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EFFECTS OF STRESS DUE TO DEPRIVATION AND TRANSPORT IN DIFFERENT GENOTYPES OF HOUSE MOUSE

by

MARGARET E. WALLACE

Department of Genetics, University of Cambridge, Milton Road, Cambridge, CB4 1XH

SUMMARY

The importance of various stress factors involved in boxing and transit of wild and laboratory mice on a 28 hour journey was studied. Transference from laboratory cage to transit box alone caused weight loss; under the best conditions the laboratory mice lost 5% of their initial weight and wild ones 8%. Deprivation of food resulted in absolute loss of weight; from this death ensued when 20% of initial weight was lost: the smaller wild mice died sooner than the larger laboratory ones. Water deprivation resulted in retarding recovery of weight lost; wild mice took longer to recover than laboratory ones. It also caused weight loss and, in conjunction with deprivation of food, poor condition and death. Transit itself affected percentage weight loss and wheat consumption; wild mice were affected differently from laboratory mice in both respects.

Genotype is seen to be important not only in controlling initial weight and activity level, but also—and independently of weight—the total food requirement.

In the light of these findings current literature giving guidance on shipment of small mammals is shown to be inadequate, and certain recommendations are given.

Current guides on the welfare of animals in transit are useful (British Standards Institution, 1961; National Academy of Sciences, 1961, 1969; King, 1970; UFAW, 1972). But without a reliable source of plain water for small rodents it is difficult to assess directly the separate contributions of various deprivations. In the spirit of the *Diseases of Animals Act* 1950, Section 22 (3), which states that ruminents and swine in transit must not be without water for 24 consecutive hours, laboratories have developed water substitutes for rodents. These include cut potato (moisture content 76%) and cakes of moist diets, both of which, however, dry out. The author therefore developed a non-spill container, small enough to pack inside transit boxes for mice and other such rodents, which provides enough water for a mouse for 1-3 days.

The present paper gives the findings of a small experiment designed to assess separately the main reactions to travel and associated stress in laboratory mice and 2 strains of wild mice, and gives guidelines for their improved condition on arrival.

MATERIALS AND METHOD

Experimental design

Altogether 4 observations were made: for 2 the mice were transported and for 2 they remained in the laboratory. The observations were made at random during January-March.

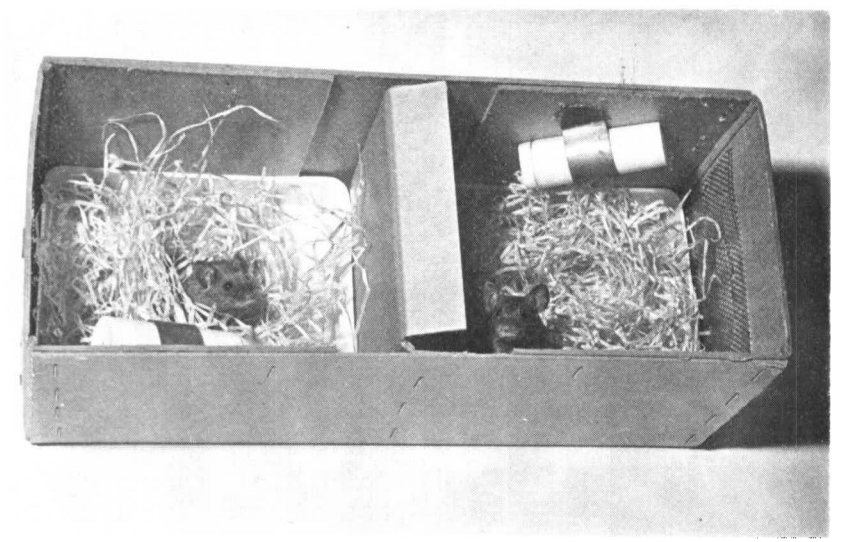

Fig. 1. Transit box (lid not shown). Each compartment is one 'box' and contains a corrugated plastic tray in the bottom, woodwool and a single mouse. Those shown have also a water container fixed to the side, with access underneath. When wheat is provided it is placed in the trays near the ventilation grids at the ends.

128 mice were used in groups of 32. Each group comprised 16 laboratory and 16 wild mice of mixed sexes, packed singly in boxes; the boxes were paired, each pair forming 2 compartments of a single unit (Fig. 1). The boxes were labelled 1-32 and arranged as follows:

Laboratory mice

	water				*no water*			
Added	nothing		glucose		potato		nothing	
Wheat	yes	no	yes	no	yes	no	yes	no
Label	1	3	5	7	9	11	13	15
	2	4	6	8	10	12	14	16

Wild mice

	water				*no water*			
Added	nothing		glucose		potato		nothing	
Wheat	yes	no	yes	no	yes	no	yes	no
Label	17	19	21	23	25	27	29	31
	18	20	22	24	26	28	30	32

The effect of stress was measured as percentage weight loss, number of half days to weight recovery or death, and as wheat consumption (grams).

An analysis of variance was performed for percentage weight loss dividing the treatments as follows:

1. moisture supply
   water in container versus no water
   moisture source (glucose water or 12 g moist cut potato) versus no moisture.
2. food supply—30 g wheat versus no wheat
3. transport—yes versus no (see below)
4. genotype—laboratory versus wild (see below)

For recovery time, the average time required by survivors was calculated for the 4 treatments above, and a χ^2 test done comparing the number of half-days to recovery for the contrasting treatments; deaths were scored as 10 half-days.

Average wheat consumption under treatments 1, 3 and 4 was similarly treated, with a subdivision of the wild mice into their 2 strains, and subjected to an analysis of variance.

Mice

The laboratory mice were of mixed genotype, derived from crosses of standard inbred strain CBA/FaCam and random-bred strains RAP and 'Swiss'. The wild derived mice were standard inbred strain San Franciscan (SF/Cam) for 2 observations, and Skokholm Island mice (then partly inbred, now standard inbred strain SK/Cam) for the remainder. These were pooled and termed 'wild' mice when contrasted with laboratory mice. They had been inbred without selection and appeared from husbandry observations to have maintained most of their wild behaviour.

Transit boxes

Each paired box was a divided cardboard 'shoebox' 30 × 12 × 11 cm high (Fig. 1). They were lined with shallow, plastic trays with regular indentations, which protected the cardboard from softening with urine and minimised the

mixing of urine and minute amounts of spilt water; this kept the water available for drinking. 6g woodwool were given but no sawdust in order to prevent absorption of water by bedding.

Food and water

Dry wheat was regarded as a source of food, and potato as a source of water. Pelleted diet was not given because it absorbs urine. The mice were accustomed to wheat given once a week in a standard Cambridge cage (Wallace, 1963). Water was provided in a hard plastic container of the author's design, holding 12·4 ml of water, and fixed tightly inside the transit box by strong elastic or a metal strip (Fig. 1). These containers had been shown, on a series of tests, to lose less than $\frac{1}{6}$ their capacity on jolting rail and van journeys without mice; they had also been shown to retain water in excess of need on journeys 1-5 days with mice (daily need is 2·4-10·0 ml/24h depending on weight, age and strain, see Green, 1966).

Method

In this country most journeys by rail take less than 24h, but some take longer when connections are missed. Accordingly a 28h journey was chosen —28h from boxing up the mice until unpacking them.

On the dispatch morning, the mice were weighed after urination and put in the boxes, 1 per compartment, prepared according to the experimental design.

The transit boxes were taken by van to Cambridge station (6 km, 4 miles), where they were dispatched to Norwich (113 km, 70 miles); they spent the night in Norwich parcels office and were returned by rail and van next day. The mice were then removed in the order in which they had been packed; each was again weighed after urination and returned to its cage. The state of the boxes' contents was noted and the remaining wheat weighed. Each mouse was weighed daily in the morning and evening until its weight returned to within 0·5 g of its pre-journey weight; in all survivors this was reached within 3 days.

Mice not sent on the journey were kept in their transit boxes on the laboratory shelves normally occupied by their cages.

RESULTS

The initial weights of the laboratory mice, and of the 2 strains of wild mice together and separately, were:

	Laboratory	*Wild*	*Wild SF/Cam*	*Wild SK/Cam*
Mean weight (g)	25·92	18·84	20·05	17·571
Standard error	±0·979	±0·979	±1·384	±1·384

Thus the wild and laboratory mice differed significantly from each other ($P<0{\cdot}001$), but there was no statistical difference between the wild strains.

The data for the absolute weight losses after the 28h journey, the state of health and time for weight recovery, are given in Table 1.

The main effects of genotype, travel, and wheat and water treatments on weight loss, wheat eaten and time to recover weight, are given in Table 2.

There were 3 significant 1st-order interactions in the analysis for percentage weight loss; these are given in Table 3. A 4th 1st-order interaction is included there because, while not significant, it is of interest in the context of wheat consumption.

There were 2 significant 1st-order interactions in the analysis for wheat consumption; these are given in Table 4.

A general impression was gained of the amount of chewing of cardboard inside the boxes, and of the plastic water-containers; it was impossible to weigh these shreds accurately. The laboratory mice, whether travelled or stationary, chewed a little; the stationary wild mice chewed somewhat more; and the travelled wild mice chewed the most.

Weight loss

Table 1 shows that all treatments caused loss of weight (only 1 of the 128 mice gained). Table 2B shows that the biggest factor is lack of food; the provision of wheat can halve weight loss (from 17% to 9%). The effect of genotype is almost as large (Table 2A and B); wild mice lost no more than laboratory ones in absolute terms (2·88 and 2·86 g), but since wild mice had a small initial weight they lost relatively nearly half as much again as laboratory mice (15 versus 11%). Water treatments had a slightly smaller effect—since mice drink little in relation to their total bodyweight in 24h (about 10% bodyweight for laboratory mice), this is not surprising. The main loss (Table 2B) was where there was no source of water supplied, plain or with glucose (16 versus 12%). This was slightly reduced with the provision of potato as a source of moisture (Table 3B). It is worth noticing (Table 3A) that when no food (wheat) was given, the addition of water (plain or with glucose) did nothing to alleviate weight loss; only when wheat was given was the addition of water beneficial.

Since the environment outside the box is constantly changing during transport, it is perhaps surprising that transport itself had a barely noticeable effect on weight; there was in fact (Table 2B) a just significant ($P<0{\cdot}05$) lesser loss for travelled mice than for stationary ones. However, this was mainly due to the reaction of the wild mice (Table 3C), who lost less when they travelled than when stationary. This odd finding persisted even when wheat was given (Table 3D), although the interaction was not significant.

Table 1. Response of mice to treatments in terms of weight loss, health, and time to recover weights.

		Laboratory mice				*Wild mice*				*Totals*
		wheat		*no wheat*		*wheat*		*no wheat*		
	Columns*:	1	2,3	1	2,3	1	2,3	1	2,3	
Untravelled	Plain water	1·60/24·98	1·5	4·27/25·42	1·0	2·08/21·24	2·5	4·20/20·91	2·25	12·15/92·55
	Glucose and water	0·67/26·42	1·5	4·20/29·15	1·0	2·22/20·02	2·25	4·03/17·83	PNN 3·0	11·12/93·42
	Potato	2·45/27·88	1·76	3·74/27·44	1·0	2·24/19·08	2·0	3·37/17·95	P 2·75	11·00/92·35
	Nothing	2·66/25·14	1·50	4·68/28·41	1·5	3·00/18·73	1·5	4·09/19·08	P 2·75	14·43/91·36
	Totals	7·38/104·42	6·26	16·89/110·42	4·5	9·54/79·07	8·25	15·69/75·77	PPP NN >10·75	49·50/369·68
Travelled	Plain water	1·03/22·86	PP 1·0	2·96/22·47	1·0	1·03/18·66	1·5	3·75/18·95	P 2·0	8·77/82·94
	Glucose and water	1·20/26·85	1·0	3·74/24·87	1·5	1·59/17·10	2·5	3·08/17·38	PAA 2·0	9·61/86·20
	Potato	2·52/24·62	2·25	3·40/25·49	P 1·5	1·59/17·47	2·5	3·19/19·08	PA 2·0	10·70/86·66
	Nothing	2·42/26·31	P 2·0	4·32/26·32	2·0	2·29/17·31	2·75	4·36/20·66	NN AA	13·39/90·60
	Totals	7·17/100·64	P >6·25	14·42/99·15	PPP 6·0	6·50/70·54	9·25	14·38/76·07	PPP NN AAAAA >6·0	42·47/346·40

*Column 1. Each entry is an average for 4 mice: 1st value is average weight loss (g), 2nd average initial weight (g). Column 2. Each entry is the average number of days each mouse which survived (out of 4) took to recover. Column 3. Each entry records the state or fate of each mouse not in good condition on arrival. Each *P* signifies a mouse in poor condition but survived, each *N* a mouse that died next day, each *A* a mouse dead on arrival. (2 of these were too decomposed for weight to be meaningful: they were assigned the average weight of survivors of the same treatment).

Table 2. Effects of mouse genotype, travel and wheat and water treatment, on weight loss, wheat eaten and recovery time (means).

	Genotype		*Travelled*		*Wheat*		*Source of water*				*s.e.*
	lab.	*wild*	*yes*	*no*	*yes*	*no*	*plain*	*gluc.*	*potato*	*none*	
A. Weight loss per mouse (g)	2·86	2·88	2·65	3·09	1·91	3·84	2·61	2·59	2·81	3·47	
B. Percentage weight loss per mouse	10·84	15·35	12·38	13·81	8·99	17·20	11·94	11·95	12·91	15·58	± 0·409
C. Wheat eaten per mouse (g)	4·92	5·36	4·53	5·62			5·19		5·08		± 0·194
D. Days to recover weight (survivors)	1·44	2·27	1·82	1·82	1·88	1·76	1·59	1·75	1·97	2·00	
E. Number died	0	9	2	7	0	9	0	4	1	4	
Probability*	$<0·001$		$>0·05$		$<0·02$			$<0·02$			

*The probabilities pertain to the differences between the members of each pair of columns (line D) when calculations are based on the number of half-days to recover, mice which died counted as taking 10 half-days.

Table 3. First-order interactions in percentage weight loss data.

Mean percentage loss per mouse.

						s.e.
A.	Whole data used	water + gluc.		potato + nothing		
		wheat	no wheat	wheat	no wheat	
		6·69	17·20	11·28	17·20	± 0·575
B.	Whole data used	water	gluc.	potato	nothing	
		11·94	11·95	12·91	15·58	± 0·575
C.	Whole data used	travelled		stationary		
		lab.	wild	lab.	wild	
		10·72	14·04	10·96	16·65	± 0·575
D.	'No wheat' ignored	travelled		stationary		
		lab.	wild	lab.	wild	
		7·03	9·91	6·95	12·05	± 0·813

Table 4. First-order interactions for wheat consumption data.

Mean wheat consumption (g) per mouse.

						s.e.
A.	Whole data used	travelled		stationary		
		lab.	wild	lab.	wild	
		5·47	3·84	4·36	6·87	± 0·27
B.	'Wild' only	travelled		stationary		
		SK/Cam	SF/Cam	SK/Cam	SF/Cam	
		4·50	3·18	9·83	3·91	± 0·39

Recovery time

Since 9 of the 128 mice died, it is difficult to devise a realistic test of significance of the differences between treatments. The last line of Table 2 ('Probability') is therefore to be regarded as a rough test only, and judgement should be based on the lines D and E taken in conjunction. It appears that the availability of wheat is of some importance as regards mortality, but barely affects survivors' time to recover weight. The most important factor is genotype; the laboratory mice took about half as long to recover as wild ones.

The form in which water is available is also important: 100% survival occurred only where plain water was supplied.

There is virtually no travelled/stationary difference in recovery time. Most deaths occurred among the stationary mice (where, as shown above, weight loss was slightly greater).

The factors concerned with the deaths, however, can best be seen from the whole data (Table 1): all deaths occurred among wild mice and then only

among those with no wheat or plain water. Death is a result of accumulated stress; it occurred when percentage weight loss reached 17-23%.

Wheat eaten

Both genotype and travel are important in wheat consumption (Table 2C; probabilities of no difference are $<0{\cdot}01$). The very significant interactions between them (Table 4A: probability of no effect $<0{\cdot}001$) show how this works: laboratory mice ate more when they travelled than when they remained stationary, while for the wild mice this situation was reversed. Moreover, the 2 wild strains (Table 4B) differed greatly in their response to travel, the SK/Cam mice eating far less when they travelled than did the SF/Cam.

Despite the interaction in terms of weight loss observed (see *Weight loss*) between food and water treatments, wheat consumption (Table 2C) varied insignificantly with water source, i.e. given wheat, the addition of plain or glucose water induced only slight further consumption. Conversely, mice did not eat more wheat when the water source was poor; wheat itself must therefore be a poor water source.

DISCUSSION

The effects of boxing, of food and water deprivation and of transport can now be integrated to give a profile of reaction for the 2 broadly different types of mice used, and where possible for the 2 wild subtypes.

Boxing

The weight loss when stationary (untravelled) and under the best conditions (plenty of wheat and plain water) gives an estimate of the reaction to transference from cage to transit box. Thus (Table 1) the relevant 8 laboratory mice lost an average of 1·32 of 23·92 g initial weight, or 5% per mouse, and the 8 wild mice 1·55 of 18·89 g, or 8% per mouse. Since the food and bedding were familiar to the mice, the loss was caused by the unfamiliar box and its fittings. However, as the mice were confined singly, it cannot be determined how much of the trauma was due to sudden isolation.

Food deprivation

The biggest effect of food deprivation is on short-term absolute loss of weight. This was the same for laboratory and wild mice, but much larger relatively for the wild ones because of their smaller initial weight (9 and 17% loss).

Food deprivation also delays recovery and can, with further stress, cause death. Wild mice are particularly at risk, probably mainly because of their small initial weight.

Indeed, initial weight is probably the most important expression of genotype here. Death occurred only in wild mice and then when percentage weight weight loss reached 17-23%. (In the 4 laboratory mice which survived a 17% weight loss, a different aspect of genotype must be important).

With this loss of about 20% initial weight as critical for death, and 5% loss on change to an unfamiliar transit box, it is to be expected that other stresses, however small, can cumulatively result in death. Thus, even for mice which are transported under the best conditions, subjection on arrival to further environmental stresses, may be sufficient to kill them.

Water deprivation

Complete deprivation of water resulted in weight loss in both laboratory and wild mice. It thus contributes, with food deprivation, to loss of health and to death. Solid sources of moisture may be expected to be better than no supply (loss with potato was less than with nothing).

The greatest effect of water deprivation was, however, on time to recover weight (1·57-1·75 days versus 1·97-2·00 days). This is no doubt partly its effect on weight loss, but it is also independent of it: wheat deprivation which did cause weight loss did not increase recovery time (Table 2).

Wild mice took longer to recover than laboratory mice (2·27 vs 1·44 days). All deaths occurred among wild mice with no water or wheat, and wild mice given wheat took longer to recover with a poor source of water than laboratory ones under the same conditions (Table 1, but the numbers are too small for significance tests). This suggests that some part of the difference in genotype between wild and laboratory mice is concerned with response to water source.

This response is at variance with the frequently made statement that wild mice live in grain stores indefinitely with no source of water other than that in the grain: one wonders whether a small supply, such as condensation on walls, has been overlooked.

These findings suggest that where mice have an unusual need for water —as in mildly polydipsic strains, in diabetic and hydronephrotic genotypes (Wallace & Spickett, 1967), in various surgically treated animals and in ordinary lactating mice—water deprivation alone could cause death in a short time and is certain to delay recovery in survivers. Since sources other than plain water have shown themselves in the present work to be in some degree inadequate, there is a case for provision of water in routine shipments of mice whether their water need has been established accurately or not.

Other experiments by the author (unpublished) show that most normal laboratory strains are distressed after 16h deprivation of water. Published recommendations which imply that mice need not be given water on journeys up to 24 h are thus inadequate.

Transport

Travel made little difference to weight loss or to recovery time. This is surprising since the environment outside the transit box changes constantly during travel, and the box itself is subject to movement and jolting. The only effect was on wheat consumption (Table 4). The finding that laboratory mice ate significantly more when they travelled than when they were stationary, and that wild mice ate significantly less, the 2 wild strains differing in this, was unexpected. It is made more puzzling by the observation that laboratory mice tended to lose more weight when they travelled than when stationary, and that wild mice lost less; this may reflect their different metabolic rates.

The appetite increase in the laboratory mice could be due to constant disturbances—wakeful periods in laboratories must habitually be concerned with eating; they must also be concerned with exploratory activity, which in turn could result in weight loss. If one supposes an overall increase of metabolic rate, then the weight loss might well fail to be compensated for by the increased intake.

Chewing of cardboard and plastic in the wild mice was unrelated to whether wheat was provided or not, and so may be a stress symptom. Wild mice of all strains in ordinary cages crumble a large amount of their pelleted diet without consuming it (unpublished). Thus gnawing appears to be a response of very active mice to the stress of confinement and unfamiliar container.

A possible explanation of their reaction to travel—eating less and losing less weight than when stationary—is that confinement and disturbance cause an initial gnawing reaction, followed by a long inactive period in a state of shock such that their overall level of metabolism is lowered.

Whatever the explanation, the observed differences in wheat consumption and cardboard chewing between laboratory and wild mice, and between the 2 wild strains, must be ascribed to differences in genotype. Differences in adrenal size as between wild and laboratory rats are well known (Richter, 1954) and thought to account for differences in behaviour, yet recommendations on acclimatisation time are extrapolated from the study of a single rat strain (Grant, Hopkinson, Jennings & Jenner, 1971). Work on adrenals comparing wild and laboratory mice (Dunn & Andervent, 1963; Badr, Shire & Spickett, 1968) using an unusual wild Peru strain (Wallace, 1971), and of Badr & Spickett (1971) suggests that differences in adrenal function rather than in size may explain observed differences between strains, whether of wild or laboratory mice. This would be relevant to any explanation for differences in response to the stresses associated with transport.

RECOMMENDATIONS

Genotype differences found here indicate that guidance on shipment of mice which gives standard recommendations irrespective of varying needs, is in-

adequate. Indeed some recommendations disregard differences between species (e.g. lumping 'small rodents' together). Others give unhelpful general guidance only, such as recommending 'food sufficient for the journey'. Only one of the references cited in the introduction mentions amounts to be provided for journeys of a specific length; but one other (National Academy of Sciences, 1969) has a recommendation that is at least on the humane side in that it considers a journey of only 3h.

The author's findings suggest that guidance in packing small animals for transit must specify the amount and type of food and water source, taking into account:

the expected duration of the journey
the initial weight of the animals
the strain and species and degree of domestication of the animals
the degree of stress the transit box and crowding or isolation is likely to impose.

The present work shows that water supplied in a pure form is a better source than moist solids. Most shippers provide water by means of sliced washed potatoes, carrots or cabbage, according to the ILAR survey (National Academy Sciences, 1961), which comments 'Generally animals will crowd around the watering devices' after a journey. This is an all too familiar observation, which, in the author's opinion is seldom necessary.

In view of the present observations on accumulated stress, it might be worth considering spacing the sources of stresses, e.g. by acclimatisation to isolation and strange transit box a day or so before the journey.

In the absence of precise information on different needs of different genotypes, better conditions should be supplied than would be thought necessary for average animals.

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