reduced. It is noteworthy that Ca^{2+} sedimentation of microsomes from the insects *Musca domestica* and *Prodenia eridania* resulted in reduced activity of the mixed function oxidase enzymes[20]. PEG treatment facilitated the isolation of proteins, certain enzymes, nucleic acids, bacteria and viruses[21]. This polymer was also used to obtain active hepatic microsomal enzymes[22] and to precipitate ligand-receptor complexes[23]. It has been demonstrated that the acid precipitation procedure was useful in obtaining active rat liver microsomal enzymes[24,25]. The *Tribolium* CS enzyme complexes obtained by either acidification (II) or PEG precipitation (IV) were relatively less active, with specific activities of 65.5% and 45.1% of the control (I), respectively.

In the inhibition study (table 2), diflubenzuron and IKI-7899 did not affect the various CS enzymes at 280 µM. This result confirms previous reports which suggest that insecticidal benzoylphenyl ureas do not act directly on the chitin polymerization step[10,14]. Polyoxin-D which is an established competitive CS inhibitor in insect[10] and fungal[3] systems affects all the CS preparations. However, for unknown reasons the CS in PEG precipitated microsomes is less inhibited (34.6%) by polyoxin-D compared with the other enzyme systems (59.2–74.4%). The benzimidazole compound at 280 µM is inhibitory almost to the same degree in all the CS preparations examined. This novel compound blocks the molting process and was found to be insecticidal in silkworm larvae[26]. Extensive structure-activity relationship study on a series of benzimidazoles having a terpenoyl moiety has been recently conducted discovering compounds with considerable inhibitory effects on the insect CS (unpublished).

The available insect CS radioassay can serve as a useful tool for massive screening and evaluation of bioactive compounds which interfere with chitin polymerization. This assay coupled with in vivo studies might yield valuable information for developing potential pesticides. Although the activity of CS obtained by Ca^{2+} aggregation is high, the small yield of microsomal proteins is disadvantageous for large screening programs (table 1). It appears that despite a certain loss in specific activity (⅔ of the control), the acid precipitation alternative might be considered as the procedure of choice for massive screening and for quantitative structure-activity relationship studies of insect CS inhibitors.

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Hepatic acid hydrolases of albino rats, *Mastomys natalensis* and albino mice during *Plasmodium berghei* infection

J.K. Saxena, S. Khare, A.K. Srivastava[1], A.B. Sen and S. Ghatak

Divisions of Biochemistry and Parasitology, Central Drug Research Institute, Lucknow 226001 (India), 23 December 1983

Summary. Changes in liver acid hydrolase activities during the infection of albino rats, *Mastomys* or mice with *Plasmodium berghei* are described. B-Glucosidase, B-galactosidase and N-acetyl-B-D-glucosaminidase exhibited widely different responses with acid phosphatase and cathepsin-B the least responsive and are likely to be causally related to immunity of animals.
Key words. Mouse, Swiss; *Mastomys natalensis;* rat, C.F. strain; acid hydrolases, liver; *Plasmodium berghei* infection.

Liver plays an important role in recovery from blood stage malaria infection and also in the modification of the immune system of the host[2,3]. The role of phagocytic cells of the liver (Kupffer cells) in host defense inflammatory processes, immune mechanisms and erythrocyte destruction has been amply documented[4–6]. Studies made with an electron microscope have also confirmed that Kupffer cells of the liver, during malaria infection, contained phagocytized hemozoin and parasitized erythrocytes[7,8]. The function of these cells, which contain a variety of hydrolases is considered to be intracellular breakdown and disposal of phagocytized materials[9]. Reports regarding increase in the levels of various tissue acid hydrolases during *Mycoplasma*

Table 1. Acid hydrolases of liver of albino rats during *Plasmodium berghei* infection*

Enzyme	Control	4th day (1–3%)**	7th day (5–8%)**	11th day (15–18%)**	15th day (immune)
Acid phosphatase	16.78 ± 3.02	16.43 ± 3.29	24.46 ± 4.53	21.62 ± 1.27	16.57 ± 0.79
B-Glucuronidase	279.42 ± 30.61	363.87 ± 49.21***	737.14 ± 108.65***	253.87 ± 21.36	74.62 ± 14.39***
B-Glucosidase	145.19 ± 64.53	291.58 ± 86.04***	593.91 ± 11.13***	203.94 ± 28.84	111.73 ± 16.13***
B-Galactosidase	624.58 ± 27.58	1732.75 ± 411.08***	ND	739.30 ± 94.40	445.33 ± 34.25***
N-Acetyl-B-D-glucosaminidase	21.88 ± 3.89	55.60 ± 8.80***	69.76 ± 6.75***	18.86 ± 4.20	11.79 ± 2.32***
Cathepsin-B	158.87 ± 27.31	178.41 ± 36.95***	171.34 ± 34.51***	122.21 ± 19.45	66.07 ± 7.65***

Values are mean ± SE. *Specific activity = units per mg protein. Enzymes units are expressed as nmoles product formed/min for acid phosphatase, B-glucuronidase, N-acetyl-B-D-glucosaminidase and cathepsin-B and pmoles product formed/min for B-gluco and galactosidases. ** % erythrocytes infected with *P. berghei*; ND, not done; ***$p < 0.01$ for all, on applying Student's t-test.

Table 2. Acid hydrolases of liver of *M. natalensis* and albino rats during *Plasmodium berghei* infection*

Enzyme		Control	4th day (1–3%)	7th day (5–8%)**	11th day (20–25%)**	15th day (40–45%)**
Acid phosphatase	A	11.06 ± 0.85	12.29 ± 3.29	18.91 ± 1.85	20.72 ± 2.81	ND
	B	10.54 ± 2.70	12.22 ± 3.71	13.99 ± 3.39	16.81 ± 5.83	15.12 ± 2.95
B-Glucoronidase	A	27.52 ± 3.10	72.30 ± 8.62	90.40 ± 11.68	92.67 ± 12.31	198.01 ± 25.69***
	B	47.82 ± 6.13	77.07 ± 11.63***	129.07 ± 19.14	135.56 ± 24.67	190.65 ± 32.67***
B-Glucosidase	A	197.17 ± 56.3	542.5 ± 90.82***	371.20 ± 87.23	117.90 ± 13.58	1026.5 ± 135.04***
	B	382.41 ± 70.50	662.05 ± 136.23***	335.4 ± 71.20	227.88 ± 24.74	1027.73 ± 212.71***
B-Galactosidase	A	881.91 ± 81.26	1597.70 ± 180.45***	520.30 ± 26.29	554.90 ± 20.40	2908.6 ± 210.35***
	B	466.60 ± 115.91	1473.70 ± 156.40***	839.6 ± 132.20	215.12 ± 90.82	1367.08 ± 229.60***
N-Acetyl-B-D-glucosaminidase	A	14.41 ± 1.64	42.10 ± 2.5***	16.40 ± 3.91	21.45 ± 5.92	25.12 ± 1.56***
	B	17.26 ± 1.83	40.67 ± 7.72***	19.41 ± 4.27	24.74 ± 5.32	58.10 ± 11.59
Cathepsin-B	A	150.00 ± 19.34	148.71 ± 16.71	101.56 ± 13.34	ND	231.17 ± 38.30***
	B	141.34 ± 22.12	166.27 ± 28.61	100.01 ± 16.35	70.24 ± 13.67	154.20 ± 27.3

Values are mean ± SE. *Specific activity = units/mg protein. Enzyme Units – same as described in table 1. ** % erythrocytes infected with *P. berghei*; ND, not done; A, *M. natalensis*; B, albino mouse; ***$p < 0.01$ for all, on applying Student's t-test.

fermentans and BCG infection in mice and in endotoxemia are available[10–12]. In the present communication, the activities of some of the liver lysosomal acid hydrolases of three different hosts, albino rats, *Mastomys natalensis* and albino mice during *Plasmodium berghei* infection are reported.

Materials and methods. Eight-week-old male albino rats (C. F. strain), albino mice (Swiss strain) and *M. natalensis* (GRA Geissen strain) were infected with *P. berghei* as reported earlier[13]. Livers of varying groups of animals were excised at the desired level of infection, washed in cold KCl (150 mM), and homogenized in the same medium to give a 10% (W/V) homogenate. Homogenization required 60–120 s with a motor driven Teflon pestle. The suspension was centrifuged at 15,000 × g for 20 min, and the supernatant solution was transferred to clean tubes. Freezing and thawing of the supernatant was done a number of times to release all the membrane-bound hydrolases before assays.

Enzyme assays. Activities of acid phosphatase (E.C. 3.1.3.2) and B-glucuronidase (E.C. 3.2.1.31) were determined according to Wootan[14] and Dodgson, Lewis and Spencer[15] respectively, while the activities of B-glucosidase (E.C. 3.2.1.21), B-galactosidase (E.C. 3.2.1.23) and N-acetyl B-D-glucosaminidase (E.C. 3.2.1.30) were analyzed according to Beck and Tappel[16]. Cathepsin-B (E.C. 3.4.22.1) activity was determined according to Anson[17]. Protein content of the enzyme preparations was estimated according to Lowry et al.[18].

Results. Tables 1 and 2 depict the activities of various lysosomal acid hydrolases in the post-mitochondrial supernate fraction of liver of albino rats, *M. natalensis* and albino mice respectively during the course of *P. berghei* infection.

In albino rats on days 4 and 7 post-infection, the activities of all the enzymes, i.e. B-glucuronidase, B-glucosidase, B-galactosidase, N-acetyl-B-D-glucosaminidase and cathepsin-B were increased considerably as compared to control values. However, on days 11 and 15 post-infection, the activities were depressed. On day 15 post-infection, when there was no detectable parasite in blood circulation, decreases from control values in the activities of B-glururonidase, B-glucosidase, B-galactosidase, N-acetyl-B-D-glosaminidase and cathepsin-B of 73, 23, 29, 46 and 50% respectively were observed, while the level of acid phosphatase was not considerably depressed (table 1).

In *M. natalensis*, activities of all of the above-mentioned enzymes except acid phosphatase, and cathepsin-B raised several fold on day 4 post-infection followed by a decline on days 7 and 11 post-infection. However, activities again increased significantly on day 15 post-infection (42–45% parasitemia), as increases of 421, 230, 74 and 54% over control values were recorded for B-glucosidase, B-galactosidase, N-acetyl-B-D-glucosaminidase and cathepsin-B respectively. Acid phosphatase and B-glucuronidase activities showed a marked increase throughout the infection (table 2).

As in *M. natalensis*, a similar pattern in the activities of acid hydrolases of liver of albino mice was observed. On day 15 post-infection, increases of 43, 299, 168, 192, 236 and 10% over control values were recorded for the activities of acid phosphatase, B-glucuronidase, B-glucosidase, B-galactosidase, N-acetyl-B-D-glucosaminidase and cathepsin-B (table 2).

Discussion. Histological studies have clearly demonstrated that malarial infection causes an increased accumulation of lymphocytes and Kupffer cells (mainly macrophages) in liver. An increase in both the number of macrophages and in their state of activation has been found in the liver during *P. yoelli* infection, as accessed by acid phosphatase staining[19]. Reports from various laboratories have also shown the accumulation profile of liver macrophages during the course of *P. berghei* and *P. yoelli* infections[2,3] and it was also found that throughout the course of infection, accumulation of these cells varies from one parasitemia to another parasitemia. The accumulation profile was very thoroughly studied by Playfair group[2,20,21] in malaria vaccinated-infected mice and unvaccinated-infected mice. However, it was found that vaccinated-infected mice recover from infection after an increase in parasitemia while unvaccinated-infected mice succumb to the infection. In both these types of mice, an increased accumulation of the cells in the liver is found on days 4 and 7 post-infection as compared to controls. In unvaccinated-infected mice, an increase in accumulation of cells (phagocytic in

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.

1 To whom correspondence and requests for reprints should be addressed. Present address: Department of Pharmacology, University of Vermont, Burlington, VT 05405 (USA).
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Tricyclic antidepressants antagonize prostaglandin (PG) E_2-induced contractions of the guinea pig ileum and hypomotility in the mouse

'Fola M. Tayo[1]

Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan (Nigeria), 16 March 1984

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