Dopamine and norepinephrine brain levels (ng/g of brain) at 760 mm Hg, 300 mm Hg and return to 760 mm Hg

Treatment	760 mm Hg		300 mm Hg		Return to 760 mm Hg	
	DA	NE	DA	NE	DA	NE
Control group	242 ± 58	55 ± 10	227 ± 43	52 ± 14	263 ± 65	36 ± 9
Treated animals (benserazide 50 mg/kg and L-Dopa 100 mg/kg)	3488 ± 648	299 ± 60	1551 ± 566[a]	196 ± 80	2135 ± 700	296 ± 106

[a] $p < 0.05$ (difference between levels at 760 and 300 mm Hg).

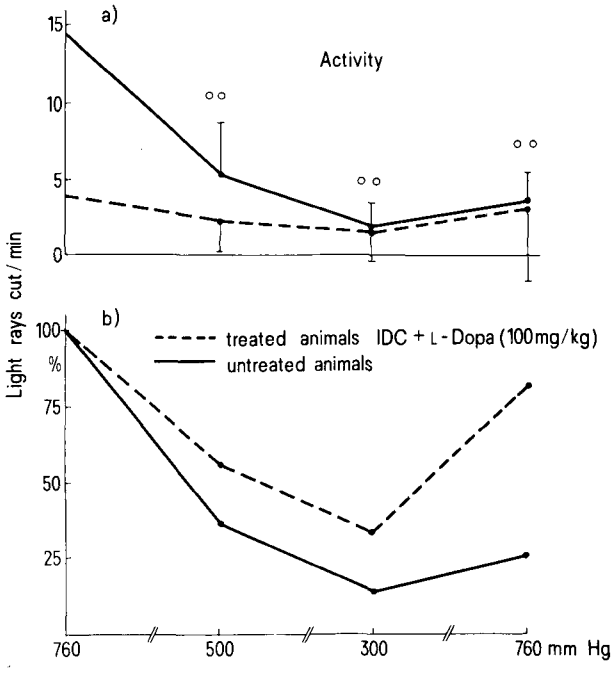

Fig. 2. Spontaneous activity measured at different pressures (10 animals per group). a) The parameters are represented in counts/min (± SE). $^{o}p < 0.05$. $^{oo}p < 0.01$ (appared couples and t-test). b) The parameter was represented in percent of its initial value.

In control animals, dopamine and norepinephrine central levels are unchanged by hypoxia or return to atmospheric pressure (Table) according to previous results[6]. In treated animals, the dopamine level falls down during and hypoxia and the norepinephrine level comes back to its initial values after return to atmospheric pressure. This high level of norepinephrine during post-hypoxic period is probably responsible for return go initial value of the motility[7]. The results of treatment by benserazide and L-Dopa indicate a stabilization of the rheoencephalogramm and a decrease in the reduction of spontaneous motility induced by hypoxia. Overdoses of cerebral dopamine and norepinephrine seem to be the origin of these phenomenons.

Summary. Pretreatment by benserazide (50 mg/kg i.p.) and L-Dopa (100 mg/kg i.p.) in rats induces a reduction of the diminution of motility after hypoxia and a stabilization of cerebral blood flow during and after hypoxia. An overload of cerebral dopamine and norepinephrine seems to be the original process of this phenomenon.

F. Boismare, M. le Poncin, J. P. Belliard and L. Hacpille

Department of Pharmacology, Hôtel-Dieu, rue de Lecat, F-76036 Rouen (France), 5 December 1974.

[6] J. N. Davis and A. Carlsson, J. Neurochem. *21*, 783 (1973).
[7] T. H. Svensson and B. Waldeck, Eur. J. Pharmac. *7*, 278 (1969).

Prostaglandin-Induced Choleresis in the Rat

Although prostaglandins (PG) affect many biological processes, PG E_2 failed to alter bile flow in the dog[1] and in the isolated perfused rat liver[2]. In view of its ATPase inhibitory properties[3] and the hypothetical role of Na^+-K^+-ATPase in bile formation[4], PG A_1 would appear to be a more likely candidate to influence bile secretion. The effect of PG A_1 on bile flow was, therefore, studied in anesthetized rats. The results demonstrate that intraportal infusion of PG A_1 significantly increases bile salt independent bile formation.

Materials and methods. Studies were performed in 6 male Wistar rats weighing 232 to 263 g which had free access to food (Altromin R) and water. Under pentobarbital anesthesia (5 mg/100 g, i.p.) the femoral vein and artery were cannulated with PE 50 polyethylene tubing for infusion of taurocholate or saline and recording of the blood pressure, respectively. From a midline incision the common bile duct was cannulated with PE 10 tubing just below the bifurcation in order to prevent drainage of pancreatic secretion. Bile was collected in 10 min periods and weighed. For the infusion of PG A_1, a PE 10 catheter was inserted into the portal vein via the gastroduodenal vein. After a control period of 30 min during which 0.9% NaCl (3.3 ml/h) was infused into the portal vein, a solution of PG A_1 was infused for 30 min at a similar rate in a concentration yielding 1 µg PG A_1/min/100 g body wt. The portal vein was chosen as the

[1] J. Sokoloff and R. N. Berk, Invest. Radiol. *8*, 9 (1973).
[2] R. A. Levine, in *Prostaglandins and Cyclic AMP* (Eds. R. H. Kahn and W. E. M. Lands; Academic Press, New York, London 1973), p. 75.
[3] J. J. Lafferty, H. Kannegiesser, J. B. Lee and L. W. Parker, in *Advances in the Biosciences* (Eds. G. Raspe and S. Bernhard; Pergamon Press, Vieweg, Germany 1972), vol. 9, p. 293.
[4] S. Erlinger, in *Progress in Liver Diseases* (Eds. H. Popper and F. Schaffner; Grune and Stratton, New York, London 1972), p. 63.

Bile flow and bile salt excretion before and during infusion of PG A_1 (Means ± SEM of three 10 min sampling periods)

	0.9% NaCl, i.v. ($n = 3$)		Taurocholate, i.v. (150 nmol/min/100 g) ($n = 3$)		Equation for linear regression ($y = a + bx$[a])	
	Bile salt output (nmol/min/100 g)	Bile flow (µl/min/100 g)	Bile salt output (nmol/min/100 g)	Bile flow (µl/min/100 g)		
Control period	224.5 ± 5.3	7.66 ± 0.23	404.4 ± 6.3	9.32 ± 0.18	$y = 5.6 \pm 0.009x$	0.82
PG A_1 infusion (1 µg/min/100 g)	222.0 ± 6.7[b]	8.51 ± 0.17[c]	410.5 ± 13.7[b]	10.12 ± 0.16[d]	$y = 6.6 \pm 0.009x$	0.92

[a] y = bile flow; a = bile salt independent fraction; x = bile salt output; b = amount of water obligated per nmol of bile salts. [b] Not significantly different from control period. [c] Significantly different from control period ($p < 0.05$). [d] Significantly different from control period ($p < 0.001$).

route of administration to minimize extrahepatic effects of PG A_1. It has been demonstrated that 55 to 90% of the vasodepressor effect of PG A_1 are destroyed by one passage through the liver[5]. In order to vary bile salt output, either 0.9% NaCl (3 animals) or sodium taurocholate (Maybridge, Cornwall, U.K.), 150 nmol/min/100 g (3 animals), was infused in addition to PG A_1 at identical rates throughout the experiment into the femoral vein. During the whole procedure rectal temperature was kept between 35.5 and 36.5 °C with a heating lamp and arterial blood pressure was recorded electromanometrically.

PG A_1 was kindly supplied by Dr. John E. PIKE, The Upjohn Company, Kalamazoo, Mich., USA, and was kept at −20 °C in 95% ethanol. Prior to each experiment, a fraction of this stock solution was diluted with 0.9% NaCl in such quantities that 1 µg PG A_1/min/100 g could be infused at a rate of 3.3 ml/h. This infusion rate was chosen on the basis of pilot studies which had shown that lower infusion rates (0.01 and 0.1 µg/min/100 g) of PG A_1 had no significant effect on bile flow while higher doses (10 µg/min/100 g) caused marked hypotension. The final ethanol concentration (< 0.5%) has been reported to be without effect on bile flow[2].

Bile flow was determined from the weighed bile samples, assuming a specific gravity of 1. Bile salts were determined enzymatically as described previously[6]. Differences between means were assessed using a paired t-test. Analysis and comparison of the regression lines were performed using current statistical methods[7].

Results. Following the infusion of PG A_1, arterial pressure dropped in each experiment, averaging 100 mm Hg (range 90 to 125) before and 80 mm Hg (range 70 to 95) 10 min after onset of PG A_1 administration, as a manifestation of the well-known vasodilatation brought about by PG A_1[8]. Blood pressure recovered to 86 mm Hg (range 80 to 95) at 20 min and to 93 mm Hg (range 80 to 100) at 30 min after start of the infusion.

During the 30-min control period, bile flow averaged 9.33 and 7.66 µl/min/100 g in the animals with and without taurocholate infusion, respectively. During PG A_1 infusion bile flow (mean of the 3 sampling periods) increased by 0.80 (range 0.76 to 0.83; $p < 0.001$) and 0.85 (range 0.63 to 1.16; $p < 0.05$) µl/min/100 g above control values in the 2 groups. This choleresis was not accompanied by a significant change in bile salt excretion (Table), suggesting an increase in the bile salt independent fraction of bile flow or altered 'osmotic properties' of the excreted bile salts.

A linear relationship between bile salt excretion and bile flow has repeatedly been demonstrated in the rat[9–12]. Extrapolation of the regression line towards zero bile salt excretion yields the bile salt independent fraction of bile flow, while the slope of the regression line represents the amount of bile water obligated per nmol of bile salts excreted. Regression analysis of the relationship between bile salt excretion and bile flow during the control period revealed a slope of the regression line of 9 µl/µmol and a bile salt independent fraction of 5.6 µl/min/100 g. These values are in close agreement with those obtained in a previous study[12], in which taurocholate had been infused at 3 different rates. Infusion of PG A_1 resulted in a statistically significant ($p < 0.01$) parallel shift of the regression line suggesting that the 'osmotic properties' of the excreted bile salts were unchanged and that the mean increase in bile flow of 0.83 µl/min/100 g was due to an increase of the bile salt independent fraction of bile flow. The equations for the 2 regression lines are given in the Table.

Discussion. Bile flow in the rat is thought to be determined by the 'osmotic properties' of secreted bile salts and a bile salt independent fraction generated by the hepatocytes[4]. Our investigation demonstrates that intraportal infusion of PG A_1 in rats leads to a significant increase in bile flow. Analysis of the data based on previous demonstrations of a linear relationship between bile salt excretion and bile flow suggests that this choleresis is due to stimulation of bile salt independent bile formation.

Our experiments do not define the mechanisms responsible for the observed choleresis. It appears unlikely that the increment in bile flow is secondary to hemodynamic changes following PG A_1 infusion since wide variations in perfusion rates produce very little change in bile flow[13]. Although an indirect action of PG A_1 via the release of gastrointestinal hormones cannot be ruled out, the mode of application and the demonstration

[5] E. W. HORTON and R. L. JONES, Br. J. Pharmac. *37*, 705 (1969).
[6] G. PAUMGARTNER, W. HORAK, P. PROBST and G. GRABNER, Naunyn-Schmiedeberg's Arch. Pharmak. *270*, 98 (1971).
[7] K. DIEM, *Scientific Tables*, 6th ed. (Documenta Geigy, Ardsley, New York 1962), p. 173.
[8] J. NAKANO and J. R. MCCURDY, Proc. Soc. exp. Biol. Med. *128*, 39 (1968).
[9] P. BERTHELOT, S. ERLINGER, D. DHUMEAUX and A. M. PREAUX, Am. J. Physiol. *219*, 809 (1970).
[10] J. J. GUMUCIO and V. D. VALDIVIESO, Gastroenterology *61*, 339 (1971).
[11] W. HORAK, G. GRABNER and G. PAUMGARTNER, Gastroenterology *64*, 1005 (1973).
[12] G. PAUMGARTNER, R. HERZ, K. SAUTER and H. P. SCHWARZ, Naunyn-Schmiedeberg's Arch. Pharmak. *285*, 165 (1974).
[13] R. W. BRAUER, in *The Biliary System* (Ed. W. TAYLOR; Blackwell, Oxford 1965), p. 41.

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).