5-HT in chick retina

Treatment	5-HT (ng/g wet wt)
None	176 ± 12
Pargyline	223 ± 19*
Reserpine	109 ± 7**
L-Tryptophan	163 ± 22

Pargyline-HCl (100 mg/kg) was injected i. p. 2 h, reserpine (5 mg/kg) 5 h and L-tryptophan (200 mg/kg) 2 h before killing. Means ± SD are given. Each value was obtained from 4 experiments. Significance from the value obtained without treatment (Student's t-test); *$p < 0.02$; **$p < 0.001$.

centrifuged (3000 rpm, 5 min). The supernatant was decanted into a glass centrifuge tube, shaken vigorously with 5.0 ml of the borate buffer solution[19] (pH 10.0) saturated with NaCl, and centrifuged (3000 rpm, 5 min). A 3.0-ml aliquot of the supernatant was transferred to another centrifuge tube containing 1.5 ml of 0.05 M sodium phosphate buffer (pH 7.0) and 6.0 ml of n-heptane, shaken vigorously and centrifuged (3000 rpm, 5 min). 1 ml of the aqueous phase was transferred to a test tube containing 0.2 ml of 0.1 M ninhydrin solution, heated at 75 °C for 30 min in an oil bath, cooled with tap water, and left at room temperature for 15 min. The fluorescence of the solution was measured with excitation at 385 nm and emission at 490 nm[16].

Results and discussion. The result is shown in the table. An appreciable amount of 5-HT was found in the retina of the untreated chick, though the level is much lower than that in the brain. The 5-HT level was increased by the administration of pargyline, a potent inhibitor of monoamine oxidase. This should be compared with the result obtained with the pineal body, because of the similarity in embryological origins of the retina and pineal body[20]. In the pineal body, 5-HT localizes in pinealocyte cytoplasm and plays exclusively the role of the precursor of melatonin[21]. The level of 5-HT in pineal body is not increased by the administration of a monoamine oxidase inhibitor[22]. Thus, the increase in 5-HT found in chick retina after the administration of pargyline suggests the role of 5-HT in the retina other than the precursor of melatonin synthesis[23].

5-HT in the retina was decreased by the administration of reserpine. This result indicates the presence of the reserpine-sensitive storage mechanism[24] of 5-HT in chick retina. This result also supports the idea that 5-HT in the retina might act as a neurotransmitter.

5-HT in chick retina remained unchanged after the administration of tryptophan. This result indicates that the level of 5-HT in chick retina is not dependent on the level of tryptophan, probably due to the higher concentration of tryptophan in untreated chick retina[25] than that in the brain, which may cause the saturation of tryptophan hydroxylase with its substrate.

Chick retina seems to be an appropriate material for the study of the physiological role of 5-HT in the retina, since 5-HT level in chick retina seems to be much higher than that in rat or rabbit retina, and in addition, chick retina is relatively large in size and can be easily excised. The possible role of 5-HT in the retina is under investigation in our laboratory.

19 D. F. Bogdanski, A. Pletscher, B. B. Brodie and S. Udenfriend, J. Pharmac. exp. Ther. *117*, 82 (1956).
20 R. J. Wurtman, J. Axelrod and D. E. Kelly, in: The Pineal, p. 2. Academic Press, New York 1968.
21 W. B. Quay, in: Pineal Chemistry, p. 143. Charles C. Thomas, Springfield 1974.
22 S. H. Snyder, J. Axelrod and M. Zweig, J. Pharmac. exp. Ther. *158*, 206 (1967).
23 D. P. Cardinali and J. M. Rosner, J. Neurochem. *18*, 1769 (1971).
24 R. J. Baldessarini, in: Handbook of Psychopharmacology, Biochemistry of Biogenic Amines, vol. 3, p. 37. Ed. L. L. Iversen, S. D. Iversen and S. H. Snyder. Plenum Press, New York 1975.
25 O. Suzuki, E. Noguchi and K. Yagi, unpublished result.

Polypeptides of cerebral subcellular fractions of differentially-housed mice[1]

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Summary. The difference in protein of cerebral nerve-ending fractions caused by differential housing of male mice is of a quantitative nature and might reflect a change in the number of nerve-endings.

Recent studies have revealed that synaptosomal fractions of the brains of aggressive 'isolated' mice contain less protein than those of their 'aggregated' counterparts[2,3]. This environmentally-induced change has been correlated with changes in the 'binding' of putative central neurotransmitters (e.g., γ-aminobutyric acid, glycine, acetylcholine[4,5]) and psycho-active agents (e.g., d-amphetamine and Li^+[6,7] to subcellular structures of the brain. Such findings have led to the contention that individual housing of mice causes a decrease in the number, size, or development of cerebral nerve-endings[8]. To elucidate further this postulated morphological change, the protein contents and polypeptide profiles of cerebral nerve-ending fractions of differentially-housed mice have been examined.

Materials and methods. Male, Swiss albino mice were differentially-housed from weanling age (21–22 days) for 6–7 weeks[9]. After decapitation, their brains (rostral to the inferior colliculi; excluding cerebellum) were excised, weighed, and homogenized at 0 °C in 20 vol. isomotic (0.32 OsM) sucrose solution. Portions (5 ml) of homogenates were used to prepare 'synaptosomal' (P_2) fractions[10]. These were resuspended in 1.5 ml of 0.32 M sucrose solution, and 1.2-ml aliquots were centrifuged at 53,000 × g, 1 h, on discontinous gradients consisting of (from top to bottom): 3.0 ml 0.6 M, 3.0 ml 0.8 M, 3.0 ml 1.0 M, 3.0 ml 1.2 M and 4.5 ml 1.6 M sucrose solutions. Gradient fractions (0.5 ml) were collected and their protein contents were estimated[11]. For polyacrylamide gel electrophoresis, the apparatus described by Studier[12]

and the discontinuous procedure of Laemmli[13] were employed. Samples were boiled at 100 °C for 4 min[14,15] in the presence of 2-mercaptoethanol (0.3 M) and 2% sodium lauryl sulfate (w/v), then mixed with sample mixture[13], and then slabs were run at 10 mA until a Bromophenol blue marker reached about 1 cm below the origin of the 10% gel and thereafter at 20 mA until the marker reached 6–7.5 cm from the origin of the 10% gel. Gels were stained with Coomassie Brilliant Blue R250[16] and de-stained by diffusion at 56 °C in 10% acetic acid (v/v). Bovine serum albumin, human IgG3, and cytochrome-C (cyt-C) served as markers.

Results and discussion. Samples separated by gradient fractionation were frozen and thawed before protein analyses. Gradient profiles of total protein possessed several peaks; i.e., at the interfaces of 0.6:0.8 M, 0.8:1.0

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2 F. V. DeFeudis, Experientia, *28*, 1427 (1972).
3 F. V. DeFeudis, A. Ojeda, P. Madtes and P. A. DeFeudis, Naturwissenschaften, *62*, 585 (1975).
4 F. V. DeFeusis, Neuropharmacology, *11*, 879 (1972).
5 F. V. DeFeudis, P. Madtes and J. Gervas-Camacho, Exp. Neurol. *50*, 207 (1976).
6 F. V. DeFeudis, Brain Res. *43*, 686 (1972).
7 F. V. DeFeudis and J. H. Marks, Experientia, *29*, 1518 (1973).
8 F. V. DeFeudis, *in*: Current Developments in Psychopharmacology, vol. 1, p. 144. Ed. W. B. Essman and L. Valzelli. Spectrum Publ., New York 1975.
9 F. V. DeFeudis, Life Sci., *10* (Part II), 1187 (1971).
10 E. G. Gray and V. P. Whittaker, J. Anat. (Lond.) *96*, 79 (1962).
11 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).
12 F. W. Studier, J. mol. Biol. *79*, 237 (1973).
13 V. K. Laemmli, Nature (Lond.) *227*, 680 (1970).
14 I. B. Levitan, W. E. Mushynski and G. Ramirez, J. biol. Chem. *247*, 5376 (1972).
15 J. V. Maizel, in: Fundamental Techniques in Virology, p. 334. Ed. K. Habel and N. P. Salzman. Academic Press, New York 1969.
16 K. Weber and M. Osborn, J. biol. Chem. *244*, 4406 (1969).

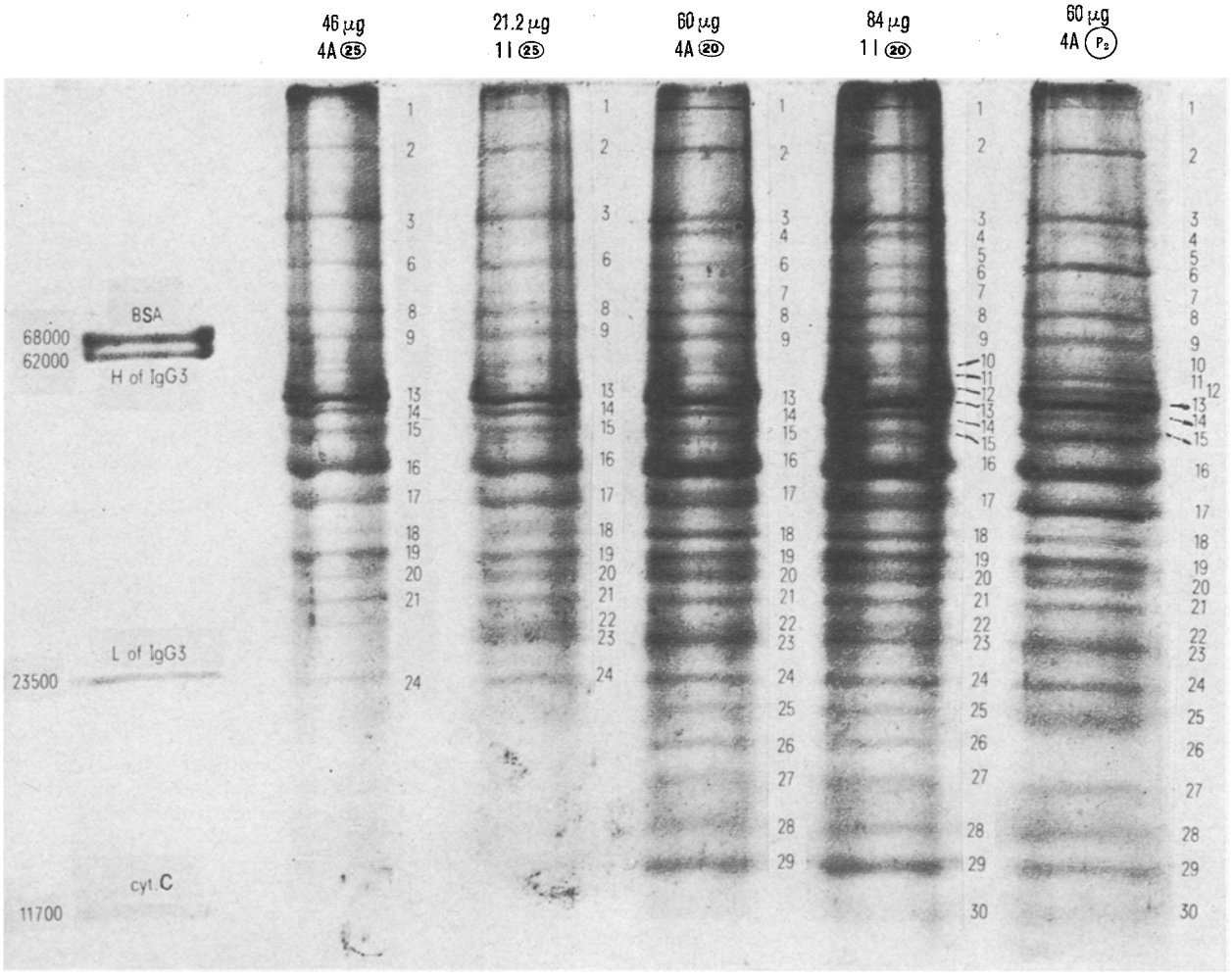

Fig. 1. Representative results of polyacrylamide slab gel electrophoresis of polypeptides of subcellular fractions of the brains of differentially-housed mice. Gradient fractions Nos. 20 and 25 of an 'isolated' (1I) and an 'aggregated' (4A) mouse were analyzed. The P_2 fraction of an 'aggregated' mouse (4A) is included for comparison. The total amounts of protein (in µg) in 40 µl of these fractions, and the markers, BSA, light (L) and heavy (H) chains of IgG3, and cytochrome C (cyt C), are indicated; bands are numbered. No qualitative differences existed between bands from 'isolated' and 'aggregated' mice.

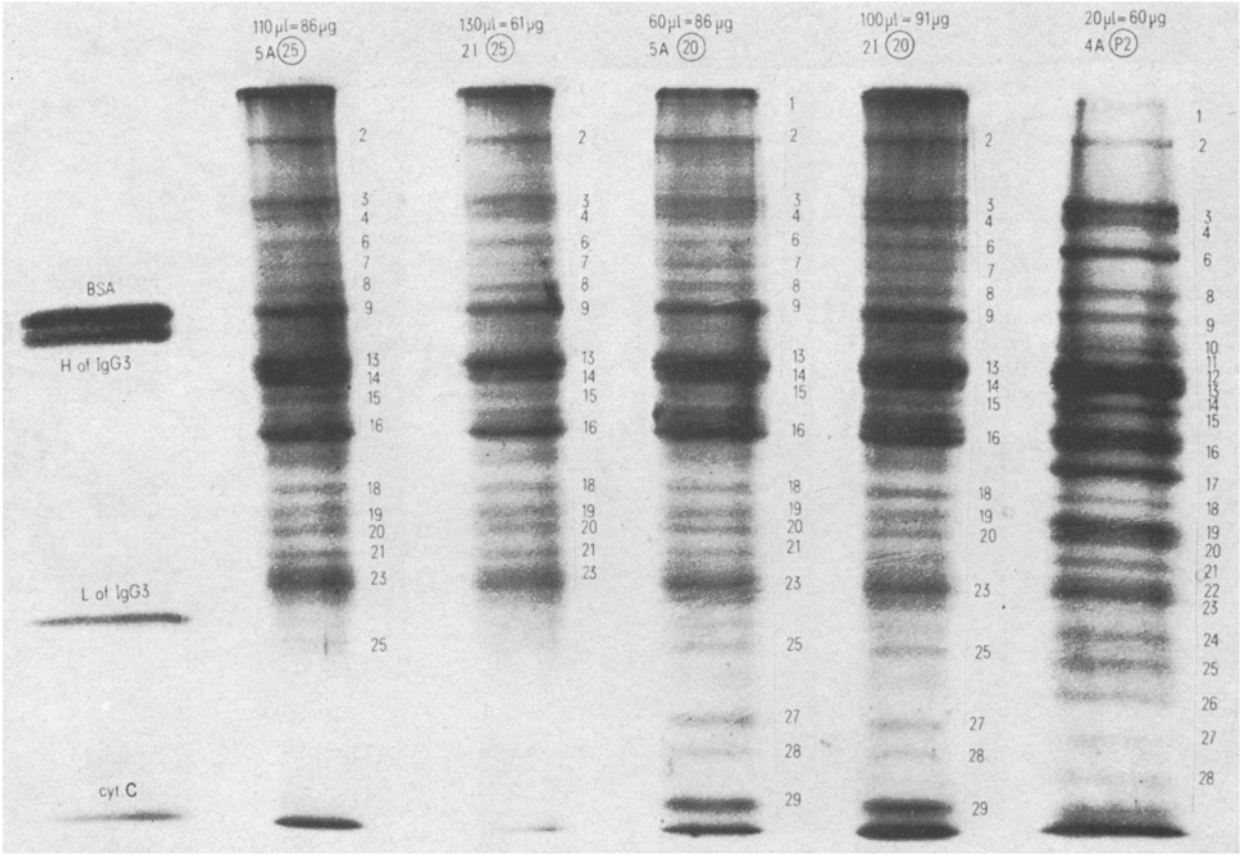

Fig. 2. Representative results of polyacrylamide slab gel electrophoresis of polypeptides of subcellular 'juctional complexes' prepared from the brains of 'isolated' (2I) and 'aggregated' (5A) mice. 20 µl of a P_2 fraction from an 'aggregated' mouse (4A), which was not treated with Triton X-100, is presented for comparison. The volumes (in µl) and the total protein contents (in µg) of samples, and the markers, BSA, light (L) and heavy (H) chains of IgG3, and cytochrome C (cyt C), are indicated. The amounts of markers used were: cyt C (2.4 µg); IgG3 (3 µg); BSA (1.2 µg). Bands are numbered, and some estimates of molecular weights are provided. Note that no qualitative differences existed between samples prepared from the brains of 'isolated' and 'aggregated' mice.

M, and 1.0:1.2 M sucrose and just above the 1.2:1.6 M interface. The only significant difference occurred in the region of the 1.2:1.6 M interface at which a greater amount of protein was present in particles from the brains of 'aggregated' mice than in those from 'isolated' mice (combined fractions Nos. 24–26 contained 1.09 ± 0.16 mg protein for 'aggregated' mice vs 0.63 ± 0.08 mg protein for 'isolated' mice; means ± SEM, n = 5 in each case; $p < 0.02$). Fractions Nos. 20 and 25, representing protein peaks localized at the 1.0:1.2 M interface and above the 1.2:1.6 M interface, were analyzed by gel electrophoresis. For results shown in figure 1, 40 µl of samples were mixed with 20 µl of a solution which provided a final concentration of 2.25% SDS and 0.284 M 2-mercaptoethanol, and the samples were run on a single slab. A good correlation existed between total Lowry protein and the general intensity of polypeptide staining in the slabs. Although fraction No. 25 from 'aggregated' mice contained more protein than that of 'isolated' mice, no qualitative differences existed in any of the 30 bands that were resolved. Also, though fractions Nos. 20 and 25 from 'isolated' and 'aggregated' mice were of similar polypeptide composition, not all bands present in fraction No. 20 were present in fraction No. 25. Figure 2 provides results obtained with samples prepared for the non-soluble Triton X-100 fraction ('junctional complex[14,17]. Much less protein was present in many of the bands after extraction with Triton X-100, and some bands between the light chain of IgG3 (L) and cyt-C markers were present in fraction 20 but absent from fraction 25.

Although quantitative differences in total protein were evident, no qualitative differences in protein subfractions were observed between cerebral samples prepared from 'isolated' and 'aggregated' mice. Since fraction No. 25, localized at a region of the gradients known to contain nerve-endings[10], differed quantitatively, but not qualitatively, between 'isolated' and 'aggregated' mice, this difference appears to be due to a change in the *number* of nerve-endings rather than to a modification of their molecular composition. This finding might be due to a decrease in the synthesis and/or axoplasmic flow of protein in the brains of 'isolated' mice, which have been shown previously to have a decreased metabolic rate[8].

17 C. W. Cotman, W. Levy, G. Banker and D. Taylor, Biochim. biophys. Acta, *249*, 406 (1971).