Long-Term Isolation Stress in Rats

Abstract. *Rats isolated for long periods became nervous and aggressive and developed caudal dermatitis (scaly tail). After 13 weeks of isolation, rats had heavier adrenals and thyroid and lighter spleen and thymus compared with rats kept in community cages. This indicates an endocrinopathy with hyperfunction of the adrenal cortex.*

Toxicity and nutritional studies on rats and mice are often long term procedures involving the use of animals confined in individual cages. This arrangement facilitates clinical observation and allows food consumption data to be taken on an individual basis. Although this practice may be desirable and necessary, it is probable that the data derived from such studies do not reflect the functionings of a normal animal. In recent years evidence has accumulated which shows that animals isolated for long periods of time have altered physiological and behavioral characteristics. This condition has been referred to as "isolation stress" by several investigators (*1, 2*).

In short-term experiments (up to 10 days) isolated mice or rats have lowered resistance to stress (*3*), lower food consumption and weight gain (*4*) and smaller adrenals (*5*) as compared with animals kept in groups of two or more. Long-term isolation (usually longer than 1 month) may bring about just the opposite effects. The mouse subjected to long-term isolation has greater food consumption, and a tendency toward larger adrenals (*2*). In addition, lower thyroid, spleen and ovary weights, increased oxygen consumption, and absolute leukopenia and eosinopenia have been observed (*2*). The last mentioned is suggestive of hyperadrenocorticism. Mice of the C_3H strain kept in individual cages were found to have a higher incidence of convulsive seizures than that found in paired or grouped mice (*6*). Isolated mice consistently develop a head twitch similar to that observed in mice treated with lysergic acid diethylamide (*7*). The aggressiveness of the isolated mouse has been used in the testing of tranquilizers (*8*). Also, an increase of plasma 17-hydroxy-ketosteroid (sic) has been shown in isolated rats (*9*). A study of the influence of dietary fat on the cardiotoxicity of isoproterenol led to the incidental observation that the toxicity of this compound is greatly increased in isolated rats (*10*).

Over 350 weanling rats of the Wistar strain bred and raised in this laboratory were used in the following experiments. Half of these were housed individually and half were housed in groups of ten. All were fed Master Fox cubes, to which they were given free access. The isolation period did not exceed 13 weeks. Except where isoproterenol was used, rats were killed by exsanguination under light ether anesthesia.

Clinical symptoms of isolation stress became apparent after 4 to 6 weeks. At 3 months the isolated rat is a nervous, aggressive intractable animal. The tendency to bite is so pronounced that normal handling procedures are not feasible and it is necessary to use heavy leather gauntlets or to anesthetize the rats. The most prominent physical symptom is an ascending caudal dermatitis in 100 percent of isolated rats, compared to a zero incidence in community-caged animals.

The results shown in Table 1 indicate that an endocrinopathy exists in the isolated rat which probably involves the adrenal cortex, considering the increased weight of the adrenal glands. It is significant that certain aspects of adrenal cortical function regulate the pattern of protein and carbohydrate metabolism.

The marked difference in the toxicity of isoproterenol between isolated and community-caged rats provided a criterion for following the development of isolation stress. The toxicity did not change appreciably in the first 3 to 4 weeks of isolation, but by 8 weeks the LD_{50} was approximately 118 mg/kg compared with approximately 815 mg/kg in the controls in community cages. After 3 months of isolation the LD_{50} was less than 50 mg/kg. Twenty-four rats were used for each LD_{50} determination.

The reversibility of isolation stress was also established, the toxicity of isoproterenol being used as the parameter. Rats which had been returned to community cages for 19 days, after 3 months in isolation, showed a normal sensitivity to isoproterenol and no sign of their previous intractability. Earlier studies had indicated that a 1-week period of communal life was insufficient to effect this recovery (*10*).

In attempts to overcome the effects of isolation, rats were handled for 5 to 10 seconds daily for 4 months. This amount of handling was only partially successful in overcoming isolation stress as measured by absolute lymphocyte count and the plasma corticoid level (*11*). Rats kept in pairs for 3 months were found to be normal in behavior and in response to isoproterenol.

Table 1. Some differences observed between rats kept in community cages and rats kept in isolation for 13 weeks. Organ weights given in grams.

Sex	No. of rats per group	Isolated	Community
	Adrenals (relative wt)		
M	20	0.013	0.011
F	20	.030	.024*
	Spleen (relative wt)		
M	20	.213	.239*
F	20	.257	.284*
	Thyroid (relative wt)		
M	20	.007	.006
F	20	.010	.008*
	Thymus (absolute wt)		
M	20	.269	.335*
F	20	.250	.307*
	Liver glycogen (g/100 g tissue)†		
M	5	.500	.740
F	5	.450	.420

* $p = 0.01$. † Determined according to the method of J. Kahan, *Arch. Biochem. Biophys.* **47**, 408 (1953).

When the full significance of isolation stress is recognized, the use of paired or routinely gentled animals could become a standard procedure in chronic toxicity and nutritional studies.

ANITA HATCH G. S. WIBERG
TIBOR BALAZS* H. C. GRICE
Food and Drug Laboratory, Ottawa, Ontario

References and Notes

1. T. C. Barnes, *Federation Proc.* **18**, 365 (1959); E. Ziskind, *J. Am. Med. Assoc.* **168**, 1427 (1958).
2. A. S. Weltman, A. M. Sackler, S. B. Sparber, S. Opert, *Federation Proc.* **21**, 184 (1962).
3. K. Martindale, G. F. Somers, C. W. M. Wilson, *J. Pharm. Pharmacol.* **12**, 153T, (1962).
4. H. F. Harlow, *J. Genet. Psychol.* **41**, 211 (1932).
5. E. C. Grant and M. R. A. Chance, *Animal Behavior* **6**, 183 (1958).
6. J. T. King, Y. C. Lee and M. B. Visscher, *Proc. Soc. Exptl. Biol. Med.* **88**, 661 (1955).
7. D. L. Keller and W. W. Umbreit, *Science* **124**, 723 (1956).
8. H. C. Y. Yen, R. L. Stanger, N. Millman, *J. Pharmacol. Exptl. Therap.* **122**, 85A (1958); T. C. Barnes, *Federation Proc.* **17**, 347 (1958).
9. H. C. Y. Yen, C. A. Day, E. B. Sigg, *Pharmacologist* **4**, (1962).
10. T. Balazs, J. B. Murphy, H. C. Grice, *J. Pharm. Pharmacol.* **14**, 750 (1962).
11. A. M. Hatch, M. Cann, G. S. Wiberg, S. Zawidska, H. C. Grice, in preparation.

* Present address: Lederle Laboratories, Pearl River, New York.

15 August 1963