brain slices, beginning at the medial surface of a hemisphere. Thus the differences are not explainable in terms of regional variations in 5-HT concentration. Therefore reserpine added to slices *in vitro* seems to cause an initial increase in spontaneous 5-HT release. After incubation, slices from flasks containing only JB-516 measured 0·443 (0·372-0·490, 4 values) μg 5-HT creatine sulfate/g wet slices. Slices from flasks containing reserpine in addition measured 0·288 (0·213–0·360, 4 values). Unincubated slices rinsed in the incubation medium for 5 min and then extracted contained 0·624 (0·472–0·880, 4 values). In several experiments it was not possible to account for the 5-HT activity of the incubate by ascribing it to loss of original 5-HT from the slice. That is to say, the total (incubate plus slice) 5-HT was sometimes greater than the average 5-HT content of the unincubated slice. Thus the question of synthesis and/or release from bound stores as an explanation of the 5-HT activity in the incubate has not been settled at this point. Oxygenation of the flasks seemed to lower the 5-HT activity of the incubates, higher values being obtained when incubation was performed in open flasks. Fluorescence spectra obtained as described above confirmed the presence of 5-HT in the incubate, but the amounts were too small to permit accurate measurement.

Thus whole-brain slices release 5-HT spontaneously *in vitro*, and this release can be initially accelerated by adding reserpine to the medium. At this point our studies do not settle the issue of the origin of the 5-HT released, whether it be from bound stores only or also from synthesis. A recent report by Andén *et al.* suggests that 5-HT synthesis may occur in isolated frog and rat spinal cord during electrical stimulation.[11]

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Epoxidation of aldrin, isodrin, and heptachlor by rat liver microsomes

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THE CONVERSION of aldrin, isodrin, and heptachlor to their respective epoxides* in soil and in plant and animal tissues is well known, the first report being that of Radomski and Davidow.[1] The epoxides

* Aldrin: 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-endoexo-dimethanonaphthalene.
Dieldrin: 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a5,6,7,8,,8a-octa-hydro-1,4,5,8-endoexo-dimethanonaphthalene.
Endrin: 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octa-hydro-1,4,5,8-endo,endo-dinonaphthalene.
Heptachlor: 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene.
Heptachlor epoxide: 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene.
Isodrin: 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-endo-endo-dimethanonaphthalene.

are usually more toxic to the host tissue and until recently[2,3] have been considered quite resistant to further chemical change.

Liver microsomes have been shown to perform a variety of biological oxidations.[4] It seems likely that enzymes involved in epoxidation would also be found in the microsomal fraction. The results of experiments designed to test this idea are presented here.

Freshly excised livers of male or female white albino rats were homogenized for 30 sec in a Waring Blender in 9 volumes of cold 0·25 M sucrose. The subsequent isolation of the microsomes followed the procedures described by Booth and Boyland.[5] A typical reaction mixture consisted of 2 ml of Tris buffer solution (0·13 M at pH 8·2), 0·5 ml of microsomal suspension equivalent to 750 mg of rat liver, and 1 ml of NADPH-generating solution. This solution contained NADP (1·8 μmoles), glucose-6-phosphate (18 μmoles), glucose-6-phosphate dehydrogenase (0·4 Kornberg units), and nicotinamide (18 μmoles). Water was added to make a total volume of 6 ml.

The incubation mixtures containing from 10 to 40 μg of the test compound were incubated, with shaking, at 37° for 15 min. The atmosphere above the incubation mixture was air. The test compounds were added in Methyl Cellosolve (ethyleneglycol monomethylether) solution, 0·02 ml per flask. The reactions were stopped by an extraction with 10 ml of a 3 : 2 mixture of *n*-hexane:isopropyl alcohol. Two additional extractions with this solvent followed.

The combined extracts were concentrated to a small volume and analyzed directly without further purification by means of the microcoulometric gas chromatographic method. Chromatographic conditions were: block temperature 260°, column temperature 240°, and gas flow (nitrogen) 120 ml min. A 6 foot by 1/4 in. aluminium column packed with 5% silicone on Chromasorb W was used. Under these conditions aldrin, dieldrin, heptachlor, heptachlor epoxide and isodrin gave single peaks; endrin gave two peaks. At least 85 per cent of the substrate compounds were recovered in the analyses, either as epoxides or as unchanged parent compounds. There was no evidence that a product other than an epoxide was produced, although a small amount of polar products could have been overlooked by the methods used. Incubation up to 2 hr did not result in reduced recoveries.

Early experiments showed that rat liver microsomes rapidly converted aldrin to its epoxide, dieldrin. Boiling the microsomes or eliminating the NADPH-generating system prevented the conversions. The rate of conversion of aldrin to dieldrin, with microsomes equivalent to 750 mg fresh liver, was linear during the first 20 min of incubation, hence 15 min was chosen as a standard for further comparison. The conversions could be inhibited by the pyrethrin synergist, sesamex, when this agent was added to the flasks at levels of 0·0005 M.

TABLE 1. THE CONVERSION OF ALDRIN, ISODRIN, AND HEPTACHLOR TO THEIR EPOXIDES BY LIVER MICROSOMES OF MALE AND FEMALE RATS

Substrate	Substrate level (μg)	Microsomal conversion to epoxides			
		Males		Females	
		Average (%)	Range*	Average %	Range
Aldrin	10	81·3 ± 7·4	73–87 (3)		
Aldrin	20	68·9 ± 10·3	58–86 (7)	6·3 ± 4·1	4–11 (3)
Aldrin	40	43·0 ± 17·0	31–55 (2)	3·0 ± 1·4	2–4 (2)
Heptachlor	10	36·7 ± 13·5	23–50 (3)		
Heptachlor	20	29·3 ± 13·5	11–44 (6)	3·6 ± 0·6	3–4 (3)
Heptachlor	40	17·5 ± 12·1	9–26 (2)	3·5 ± 0·7	3–4 (2)
Isodrin	10	52·0 ± 12·7	43–61 (2)		
Isodrin	20	44·6 ± 13·2	31–63 (7)	5·0 ± 1·0	4–6 (3)
Isodrin	40	27·3 ± 4·7	22–31 (3)	2·5 ± 0·7	2–3 (2)

* Numbers in parentheses refer to number of animals tested.

Most of the tests utilized microsomes prepared from livers of male rats. However, when a female was used with aldrin as substrate, a surprisingly low conversion to dieldrin occurred. This experiment has been repeated several times with the three olefin compounds. The results (Table 1) show that females are much less active in the conversion.

The sex difference seen in the epoxidation of these compounds helps explain the results of Gaines,[6] who studied their acute toxicity to male and female rats. This author found that females could tolerate appreciably larger doses of aldrin and heptachlor. The present results suggest that females are less susceptible to aldrin and heptachlor because of their reduced production of the more toxic epoxides. The relationship may not hold with isodrin, where Gaines found female rats to be more susceptible than males, because of the extreme toxicity of its metabolite, endrin.

The *in vitro* results presented here may indicate that female rats metabolize aldrin, isodrin, and heptachlor less rapidly than do males. It is of interest that Ludwig *et al.*[3] used only male rats in demonstrating the complete metabolism and excretion of aldrin-^{14}C.

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