nature) was found on day 4 post-infection followed by decline on days 7 and 11 post-infection. However, on day 15, an increase in accumulation of cells again was observed as compared to controls. It is well known that lysosomes are intracellular membrane-bound collections of hydrolytic enzymes and are mainly activated in the macrophages during endotoxemia and have potential for some direct or indirect role in phagocytosis[22].

In view of these findings in our studies, we have tried to evaluate the activity profile of various hydrolytic enzymes in various hosts having their different susceptibilities towards *P. berghei* infection. In albino rats, sterile immunity develops after a few days while *M. natalensis* and albino mice succumb to infection. The result of the present study indicates that the activity profile of various acid hydrolases in albino rats follows a similar pattern as described for accumulation of cells in vaccinated-infected mice[20] which recover from infection, i.e., activities of all the enzymes increased on day 4 and 7 post-infection followed by decline on days 11 and 15 post-infection. On day 15, when the parasitemia was negligible and the animals became fully recovered from the infection, the activities of all the enzymes came below the control values. Similarly, the activity profile of various hydrolases except acid phosphatase and B-glucuronidase in *M. natalensis* follows the pattern observed in unvaccinated-infected mice which succumb to the disease, i.e., an increase in activities was observed on day 4 post-infection followed by a decline on days 7 and 11 post-infection. Activities again increased considerably on day 15 post-infection. However, activities of acid phosphatase and B-glucuronidase increased regularly throughout the infection. The above results indicate that during the course of *P. berghei* infection, the pattern of lysosomal enzymes in liver of albino rats differ widely from that of albino mice and *M. natalensis*. The most striking feature is that the activities of all the enzymes declined below the normal values on day 15 post-infection in albino rats when animals became immune while in *M. natalensis* and albino mice, which succumb to infection, activities showed several-fold increase over control group of animals. These results suggest a close correlation between host's resistance to infection and level of these enzymes.

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2 Playfair, J. H. L., DeSauza, J. B., Dockrell, H. M., Agomo, P. U., and Taverne, J., Nature *282* (1979) 731.
3 Dockrell, H. M., DeSauza, J. B., and Playfair, J. H. L., Immunology *41* (1980) 421.
4 Crofton, R. W., Diesselhoff Den Dulk, M. M. C., and VanFurth, R., J. exp. Med. *148* (1978) 1.
5 Van Furth, R., Longevoort, R. H. L., and Scharberg, A., in: Mononuclear phagocytes in Immunity, Infection and Pathology. Blackwell Scientific Publications Ltd, Oxford 1975.
6 North, R. J., J. exp. Med. *130* (1969) 315.
7 Jap, P. H. K., and Jerusalem, C., Adv. exp. Med. Biol. *15* (1971) 183.
8 Wedderburn, N., Turk, J. L., and Hutt, M. S. R., Trans. R. Soc. trop. Med. Hyg. *69* (1975) 468.
9 Verity, M. A., Ann. int. med. *78* (1973) 725.
10 Gabridge, M. C., Dickman, Y., and Hedges, K., Infec. Immunity *12* (1975) 233.
11 Saito, K., and Suter, E., J. exp. Med. *121* (1965) 727.
12 Janoff, A. G., Weissmann, B. W., und Zweifach, T. L., J. exp. Med. *116* (1962) 451.
13 Saxena, J. K., Ghatak, S., and Sen, A. B., Indian J. Malar. *18* (1981) 80.
14 Wootan, I. D. P., Microanalysis in Medical Biochemistry, 4th edn. J. & A. Churchill Ltd, London 1964.
15 Dodgson, K. S., Lewis, J. I. M., and Spencer, B., Biochem. J. *55* (1953) 253.
16 Beck, C., and Tappel, A. L., Biochim. biophys. Acta *151* (1968) 159.
17 Anson, M. L., J. gen. Physiol. *22* (1938) 79.
18 Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J., J. biol. Chem. *193* (1951) 265.
19 Karnovsky, M. F., and Lazdins, J. K., J. Immun. *121* (1978) 809.
20 Playfair, J. H. L., and DeSauza, J. B., Parasite Immun. *1* (1979) 197.
21 Letchuk, R., Taverne, J., Agomo, P. U., and Playfair, J. H. L., Parasite Immun. *1* (1979) 61.
22 Weissmann, G., and Thomas, L., J. exp. med. *116* (1962) 433.

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Tricyclic antidepressants antagonize prostaglandin (PG) E_2-induced contractions of the guinea pig ileum and hypomotility in the mouse

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Summary. The interactions of PGE_2 and 2 tricyclic antidepressants were tested both on the guinea pig ileum and motility in the mouse. PGE_2-induced contractions of the guinea pig ileum were irreversibly blocked by amitriptyline and desipramine. Chronic administration of amitriptyline and desipramine blocked PGE_2-induced hypomotility in the mouse.
Key words. PGE_2; contraction; hypomotility; tricyclics.

Prostaglandins have a depressant action on behavior which varies in intensity depending on the route of administration and the compound used[2]. PGEs are the most active[3]; little or no effect is seen with PGF_2[4]. In the present work, the interactions of PGE_2 and the tricyclic antidepressants, amitriptyline and desipramine have been studied.

Materials and methods. Male guinea pigs (420–500 g) were stunned and exsanguinated. The terminal ileum was removed and set up in a 20-ml tissue bath. Some animals received reserpine (4 mg/kg, i.p. 16 h before sacrifice to test whether the actions of PGE_2 are direct. The bath contained Tyrode solution of the following composition (mM/l): NaCl 137, KCl 2.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.2, $NaHCO_3$ 11.9 and glucose 5.5 at 37 °C and gassed with 95% oxygen plus 5% CO_2. After equilibration, contractions were recorded on a smoked paper via a frontal writing lever and magnified × 5. Agonists were allowed 1 min contact time before washing and antagonists were added 30 min before agonists. Control tissues received the agonists but not antagonists. Whenever possible, antagonists were characterized by pA_2[5]. A slope of the A–S plot not significantly different from unity was taken as a satisfactory criterion for competitive antagonism[5]. Male mice (28 g–35 g) were injected with PGE_2 (100 µg/kg) i.p.; control animals received the vehicle (equivalent volume and dilution of ethanol) in which PGE_2 was contained. Others received amitriptyline (5 mg/kg/day) or desipramine (10 mg/kg/day) i.p. for 10 days before PGE_2. The effect of drugs on motility was monitored using an activity counter. Results are expressed as means

± SE. The difference in the mean was regarded as significant when on analysis by Student's t-test $p < 0.05$.

Results and discussion. PGE_2-induced contractions were blocked by amitriptyline (0.32–1.28 μM) and desipramine (0.33–1.32 μM) non-competitively (fig. 1a and b). Amitriptyline was more potent than desipramine (compare fig. 1a with fig. 1b). In constrast, histamine and acetylcholine-induced contractions were blocked competitively by amitriptyline and desipramine. The pA_2 values of amitriptyline and desipramine against histamine were 8.35 and 6.50 with slopes of 0.94 and 1.06, respectively (fig. 2a and b). Atropine (0.1 μM) (fig. 1c) and mepyramine (0.1 μM) (fig. 2d) did not reduce the effect of PGE_2. On the other hand, atropine reduced the effect of acetylcholine (fig. 1d) and mepyramine reduced the effect of histamine (fig. 2c). Methysergide (0.5–5 μM) also reduced the contractile responses to PGE_2 and 5-HT. In the reserpinized preparations, PGE_2 produced smaller responses. These responses were equally reduced by the tricyclics. PGE_2 caused a profound decrease in motor activity and the animals were fully sedated 5 min after injection. Hypomotility was reduced by chronic administration of amitriptyline or desipramine. In each case, the action of amitriptyline was quantitatively greater than that of desipramine (fig. 3).

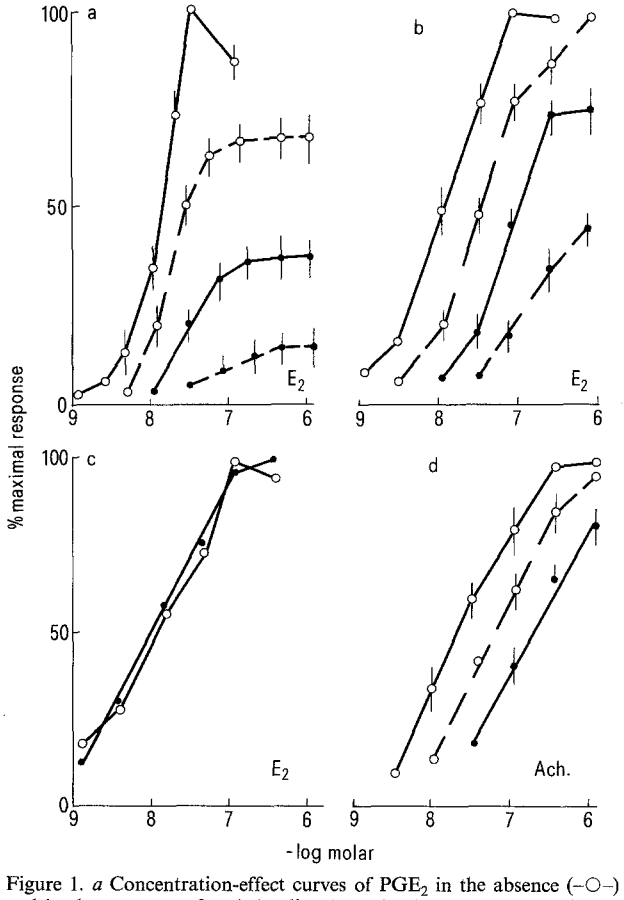

Figure 1. *a* Concentration-effect curves of PGE_2 in the absence (–○–) and in the presence of amitriptyline 0.32 μM (--○--); 0.64 μM (–●–) and 1.28 μM (--●--). Each point is the mean ± SE of 6 observations. *b* Concentration-response curves of PGE_2 in the absence (–○–) and in the presence of desipramine 0.33 μM (--○--); 0.66 μM (–●–) and 1.32 μM (--●--). Each point represents the mean ± SE of 6 observations. *c* Effect of PGE_2 alone (–○–) and in the presence of atropine 0.1 μM (–●–). Results are expressed as means. SE's are not graphed because the atropine-treated was not different from control. Number of observations = 5. *d* Concentration-effect curves of acetylcholine (–○–) alone and in the presence of atropine 10 nM (--○--) and 50 nM (–●–). Each point represents the mean ± SE of 5 observations.

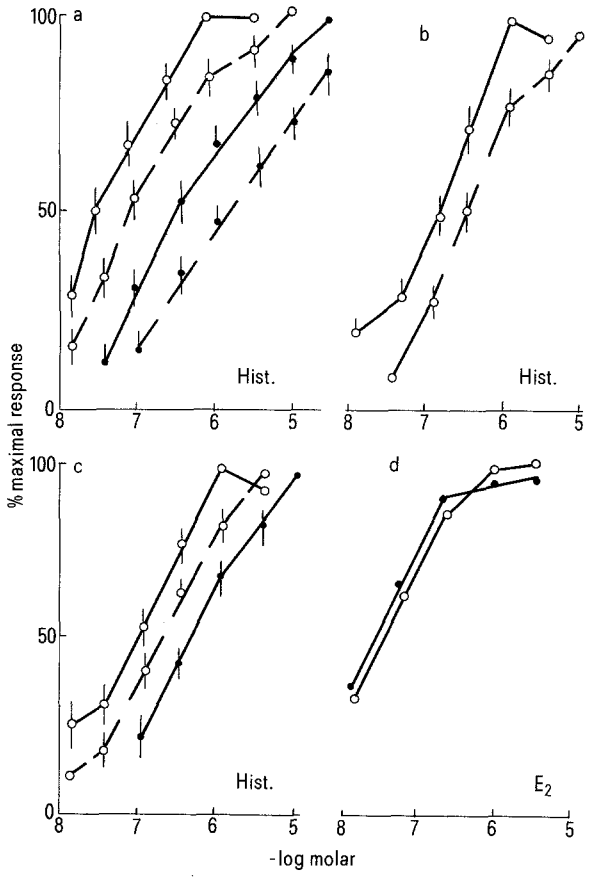

Figure 2. *a* Antagonism of histamine-induced contractions of the guinea pig ileum by amitriptyline. Control (untreated) (–○–); amitriptyline 0.32 μM (--○--); 0.64 μM (–●–) and 1.28 μM (--●--). The values plotted are the means ± SE of 6 observations. *b* Interactions of desipramine and histamine in the guinea-pig ileum. Histamine (control) (–○–); histamine treatment after desipramine 0.66 μM (--○--). Each point is the mean ± SE of 6 observations. *c* Concentration-response curves of histamine in the guinea pig ileum in the absence (–○–) and in the presence of mepyramine 10 nM (--○--) and 50 nM (–●–). Each point is the mean ± SE of 5 observations. *d* Inability of mepyramine 0.1 μM to influence PGE_2-induced contractions of the guinea pig ileum. (–○–) = PGE_2 and (–●–) = mepyramine 0.1 μM 30 min before PGE_2. The means are plotted without the SE because values are too close. Number of observations = 5.

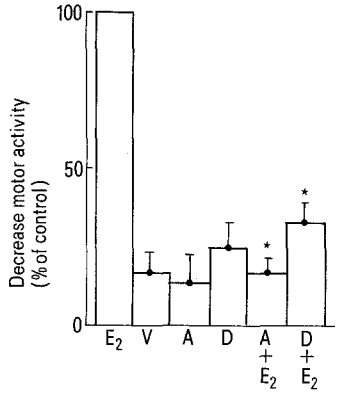

Figure 3. Effect of PGE_2 on motor activity in mice. $E_2 = PGE_2$ (100 μg/kg); V = vehicle in which PGE_2 was contained but without E_2, i.e. an equivalent volume and dilution of ethanol; A = amitriptyline (5 mg/kg/day given i.p. for 10 days; and D = desipramine 10 mg/kg/day given i.p. for 10 days before PGE_2. * indicates that value is significantly different from control PGE_2-treated (p 0.05). Control response to PGE_2 was taken as 100% and other results are expressed relative to this.

These results show that the tricyclic antidepressants reduced the effects of PGE_2 in the guinea pig ileum and in the mouse central nervous system. It was not possible to quantify the antagonism in the mouse because recovery was hardly achieved. This study indicates that interactions of PGE_2 and the tricyclics in the ileum is similar to the interactions of the tricyclics and 5-hydroxytryptamine (5-HT) in the same preparation[6]. In addition, methysergide also reduced the effects of PGE_2. Reduction of the contractile action of PGE_2 in reserpinized ileum may indicate that some of its actions are mediated via the release of a spasmogen. Since both antidepressants are also potent non-competitive antagonists of 5-HT[6], it can be speculated that some of the actions of PGE_2 may be exerted via 5-HT. The monoamine theory of depression[7] highlights the significance of 5-HT in depression. Tricyclics, more specifically, amitriptyline and clomipramine[8] are more potent inhibitors of 5-HT than they are of noradrenaline uptake. In conclusion, it would appear that PGE_2 produces contractions of the guinea-pig ileum and hypomotility in the mouse via a sertonin-like mechanism.

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2 Coceani, E., and Pace-Asciak, C.R., in: Prostaglandins: Physiological, pharmacological and pathological aspects, p. 1. Ed. S.M.M. Karim. M.T.P. Press, Lancaster, U.K., 1976.
3 Holmes, S.W., and Horton, E.W., in: Prostaglandins symposium of the Worcester Foundation for Experimental Biology, p. 21. Eds P.W. Ramwell and J.E. Shaw. Worcester Foundation, 1968.
4 Horton, E.W., and Main, I.H.M., Int. J. Neuropharmac. *4* (1965) 65.
5 Arunlakshana, O., and Schild, H.O., Br. J. Pharmac. *14* (1959) 48.
6 Akah, P.A., and Tayo, F.M., Archs int. Pharmac. in press (1984).
7 Schildkraut, J.J., Am. J. Psychiat. *122* (1965) 509.
8 Spencer, P.S.J., Br. J. clin. Pharmac. *4* (1977) 57S.

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Indoxyl derivatives of drug metabolites

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Ciba-Geigy Limited, CH-4002 Basel (Switzerland), 1 March 1984

Summary. Indoxyl derivatives were detected as minor products among the urinary metabolites of two trial drugs, a benzodiazepine (GP 55 129) and a benzophenone (CGP 11 952). Their structures were elucidated by NMR and mass spectroscopy. Presumably, metabolites containing potential aldehyde functions react spontaneously with endogenous indoxyl. Such derivatives have not hitherto been encountered in drug metabolism.
Key words. Drug metabolism; indoxyl derivatives; aldehyde intermediates; benzodiazepines; benzophenones.

Indoxyl (**I**) is a product of tryptophan degradation in the mammalian organism[3]. In urine it is excreted in considerable amounts as O-sulphate (**II**) and O-glucuronide (**III**)[4,5] (fig. 1). Healthy adult persons, for instance, excrete about 60–200 mg of **II** per day[6]. In the rat the amount is about 3 mg per day[7]. Recently we investigated the biotransformation of two trial drugs in rat and dog. GP 55 129 (**IV**)[8] is a benzodiazepine, and CGP 11 952 (**V**)[9] is a benzophenone (fig. 2). Both display central nervous activities. The two trial drugs were administered in ^{14}C-labeled form. Among the numerous radioactive compounds found in urine, some contained an indoxyl moiety as part of their structure.

Indoxyl derivatives of drug metabolites have not been described so far. In the present paper we therefore wish to report on the isolation and structure elucidation of these derivatives.

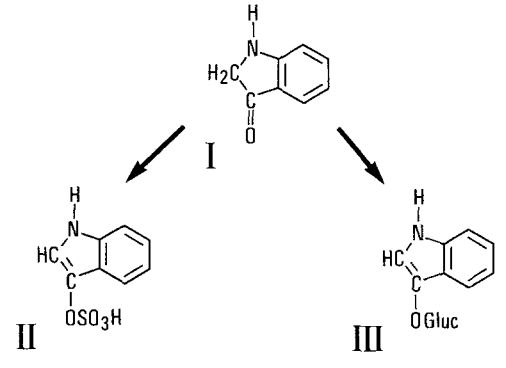

Figure 1. The structures of indoxyl (**I**), and of indoxyl conjugates excreted in urine (**II**, **III**) (Gluc = glucuronic acid residue).

Materials and methods. ^{14}C-Labeled GP 55 129[8] and CGP 11 952[9] had specific radioactivities of 18 and 23 kBq/mg, respectively. The synthetic, non-labeled indoxyl derivatives CGP 27 729 (**VI**)[10] and H.A. 2800.2.1 (**VII**)[11] were available as reference substances. Solvents and chemicals were of reagent grade and were obtained from Fluka (Buchs, Switzerland) or E. Merck (Darmstadt, FRG).

Rats (N = 12) received oral doses of 50 mg/kg of [^{14}C] GP 55 129 on two consecutive days. Dogs (N = 5) received oral doses of 5 mg/kg of [^{14}C] CGP 11 952 singly, or on two conse-

Spectroscopic data of **VI** and **VIII**

VI	NMR (CD_3OD):	7.93, 7.92 and 7,68 (H-3, H-5 and H-6 of the 4-chlorophenyl ring); 7.62, 7.56, 7.11 and 6.98 (H-4, H-6, H-7 and H-5 of the indolinone moiety); 7.32, 7.29,7.18 and 7.16 (H-6, H-4, H-3 and H-5 of the o-chlorophenyl ring); 6.14 (s, vinyl proton).
	MS m/e (%)*:	503(100) M(2 Cl); 468 (3) M-Cl; 423 (2) 468-NH_3/CO; 364(20) M-C_6H_4ClCO; 347(10) 364-NH_3; 319(6) 347-CO; 139(34) C_6H_4ClCO; 111(15) 139-CO.
VIII	NMR (CD_3OD):	7.83, 7.71 and 7.63 (H-5, H-3, and H-6 of the 4-chlorophenyl ring); 7.58 and 6.70 (AA' BB' system, p-hydroxyphenyl ring); 7.55, 7.49, 7.08 and 6.92 (H-4, H-6, H-7 and H-5 of the indolinone moiety); 5.82 (s, vinyl proton); 4.66 and 4.53 (AB system, J = 14 Hz, CH_2).
	MS m/e (%)*:	472(100) M($C_{25}H_{17}ClN_4O_4$). 351(13) M-C_6H_4OHCO.
	IR (KBr):	1705, 1655, 1631 and 1608 cm^{-1}.
	UV (CH_3OH):	λ_{max} = 279, 300, 479.

* Peaks due to ^{37}Cl are neglected.