Greater lowering of brain and adrenal catecholamines in group-housed than in individually-housed mice administered DL-α-methyltyrosine

SIR,—We have previously interpreted differences in brain and adrenal catecholamines between mice that live in groups and their individually-housed littermates to be indicative of higher basal levels of amine metabolism in the grouped mice (Welch, 1965; 1967; Welch & Welch, 1964; 1968); support for this interpretation has now been provided by experiments with DL-α-methyltyrosine, an inhibitor of the rate-limiting enzyme in noradrenaline biosynthesis (Spector, Sjoerdsma & Udenfriend, 1965).

Male white Swiss mice born within the same two-day period were weaned at 4 weeks, and littermates weighing within 2 g of the same body weight were randomly assigned to individual housing or to live in a group of 8 to 10 mice. We have found that fighting is minimal among male mice of this strain if they are grouped together from time of weaning. Grouped mice were housed in cages 10 × 10 × 6 inches, a space slightly more liberal than that recommended for maintaining laboratory mice (U.S. Dept. of Health, Education and Welfare, 1965); individually housed mice were in cages half this size. Food and water were always accessible in abundance to the animals. The room was maintained at 75–78° F and contained no other animals. Cages were changed once each week. In preliminary experiments, 80 mg/kg of DL-α-methyltyrosine was found to reduce catecholamines as effectively as higher doses. After 14 weeks and with minimal disturbance, half of the isolated mice and half of the mice in each group were administered either DL-α-methyltyrosine, 80 mg/kg, intraperitoneally in 0·2 ml of 0·9% saline at pH 3, or vehicle alone. The mice were colour-coded and were *returned to their original cages*; exactly 6·5 hr after injection they were decapitated. The left adrenal and the whole brain, exclusive of the *bulbus olfactorius*, were weighed, frozen on dry ice, and stored at −20°C. The brains were homogenized in 0·01 N hydrochloric acid and analyzed for noradrenaline and dopamine (Welch & Welch, 1968). Duplicate internal standards of each amine were added to tissue extracts at 3 concentrations and carried through the whole procedure (% recovery = 76 ± 0·3–0·6 s.e.). The adrenal was homogenized in 0·5 N perchloric acid, titrated to pH 6 with K_2CO_3, centrifuged, and analyzed for adrenaline and noradrenaline. All manipulations were patterned to balance any diurnal or other rhythmic variations. Saline and drug pairs of isolated and grouped mice were established with the assumption that the decline of catecholamines after blockade of synthesis is proportional to their concentration (Brodie, Costa, & others, 1966), and differences between them were evaluated by Student's *t*-test.

α-Methyltyrosine lowered brain catecholamines and the adrenaline content of the adrenal medulla significantly more in the grouped mice (Table 1). Body weight was significantly less in the grouped mice, but the adrenal weight was significantly greater. The adrenaline content of the adrenal medulla was significantly greater in the grouped mice when corrected for differences in body weight by covariance. Mice that received α-methyltyrosine gradually became less active than their controls, and at the time of death they were slightly sedated, the grouped mice more so than the isolates. Deep body temperature of the drug-injected mice averaged 3°C lower than controls 3 hr after injection and 5°C lower at the time of death; grouped and isolated mice did not differ in temperature. No fighting occurred during the experiment.

Brain noradrenaline and dopamine are released by nerve stimulation (Glowinski & Baldessarini, 1966), and it is well established that adrenal catecholamines are released by stimulation of the splanchnic nerve. Further, the release of

TABLE 1. EFFECT OF TYROSINE HYDROXYLASE INHIBITION ON BRAIN AND ADRENAL CATECHOLAMINES IN MALE MICE GROUP-HOUSED AND INDIVIDUALLY HOUSED FOR 14 WEEKS SUBSEQUENT TO WEANING AT AGE OF 4 WEEKS

	Isolated (± s.e.)	Group 10 (± s.e.)	P<
Number	30	28	
Body wt.	38·1 ± 0·8	33·0 ± 0·8	0·01
Left adrenal (mg)	2·08 ± 0·09	2·44 ± 0·07	0·01*
Brain noradrenaline			
Saline control (ng/g)	402 ± 14·8	456 ± 16·7	0·025*
α-Methyltyrosine decrease (% control)	58·4 ± 1·38	64·4 ± 1·96	0·01
Brain dopamine			
Saline control (ng/g)	788 ± 30·9	748 ± 29·1	n.s.
α-Methyltyrosine decrease (% control)	53·8 ± 0·99	62·0 ± 0·89	0·001
Adrenal adrenaline			
Saline control (μg/left adrenal)	3·66 ± 0·25	4·01 ± 0·33	n.s.*
α-Methyltyrosine decrease (% control)	20·9 ± 3·25	33·3 ± 4·16	0·01
Adrenal noradrenaline			
Saline control (μg/left adrenal)	0·44 ± 0·04	0·42 ± 0·10	n.s.
α Methyltyrosine decrease (% control)	11·4 ± 10·28	14·3 ± 9·84	n.s.

* Differences are enhanced when values are adjusted to body weight by covariance analysis; on this basis, the adrenals of the grouped mice contain significantly more adrenaline ($P < 0·001$.)

catecholamines after pharmacological inhibition of tyrosine hydroxylase depends upon nervous stimulus (Dahlstrom, Fuxe, & others, 1965; Andén, Corrodi, & others, 1966). Our results, therefore, suggest that the grouped mice experienced a higher general level of activation of central and peripheral catecholamine-containing neurons than their isolated littermates, even though they lived under conditions contrived to minimize fighting. Further, inasmuch as catecholamine stores were maintained at similar or at higher relative levels in the grouped than in the isolated controls, it is implicit that normal *de novo* catecholamine biosynthesis must have been proceeding more rapidly in the former.

The rate constant of exponential lowering of catecholamines after intravenous administration of 200–300 mg/kg of the L-isomer of α-methyltyrosine to rats has been used as a direct indication of the rate of catecholamine "turnover" (Brodie, Costa, & others, 1966; Costa & Neff, 1966). However, intraperitoneal doses of 150–200 mg/kg of the L and DL-isomers have been found lethal to a high percentage of rats injected in three laboratories (Hanson, 1965; Weissman & Koe, 1965; Moore, Wright & Bert, 1967). Further, two violations of the underlying assumption that the drug does not change the metabolic rate of the catecholamines (Brodie & others, 1966) are unavoidable. First, doses of 150–200 mg/kg of the DL-isomer cause a rapid fall in body temperature in rats (Udenfriend, 1966), just as 80 mg/kg in our laboratory invariably causes mice to become hypothermic. Second, impairment of catecholamine availability by inhibition of biosynthesis causes some sedation, impairs behavioural performance (Hanson, 1965; Moore & Rech, 1967), and generally decreases responsiveness to stimuli. It follows that the reduction of catecholamine stores after inhibition of tyrosine hydroxylase must slow with passing time simply because of the lowered body temperature and the decreased responsivity of the animal to its stimulus environment. It seems appropriate, therefore, to view rates of catecholamine "turnover", which are based upon their rate of disappearance following inhibition of tyrosine hydroxylase, as *minimal* rates.

In view of the fact that the grouped mice in our experiment became mildly sedated and reduced their activity earlier than the isolates, the differences between our grouped and individually-housed mice treated with α-methyl-

tyrosine were probably minimal estimates of the differences in the rate of catecholamine release from the brain and the adrenal medulla of their non-drug controls.

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Memorial Research Center and Hospital,
University of Tennessee,
Knoxville, Tennessee, U.S.A.
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BRUCE L. WELCH
ANNEMARIE S. WELCH

Andén, N.-E., Corrodi, H., Dahlstrom, A., Fuxe, K. & Hökfelt, T. (1966). *Life Sci.*, **5**, 561–568.

Brodie, B. B., Costa, E., Dlabac, A., Neff, N. H. & Smookler, H. H. (1966). *J. Pharmac. exp. Ther.*, **154**, 493–498.

Costa, E. & Neff, N. H. (1966). In *Biochemistry and Pharmacology of the Basal Ganglia.* Editors, Costa, E., Côte, L. & Yahr, M. D., pp. 141–156. New York: Raven.

Dahlstrom, A., Fuxe, K., Kernell, D. & Sedvall, G. (1965). *Life Sci.*, **4**, 1207–1212.

Glowinski, J. & Baldessarini, R. J. (1966). *Pharmac. Rev.*, **18**, 1210–1238.

Hanson, L. C. F. (1965). *Psychopharmacologia*, **8**, 100–110.

Moore, K. E. & Rech, R. H. (1967). *J. Pharmac. exp. Ther.*, **156**, 70–75.

Moore, K. E., Wright, P. F. & Bert, J. K. (1967). *Ibid.*, **155**, 506–515.

Spector, S., Sjoerdsma, A. & Udenfriend, S. (1965). *Ibid.*, **147**, 86–95.

Udenfriend, S. (1966). In *Mechanisms of Release of Biogenic Amines.* Editors, Euler, U. S. von, Rosell, S. & Uvnas, B., pp. 103–108. New York: Pergamon.

U.S. Dept. Health, Ed. and Welfare. (1965). Guide for Laboratory Animal Facilities and Care. Washington, D.C.: U.S. Govt. Print. Off.

Weissman, A. & Koe, B. K. (1965). *Life Sci.*, **4**, 1037–1048.

Welch, A. S. & Welch, B. L. (1968). *Biochem. Pharmac.*, in the press.

Welch, B. L. & Welch, A. S. (1964). In *Prog. in Brain Res.*, Vol. 8: *Biogenic Amines.* Editors, Himwich, H. E. & Himwich, W. A., pp. 201–206. Amsterdam: Elsevier.

Welch, B. L. (1965). In Proc. Symp. *Medical Aspects of Stress in the Military Climate* (April 22–24, 1964). pp. 39–99. Walter Reed Army Inst. Res. Washington, D.C.: U.S. Govt. Print. Off.

Welch, B. L. (1967). In *U.C.L.A. Forum in Medical Sciences.* Editors, Clemente, C. D. & Lindsley, D. B., Vol. 5, pp. 150–170. California: U.C.L.A. Press.