There is also a possibility that radioactive constituents from BHC are taken up by the white matter after structural changes of the BHC molecule. It is known that BHC is transformed in the liver into various chlorophenol derivatives by the splitting off of chlorine and hydrogen and uptake of oxygen. These derivatives, however, could not be found in the CNS[8] and are probably rapidly excreted.

The manner in which BHC enters into the white matter has not yet been studied systematically. Its distribution seems to occur via the blood and not via the cerebrospinal fluid, as is the case with acetazolamide.[12] Nor did we find any typical accumulation in especially well-vascularized areas as Roth and Barlow[13] did with extremely lipoid-soluble substances such as thiopental.

It is not at present possible to relate the anticonvulsive action of BHC to the distribution findings. For this purpose metabolic studies should be made on white matter specifically rather than with homogenates of whole brain.

Departments of Pharmacology,
Freie Universität Berlin, West Germany;
Royal Veterinary College,
Stockholm, Sweden.

WOLFGANG KORANSKY
SVEN ULLBERG*

* Present address: Department of Zoology, University of California, Los Angeles, Calif.

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Toxicities and myotonic activities of certain polychlorobenzoic acids

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AS PART of a toxicological investigation of the herbicide 2, 3, 6-trichlorobenzoic acid (TBA)[1] and its derivatives, a series of isomeric di-, tri- and tetrachlorobenzoic acids has been examined. They were administered subcutaneously, as 10% aqueous sodium salts, to groups of albino mice, for comparative toxicity assessment. The results are of some interest from the structure-activity aspect.

In addition to non-specific toxic effects including weakness, dyspnoea, prostration, tremors, and terminal coma, certain of the compounds also caused myotonic muscular stiffness and transient spasm, especially marked in the back legs, and on stimulation. This myotonic action is quite characteristic, is produced by a number of hormone weedkillers at high dosages, and has been fully described.[2] The results obtained are summarized in Table 1.

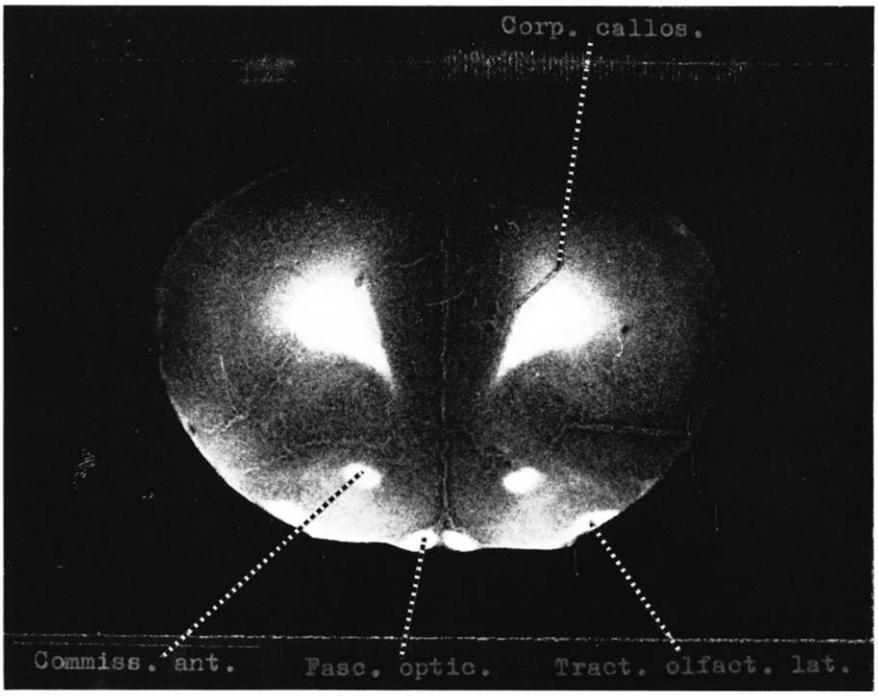

FIG. 1. Autoradiogram of frontal section through the head of a rat 24 h after intraperitoneal injection of ^{14}C-hexachlorocyclohexane. The section levels in Figures 1 through 5 are from forebrain to brain stem. Section thickness 20μ. White areas correspond to high uptake of radioactive substance.

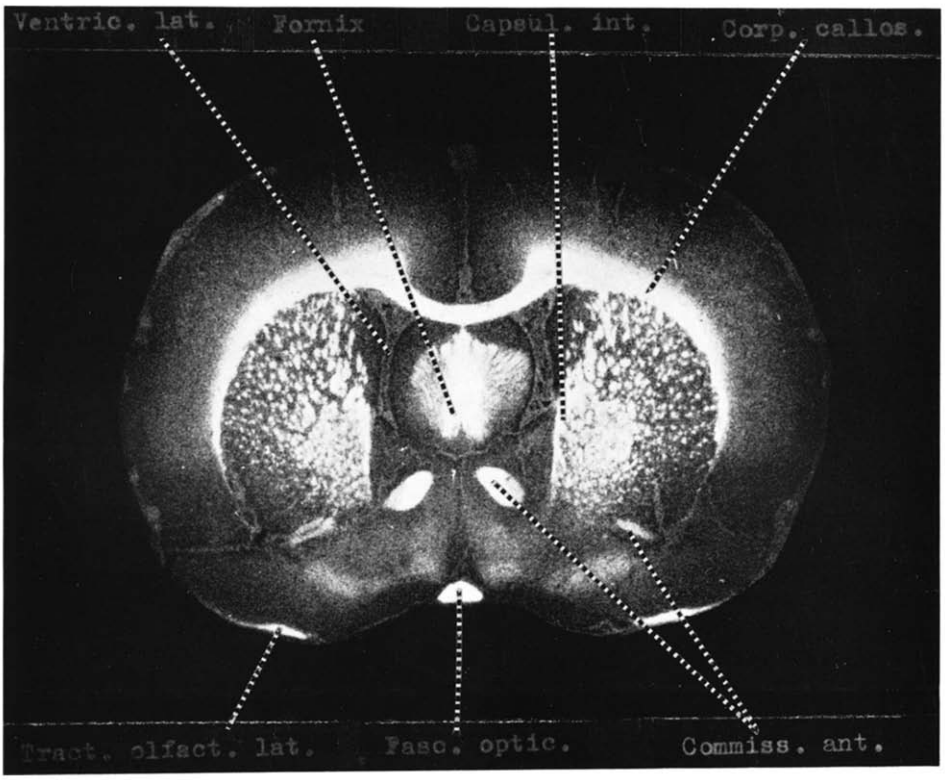

FIG. 2. Frontal section of rat brain; see legend to Fig. 1.

facing page 1538

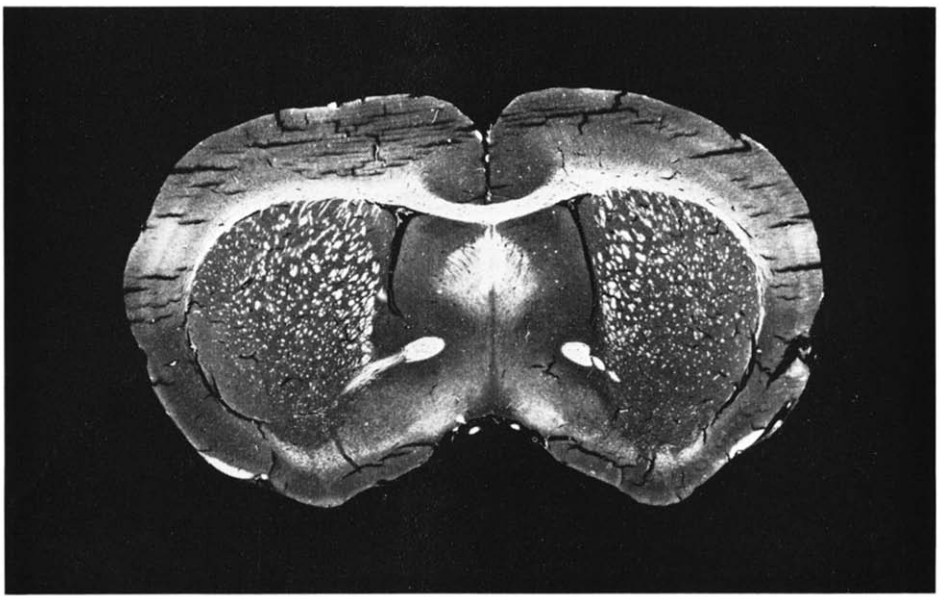

FIG. 3. Photograph (negative) of a 3-μ-thick frontal section through the brain of a rat. The white matter is stained according to Heidenhain.[11] The areas stained deep blue show up light against the dark background.

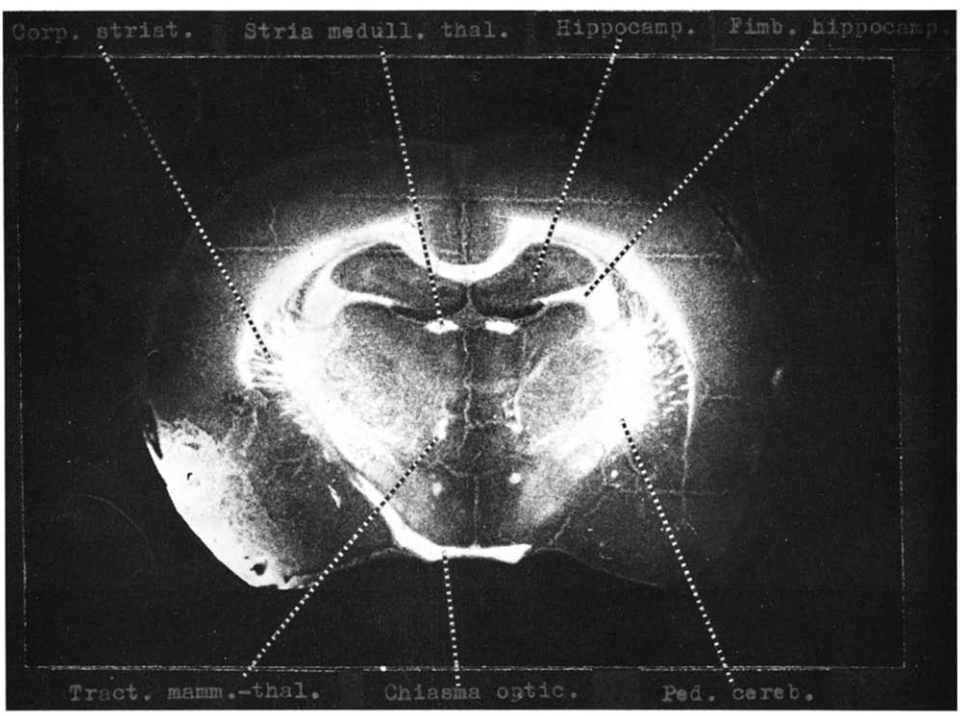

FIG. 4. Frontal section of rat brain; see legend to Fig. 1.

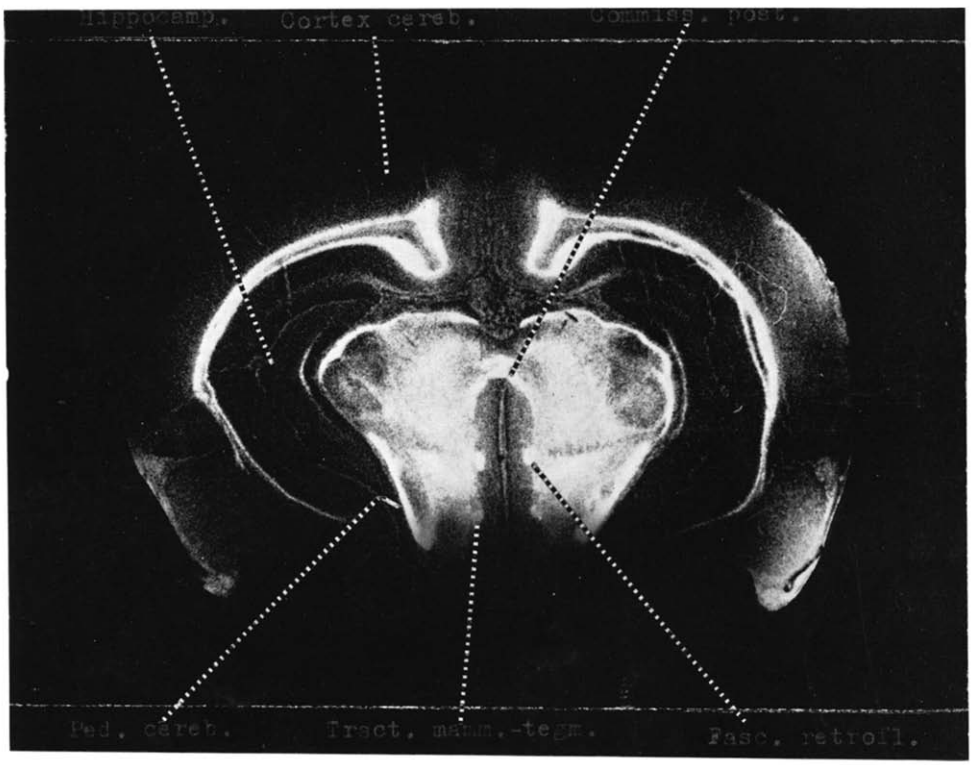

FIG. 5. Frontal section of rat brain; see legend to Fig. 1.

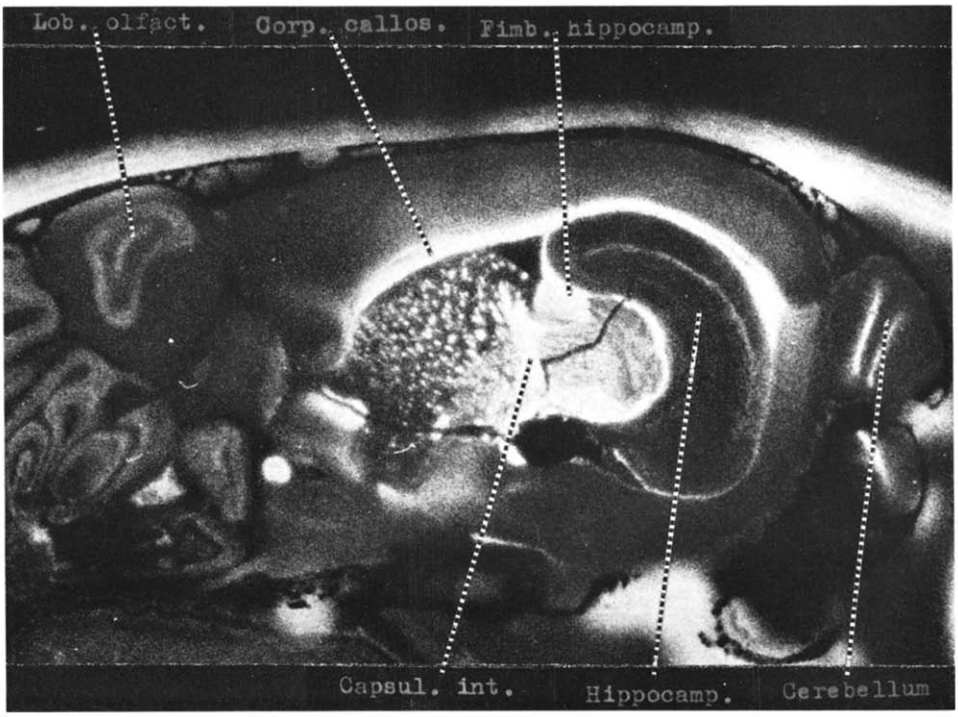

FIG. 6. Autoradiogram of a 20-μ-thick sagittal section through a rat brain 24 h after intraperitoneal injection of ^{14}C-hexachlorocylohexane. Note high uptake of radioactive substance in white matter. This section is from a more lateral area than Fig. 7.

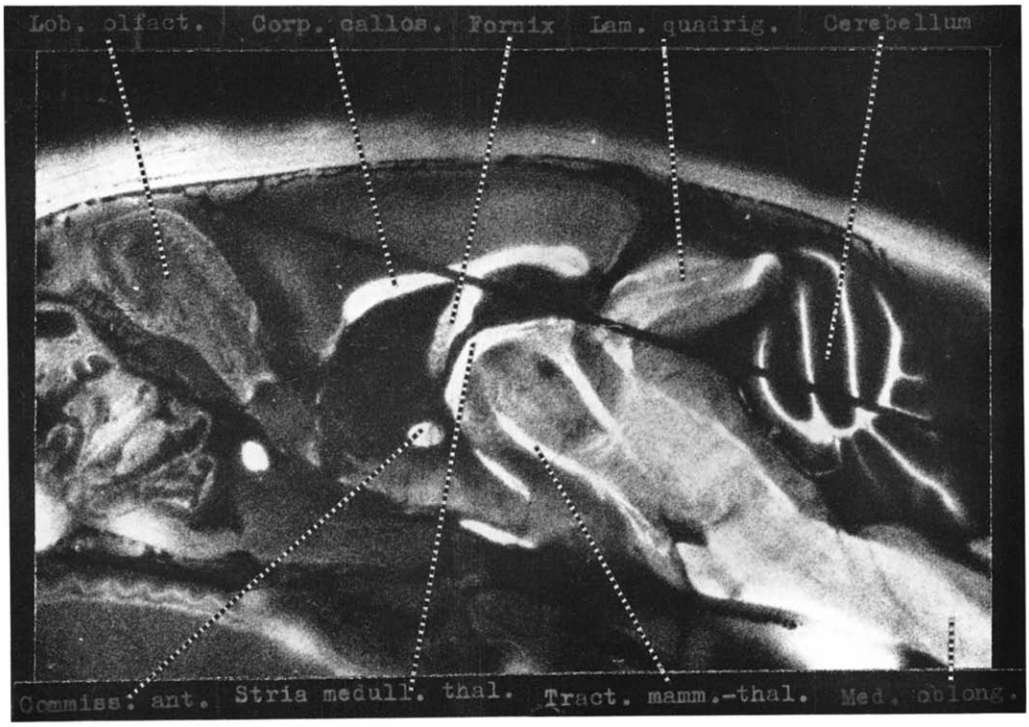

FIG. 7. Sagittal section of rat brain; see legend to Fig. 6. This section is from a more medial area than that in Fig. 6.

In the dichlorobenzoic acids myotonia occurred with the four acids having one or two chlorine atoms *ortho* to the carboxyl group, but not with the two having no *ortho* substituent. Also, these last two were of rather higher toxicity than the others.

In the tri- and probably the tetrachlorobenzoic acids, myotonia did not occur when all the chlorine atoms were adjacent, but did when any two were separated by hydrogen. Two of the trichlorobenzoic

TABLE 1. MOUSE TOXICITY AND MYOTONIC ACTION OF POLYCHLOROBENZOIC ACIDS

Acid	M.P. °C*	Mouse s/c LD50 mg/kg	Myotonia
2,6-dichloro-	140–6	1500	+ve
2,5-dichloro-	154	1200	+ve
2,4-dichloro-	164	1200	+ve
2,3-dichloro-	164–7	900	+ve
3,5-dichloro-	183–8	250	−ve
3,4-dichloro-	204–8	400	−ve
2,3,6-trichloro-	124–8	1500	+ve
2,3,5-trichloro-	163	300	+ve
2,4,6-trichloro-	163–4	1200	+ve
2,4,5-trichloro-	163–8	300	+ve
2,3,4-trichloro-	187–8	300	−ve
3,4,5-trichloro-	203–10	250	−ve
2,3,4,6-tetrachloro-	131–2	—	+ve together
2,3,5,6-tetrachloro-	178–9	—	+ve together
2,3,4,5-tetrachloro-	186–94	—	−ve

* Range of available separate values

acids were rather less toxic than the other four, and these were the ones in which both *ortho* positions carried chlorine atoms.

This effect of *ortho* substitution on the toxicity may be compared with the statement[3] that *ortho* substitution of benzoic acids always reduces conjugation *in vivo*.

As an empirical observation in all three series, the isomers of highest melting point were those which did not produce myotonia.

Medical Department,
Chesterford Park Research Station,
Nr. Saffron Walden,
Essex, England.

E. F. EDSON
D. M. SANDERSON

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Interference by physostigmine and serotonin in the colorimetric determination of acetylcholine

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IN THE course of a study dealing with acetylcholine metabolism in the spinal cord, physostigmine (eserine) was orginally used routinely as inhibitor of acetylcholinesterase (AChE; 3.1.1.7.).* When

* *Report of the Commission on Enzymes*, Pergamon Press, London (1961).