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REFERENCES

1. B. B. BRODIE, S. SPECTOR and P. A. SHORE, *Pharmacol. Rev.* **11**, 548 (1959).
2. R. SCHAYER, *Physiol. Rev.* **39**, 116 (1959).
3. W. SCHULER, *Experientia (Basel)* **8**, 230 (1952).
4. P. A. SHORE and V. H. COHN, *Biochem. Pharmac.* **5**, 91 (1960).
5. D. D. BROWN, R. TOMCHICK and J. AXELROD, *J. biol. Chem.* **234**, 2948 (1959).
6. A. GUSTAFSSON and G. P. FORSHALL, *Acta. chem. scand.* **17**, 541 (1963).
7. T. WHITE, *Fed. Proc.* **23**, 1103 (1964).
8. K. J. NETTER, V. H. COHN and P. A. SHORE, *Amer. J. Physiol.* **201**, 224 (1961).
9. P. A. SHORE, A. BURKHALTER and V. H. COHN, *J. Pharmacol. exp. Ther.* **127**, 182 (1959).
10. F. HAWKING and W. L. M. PERRY, *Brit. J. Pharmacol.* **3**, 320 (1948).
11. G. H. HITCHINGS, *Clin. Pharmacol. Ther.* **1**, 570 (1960).
12. W. C. WERKHEISER, *Cancer Res.* **23**, 1277 (1963).
13. J. HILL, in *Experimental Chemotherapy*, R. J. SCHNITZER and F. HAWKING, Eds., vol. 1, p. 513. Academic Press, New York (1963).

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Monoamines in isolated nerve ending particles*

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STUDIES on the localization and distribution of catecholamines (CA) in the adrenergic nervous system and terminals have been greatly facilitated by the specific histochemical fluorescence method developed by Hillarp and co-workers (for references, see Dahlström and Fuxe[1]). The monoaminergic nerve terminals have abundant, brightly fluorescent enlargements (varicosities).[2–4] In view especially of the studies relating endogenous CA and 5-HT to the fraction containing the isolated nerve-ending particles (NEPS) or synaptosomes,[5–7] it was of importance to determine whether the amine-containing varicosities observed in the fluorescence microscope are related to the synaptosomes.

MATERIALS AND METHODS

The brain stem of Sprague–Dawley rats was homogenized in cold 0·32 M sucrose and the P_2 residue of Gray and Whittaker[8] was obtained by differential centrifugation. This pellet was resuspended in isotonic sucrose and subfractionated by density gradient centrifugation.[6] The three well-defined subfractions of P_2 (A, B and C) were isolated and smears were prepared on micoscope slides. These slides were treated with formaldehyde gas at 80° for 1 hr. With this treatment CA and serotonin (5-HT) are converted to intensely fluorescent compounds, the former being green or yellow-green and the latter yellow. The specificity and chemical basis of this reaction have been discussed recently.[1]

RESULTS

The three subfractions (P_2–A, P_2–B and P_2–C) were examined in the fluorescence microscope. From normal rats, the P_2–B smear was covered with a myriad of intensely green to yellow-green (and possibly a few yellow) fluorescent spots of about the same size as varicosities (Fig. 1). The P_2–A and P_2–C smears had relatively much fewer numbers of fluorescent spots. The P_2–B slides showed no

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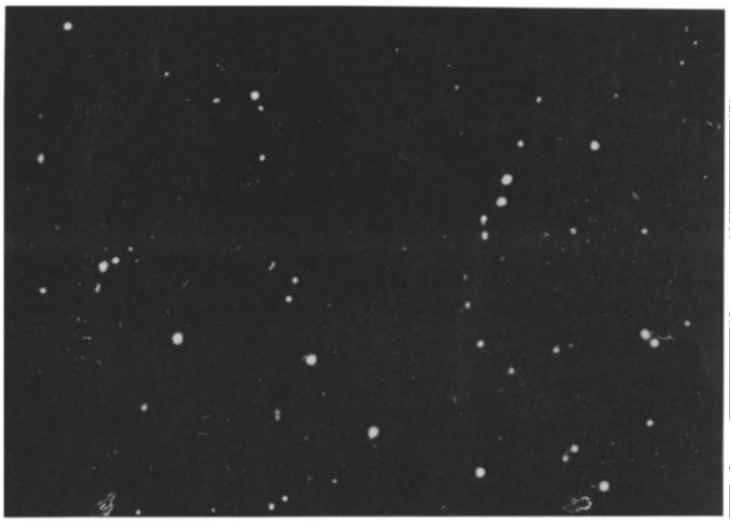

FIG. 1. Smear from the P_2–B fraction, obtained by differential centrifugation of a homogenized brain stem of a normal rat. Formaldehyde gas treatment for the visualization of monoamines. Fluorescence microphoto. The fluorescent spots have about the same size as the varicosities of nerve terminals, seen in tissue sections. (Magnification × 550)

facing page 1688—B.P.

fluorescence in the absence of formaldehyde treatment. The brain stems from rats pretreated with reserpine (5 mg/kg i.p. 12 hr) were homogenized and fractionated concurrently with those from normal animals. These smears were free of fluorescent spots.

DISCUSSION

Because of the small dimensions of the isolated particles, it was not possible to determine whether the fluorescent spots were all green to yellow-green (NA or dopamine) or whether a few had a yellow fluorescence (5-HT). Nevertheless, the size of the fluorescent spots and the specificity of the fluorescence reaction leave little doubt that they contain monoamines and indicate that they are identical with the varicosities of the nerve terminals. It is thus clear that amine-containing fluorescent structures, presumably the varicosities, follow the separation of the NEPS fraction by density gradient centrifugation, since the P_2–A fraction consists mainly of myelin fragments, P_2–B of NEPS, and P_2–C of mitochondria.[8] 5-HT and NE determinations of the subfractions of P_2[6, 7] are in general accordance with the present results.

Electron microscopic observations have shown that the synaptosomes[9] and the synaptic structures of autonomic nerves seen in tissue sections[10, 11] have the same structural characteristics. There is strong evidence that these latter structures are identical with the varicosities of the nerve terminals observed in the fluorescence microscope.[3, 4] The fluorescent spots seen in the present study are thus in all probability identical with the varicosities of the nerve terminals seen in the fluorescence microscope, with amine-containing synaptosomes obtained by density gradient centrifugation, and with the presynaptic widenings of the nerves seen in electron microscopic sections. All these structures thus in all probability represent the synaptic structures of the monoaminergic nerve terminals.

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REFERENCES

1. A. DAHLSTRÖM and K. FUXE, *Acta physiol. scand.* **62**, *Suppl.* 232 (1964).
2. A. CARLSSON, B. FALCK and N.-Å. HILLARP, *Acta physiol. scand.* **56**, *Suppl.* 196 (1962).
3. K.-A. NORBERG and B. HAMBERBER, *Acta physiol. scand.* **63**, *Suppl.* 238 (1964).
4. K. FUXE, *Z. Zellforsch. mikrosk. Anat.* **65**, 573 (1965).
5. T. CHRUŚCIEL, *Adrenergic Mechanisms.* Ciba Foundation Symposium, p. 539. Churchill, London (1960).
6. I. A. MICHAELSON and V. P. WHITTAKER, *Biochem. Pharmac.* **12**, 203 (1963).
7. R. LEVI and E. W. MAYNERT, *Biochem. Pharmac.* **13**, 615 (1964).
8. E. G. GRAY and V. P. WHITTAKER, *J. Anat.* **96**, 79 (1962).
9. V. P. WHITTAKER, I. A. MICHAELSON and R. J. A. KIRKLAND, *Biochem. J.* **90**, 293 (1964).
10. K. C. RICHARDSON, *J. Anat.* **96**, 427 (1962).
11. L.-G. ELFVIN, *J. Ultrastruct. Res.* **8**, 441 (1963).

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Release of cardiac noradrenaline by decaborane in the heart-lung preparation of guinea pig

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MERRIT *et al.*[1] reported the ability of decaborane ($B_{10}H_{14}$) to deplete noradrenaline from the rat brain. Recently Euler and Lishajko[2] have found that decaborane (10^{-5} M) releases catecholamines from isolated adrenergic nerve granules. A striking decrease of the noradrenaline content in the rabbit organs 24–48 hr after the administration of 4 mg/kg of this drug has been shown by the same authors. The depletion of the heart catecholamines was found to be 80–90 per cent. The action of decaborane was previously studied *in vivo* by i.p. or s.c. administration. With this procedure it is difficult to follow the first phase of the depletion process. In an attempt to gain further information