of PG receptors in rat liver plasma membranes[14] would favor a direct effect on the liver. Several biological actions of PG are thought to be mediated by cyclic AMP. It is, therefore, noteworthy that cyclic AMP[15], as well as substances which increase the concentration of cyclic AMP such as theophylline[15–17] and vasopressin[18], increase bile flow in the dog. In the rat, however, neither cyclic AMP[19,20] nor vasopressin[19,20] or theophylline (unpublished observation) are choleretic. It appears unlikely, therefore, that PG A_1 affects bile flow via cyclic AMP. In the kidney, PG A_1 markedly decreases Na^+-K^+-dependent ATPase activity which is thought to be responsible for the PG induced natriuresis[3]. A similar mechanism could play a role in the PG-induced increase in bile flow, since other ATPase inhibitors, such as ouabain[21,22] and certain diuretics[23,24], exhibit a similar effect on bile salt independent bile flow in the rat[21,23] and the dog[22,24]. Further studies are needed to elucidate the mechanism and site of action of PG A_1, and to define the eventual physiological significance of PG A_1 for bile formation.

Summary. It could be demonstrated that intraportal infusion of prostaglandin A_1 (1 μg/min/100 g body wt.) in Wistar rats significantly increases bile flow. An analysis of the relationship between bile salt excretion and bile flow revealed that this choleresis is due to an increase in the bile salt independent fraction of bile.

B. Lauterburg, G. Paumgartner and R. Preisig[25]

Department of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH-3010 Bern (Switzerland),
6 June 1975.

[14] M. Smigel and S. Fleischer, Biochim. biophys. Acta *332*, 358 (1974).
[15] T. Q. Morris, Gastroenterology *62*, 187 (1972).
[16] S. Erlinger and M. Dumont, Biomedicine *19*, 27 (1973).
[17] J. L. Barnhart and B. Combes, Am. J. Physiol. *227*, 194 (1974).
[18] R. Preisig, H. Strebel, G. Egger and V. Macarol, Experientia *28*, 1436 (1972).
[19] E. Hosmher and G. C. Cotzias, Nature, Lond. *208*, 687 (1965).
[20] R. A. Levine, Am. J. Physiol. *208*, 317 (1965).
[21] J. Graf, P. Korn, A. Lindner and M. Peterlik, Wien. klin. Wschr. *85*, 70 (1973).
[22] K. Kutz, G. Egger and R. Preisig, Eur. J. clin. Invest. *3*, 246 (1973).
[23] H. Shaw, I. Caple and T. Heath, J. Pharmac. exp. Ther. *182*, 27 (1972).
[24] K. Kutz, G. Paumgartner and G. Egger, Eur. J. clin. Invest. *2*, 292 (1972).
[25] Supported by the Swiss National Foundation for Scientific Research.

The Effect of Pesticides on Hydroxylation of Testosterone in Hepatic Microsomes of Rabbits

Malathion and parathion known as the organic phosphorothionate insecticides are converted in vitro and in vivo into malaoxon and paraoxon, respectively, which are potent inhibitors of cholinesterase[1]. The in vitro additions of these insecticides inhibit the metabolism of ethylmorphine[2] and testosterone[3] in rat hepatic microsomes. The single administration of these inseticidces prolonged hexobarbital sleeping time in mice[4]. The treatment of rats with chlorinated insecticides such as dichlorodiphenyltrichloroethane (DDT) for 10 days stimulates the enzyme activities involved in the metabolism of many drugs and steroids[3].

The purpose of this paper was to examine the effects of the long-term administration of ethylmercuric phosphate (EMP), 0,0-dimethyl-0-(3-methyl-4-nitrophenyl) thiophosphate (MEP, a derivative of parathion) and benzene hexachloride (BHC), which are commonly used as pesticides in Japan, on the hydroxylation of testosterone in microsomes of rabbit livers.

Methods. Male adult albino rabbits weighing about 2 kg were used. EMP, organomercury pesticide, dissolved in physiological saline, and MEP and BHC, halogenated hydrocarbon insecticide, both are non-water-soluble agents, dissolved in corn oil, were given orally every other day for 5 months. The dose per kg body weight was equivalent to the amount of the pesticide contained in 1 ml of the spray commonly used by farmers in Japan. The dosage was 0.05 mg/kg of body weight (1/800 of the LD_{50} in rats) for EMP, 0.5 mg/kg (1/1,800) for MEP or 1.5 mg/kg (1/400) for BHC. Half of the control animals were given saline and the other half were given corn oil. The animals were sacrificed by anesthetizing with thiopental sodium. The liver was removed immediately, perfused with ice-cold saline and homogenized with 4 volumes of cold, isotonic (1.15%) KCl in a Potter-Elvehjem type homogenizer. The homogenate was centrifuged to remove the nuclei and the mitochondria at $18{,}000 \times g$ for 10 min at 4 °C and the microsomal pelltes were obtained by centrifugation at $105{,}000 \times g$ for 60 min. The microsomes were suspended in ice-cold 0.1 *M* Krebs-Ringer phosphate buffer (pH 7.4) at a concentration of 5 mg protein per ml. 500 μg of NADPH dissolved in 0.5 ml of 0.1 *M* Krebs-Ringer phosphate buffer and 86 nmoles of testosterone containing 1 μCi of 4-^{14}C-testosterone (specific activity; 57.5 mCi/*mM*, the New England Nuclear Corp., Boston, Mass, USA) were added to the microsomal fraction containing 10 mg of protein. The final volume of the incubation mixture was brought to 5 ml with 0.1 *M* Krebs-Ringer phosphate buffer and the mixture was incubated for 30 min at 37 °C under the bubbling of 95% oxygen and 5% carbon dioxide. After incubation, testosterone and its metabolites were extracted from the incubation mixtures with methylene chloride twice. The pooled extracts were evaporated and an aliquot of the extract was chromatographed on a thin layer for separation of testosterone and hydroxylated testosterones[5]. The radioactivities were measured with a liquid scintillation counter[6]. The relevant activity of testosterone hydroxylation was expressed as the sum of 6β-, 7α- and 16α-hydroxytestosterones produced from testosterone per mg of protein of microsomes for 30 min. The contents of cytochrome P-450 and protein in the microsomes were determined by the spectrophotometric methods of Omura and Sato[7] and by the method of Lowry et al.[8], respectively.

[1] J. C. Gage, Biochem. J. *54*, 426 (1953).
[2] J. T. Stevens and F. E. Greene, Life Sci. *13*, 1677 (1973).
[3] R. M. Welch, W. Levin and A. H. Conney, J. Pharmac. exp. Ther. *155*, 167 (1967).
[4] J. T. Stevens, R. E. Stitzel and J. J. McPhillips, J. Pharmac. exp. Ther. *181*, 576 (1972).
[5] N. Sato, M. Ota and K. Obara, Tohoku J. exp. Med. *98*, 281 (1969).
[6] M. Ota, N. Sato and K. Obara, J. Biochem. *72*, 11 (1972).
[7] T. Omura and R. Sato, J. biol. Chem. *239*, 2370 (1964).
[8] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).

Effects of pesticides on hydroxylation of testosterone in hepatic microsomes of rabbits

	Control	EMP	MEP	BHC
Cytochrome P-450 (nmole/mg protein) [a]	0.900 ± 0.102	0.740 ± 0.082	0.554 ± 0.042	0.715 ± 0.158
Hydroxylated testosterones (nmole/mg protein) [a]	0.468 ± 0.188	0.244 ± 0.026 [b]	0.196 ± 0.022 [b]	0.214 ± 0.030 [b]
Hydroxylation (nmole/nmole cytochrome P-450) [a]	0.537 ± 0.124	0.330 ± 0.001	0.353 ± 0.013	0.304 ± 0.025
Unmetabolized testosterone (nmole/mg protein) [a]	6.036 ± 0.518 (69.4%) [c]	7.317 ± 0.044 [b] (85.5%) [c]	7.452 ± 0.048 [b] (86.1%) [c]	7.591 ± 0.013 [b] (87.3%) [c]

[a] Values are the mean ± S.D. obtained from four animals. [b] Different from the control at $P < 0.05$. [c] Percentage of the unmetabolized substrate to the substrate.

Results and discussion. The contents of cytochrome P-450 and the activities of testosterone hydroxylase in the hepatic microsomes of the control rabbits and the rabbits treated with the pesticides are shown in the Table. As the values in the 2 control groups were similar, they were combined. The content of cytochrome P-450 in the BHC- or EMP-treated rabbits was reduced to about 80% of that in the control animals and, in the MEP-treated rabbits, it was reduced to about 60%. The unmetabolized substrate, testosterone, was higher in the experimental group in comparison with that in the control group and the formation of hydroxytestosterones per mg protein or per cytochrome P-450 in the experimental groups was half as low as that in the control. These findings suggested that the pesticides inhibited steroid hydroxylase activity in liver microsomes.

STEVENS et al.[4] observed that malathion and parathion given 1 h prior to hexobarbital administration significantly prolonged hexobarbital sleeping time and suggested that the increase in sleeping time by these insecticides was not due to an inhibition of cholinesterase, but due to an impairment of hexobarbital metabolism. STEVENS and GREENE[2] found that a parallel relation between inhibition of ethylmorphine metabolism by these insecticides and the binding affinity of these agents to microsomal cytochrome P-450. The administration of chlorthion, organic phosphorothionate insecticides, to rats for 10 days inhibited the liver microsomal metabolism of testosterone, estradiol-17β, progesterone and deoxycorticosterone to highly polar metabolites, whereas chlorodane and DDT markedly stimulated the metabolism of these steroids by liver microsomes[3]. It was demonstrated that chlorinated insecticides can either stimulate or inhibit microsomal testosterone hydroxylation depending upon whether the agents are given chronically or acutely[3].

LUCIER et al.[9] suggested that the reduced content of cytochrome P-450 of rat livers occurred following the administration of methylmercury hydroxide for 2 days was due to increasing degradation of the fast-phase hemoprotein and/or decreasing synthesis of the slow-phase component and that aminopyrine demethylation, a mixed function oxidase reaction catalyzed by the cytochrome P-450, was decreased by reducing cytochrome P-450 levels.

Since no change was observed in the difference spectra (namely shift of the peak at 450 nm and appearance of the peak at 420 nm had not occurred) and the hydroxylase activity was not in parallel with the content of cytochrome P-450 in this study, the decrease in the hydroxylase activity was not only due to the decrease of cytochrome P-450 content, but also due to other factors, such as the impairment of the hepatic cells. In general, the pesticides seemed to have inhibitory effects on the drug and steroid metabolism in hepatic microsomes when given in a higher dose and/or for a long period.

Résumé. L'hydroxylation de la testostérone par les microsomes du foie de lapins adultes est inhibée par l'hexachlorobenzène, par le 0,0-diméthyle-0-(3-méthyle-4-nitrophényle) thiophosphate et par le phosphate éthylmercurique administrés par voie orale, tous les deux jours, pendant 5 mois.

N. SATO, M. OTA, Y. MURATA and K. OBARA

Department of Biochemistry, Iwate Medical University School of Medicine, Morioka (Iwate 020, Japan), 20 May 1975.

[9] G. W. LUCIER, R. KLEIN, H. B. MATTHEWS and O. S. MCDANIEL, Life Sci. *11*, 597 (1972).

High Permeability of Insect Blood-Brain Barrier to Alcohols Demonstrated by an Electrophysiological Technique

Insects are alone among invertebrates in that their central nervous system possesses an ionic diffusion barrier between the extraneuronal spaces and the blood[1], analogous to that of vertebrates. The possible influence of this barrier on the movements of organic molecules is a complicating factor in pharmacological and toxicological studies, these being of extreme interest as many insecticides act primarily on the central nervous system[2]. Previous research using radioisotopes has suggested that the insect nerve cord is surprizingly impermeant to alcohols[3] and other organic molecules[4]. Here I describe use of the electrophysiological effects of the alcohols to measure their rate of arrival at the neurone surfaces, a technique which could be extended to other molecules

[1] G. HOYLE, Nature Lond. *169*, 281 (1952).
[2] T. NARAHASHI, *Advances in Insect Physiology* (Academic Press, New York 1971), vol. 8, p. 1.
[3] M. E. ELDEFRAWI and R. D. O'BRIEN, J. Insect Physiol. *13*, 691 (1967).
[4] M. E. ELDEFRAWI and R. D. O'BRIEN, J. Insect Physiol. *12*, *1133* (1966); J. exp. Biol. *46*, 1 (1967); with A. TOPPOZADA and M. M. SALPETER, J. exp. Biol. *48*, 325 (1968).