal neutrality (Kibler and Brody 1950). Findings are similar for laboratory animals kept under different climatic conditions for a number of generations (Howard 1963).

The period of separation of the mouse stocks used in these experiments was only 10 generations. When both were exposed to 21°C it was possible to detect differences in growth potential and in the pattern of oxygen consumption, but it was not possible to detect differences in hair cover. This is in agreement with the results of Barnett and Scott (1963) and Barnett (1965) who found that random-bred mice, reared for 12 generations at -3°C, grew better than controls at 21°C, but did not differ in the weight of hair carried. Apparently changes in growth rate and in heat production appear earlier than changes in insulation in races exposed to different climatic conditions.

Since the mice used in these experiments were from a random-breeding stock the changes in growth and oxygen consumption following exposure to 34°C were due, probably, to selection of a favourable genotype. The frequency of only one gene (pink-eye) was studied. This did not change with increasing generation number so it is unlikely that this particular linkage group was intimately involved. Nineteen further linkage groups still remain to be tested. The possibility that mutations (Cowles 1965), cytological changes (Brun 1965), and cumulative maternal effects (Barnett 1965) contributed to the changes observed also remains to be investigated.

V. ACKNOWLEDGMENTS

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