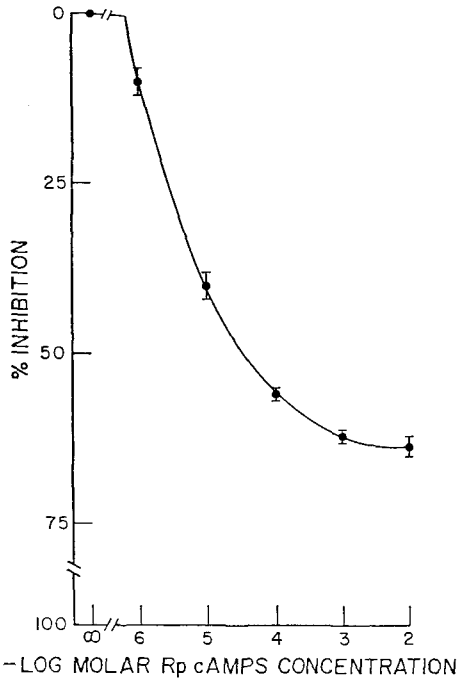

Figure 2. Dose-response curve for the inhibitory action of Rp-cAMPS on the response of the ouabain-insensitive Na efflux to the injection of 10^{-3}M-cAMP. Each plotted point represents the mean value of 3 determinations. Vertical bars indicate ±SE of mean. The fibers were isolated from the same barnacle specimen.

varying concentration. As shown in figure 2, preinjection of 10^{-6}M-Rp-cAMPS exerts a rather minimal inhibitory effect (viz 10%), whereas a 10^{-3}M- or 10^{-2}M-solution exerts a maximal inhibitory effect on the response of the ouabain-insensitive Na efflux to the injection of 10^{-3}M-cAMP. The magnitude of the latter effect is about 61%. Thus, the IC_{50} falls in the region of 5×10^{-6}M (using a log scale). The fact that Rp-cAMPS does not completely abolish the response of the Na efflux to cAMP when it is injected in an equimolar concentration cannot be readily attributed to the breakdown of the analog by cAMP phosphodiesterases (low and high K_m forms), since it is known to be resistant to hydrolysis[13,14]. However, there is an exception[8], namely the case of yeast cAMP-PDE, the apparent K_m for Rp-cAMPS being 1×10^{-4}M. If this is also true of barnacle muscle cAMP-PDE, one would then expect Rp-cAMPS to interfere with the breakdown of injected cAMP, thus resulting in a greater response of the Na efflux to cAMP. Another but simpler explanation is that Rp-cAMPS behaves as a partial antagonist in situ of the cAMP-induced activation of cAMP-protein kinase. Furthermore, it must not be overlooked that in experiments of this type the injection of a substance axially and uniformly along the entire length of the fiber cannot be claimed with certainty.

Another means of studying the efficacy of Rp-cAMPS is to determine whether it reduces the magnitude of the stimulatory response of the Na efflux to high external K^+. Experiments therefore were done in which test and control fibers were suspended suddenly in 100 mM-K^+-ASW after injection of 10^{-3}M-Rp-cAMPS and 3 mM-HEPES into them, respectively. The results obtained are as follows: 131 ± 17% stimulation, n = 5, vs 220 ± 22%, n = 5 in controls. The difference is significant ($p < 0.02$). This finding confirms an earlier conclusion that the response of the Na efflux to high external K^+ or injection of Ca^{2+} is mediated in part by the activation of cAMP-protein kinase by newly formed cAMP[15,16].

In conclusion, these observations provide evidence that Rp-cAMPS is a partial antagonist of cAMP and that it promises to be a powerful tool at the disposal of the cell physiologist. Whether it also acts as a partial antagonist in barnacle fibers when the internal cAMP level is raised as the result of activation of the membrane adenylate cyclase system in situ by specific agonists remains to be seen.

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Studies on correlations between chloroquine-induced tissue damage and serum enzyme changes in the rat[1]

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Summary. The administration of chloroquine to rats resulted in a significant elevation of serum enzymes and a corresponding decrease of these enzymes in the tissues. The changes in serum and kidney enzymes were most marked, thus indicating a primary renal dysfunction.
Key words. Rats; chloroquine; renal damage; lysosomes; acid hydrolases; membrane damage.

The deleterious effects of chronic and acute administration of chloroquine (aralene, 7-chloro-(4-diethyl amino-1-methylbutyl amino) quinoline) to animals and man have been previously reported[11,13,19,21,22]. Some of these reports have indicated the presence of membrane-bound whorl-like structures in the various tissue systems. The lysosomal, endoplasmic reticular and mitochondrial organelles have been implicated in the formation of these structures[11,21]. Results have shown that these membrane-bound whorl-like structures, which arise from chloroquine treatment, are a non-specific reaction of the cell to chloroquine molecules, involving the separation of various cell organelles from the surrounding cytoplasm and the subsequent digestion of these organelles through the action of the lysosomal acid hydrolases[11]. So far, there has been no clear demonstration of any organ-specific effects of chloroquine, although the retinal, cardiac and renal tissues have often been mentioned[11,13,19]. The present investigation was an attempt to identify the organ(s) most adversely affected following acute chloroquine treatment in the rat, by measurement of enzyme changes in the kidney, liver and heart, and to relate these changes to the levels found in the blood of chloroquine-treated animals.

Materials and methods. 4-Nitrophenyl disodium orthophosphate and sodium pyruvate were purchased from BDH Chemicals Ltd., Poole, England, while NADH was purchased from the Sigma Chemical Co., London, England. All other chemicals used were of analytical grade and were prepared in double-distilled water. All buffer solutions were prepared and adjusted to their appropriate pH at 25 °C.

Enzyme and protein measurements: Acid phosphatase (EC.3.1.3.2) and alkaline phosphatase (EC.3.1.3.1) activities were estimated as described earlier[4,23], except that in the case of alkaline phosphatase, the enzyme action was stopped by the addition of 1.0 M sodium hydroxide solution. Lactate dehydrogenase (EC.1.1.1.27) was determined using the method of Leathwood et al.[16]. Protein was estimated by the biuret method[12]. All enzyme activities were expressed as specific activities in U/mg of protein, and all determinations were carried out using a Pye Unicam SP 1800 double beam spectrophotometer. Due to some technical problems encountered during the homogenization procedure, heart LDH was not estimated in the present investigation.

Administration of chloroquine: Male Wistar rats, weighing 200–230 g, were used. They were bred in the animal holding of the Faculty of Health Sciences, Obafemi Awolowo University, Ile-Ife. Chloroquine phosphate (Imarsel Chemical Co. Ltd., Chinoin, Hungary) was dissolved in sterile physiological saline and was administered daily s.c. in a dose of 40 mg/kg b.wt for 11 days.

Preparation of serum samples: Rats were anaesthetized using diethyl ether (AR) and after careful removal of the fur under the neck, the jugular veins were exposed. The veins were then punctured with a clean and sharp mounted needle and the blood collected into a pre-warmed beaker. The animal was killed while still under anaesthesia by dislocation of the neck from the base of the skull. The beaker was placed in an incubator at 37 °C for 30 min and the serum collected after centrifugation on a bench centrifuge at 3000 g_{av}, for 15 min. This procedure if carefully followed gave clear samples of serum with no haemolysis. A sample was removed for the measurement of protein and the remainder dialyzed against ice-cold distilled water as previously described[16].

Preparation of tissue extracts: Rats were killed by dislocation of the neck from the base of the skull and the kidneys, livers and hearts rapidly taken out. The tissues were thoroughly washed in ice-cold 0.25 M-sucrose solution and dried with clean filter paper. They were then weighed and homogenized in ice-cold 0.25 M-sucrose solution using a TRI-R STIR-S Model K_{43} homogenizer (TRI-R Instruments, Rockville Center, New York). All operations were carried out at 0–4 °C. The non-ionic detergent Triton X – 100 was added to the homogenate to a final concentration of 0.1 %$^{v}/_{v}$ and the suspension stood in the cold room (0 – 4 °C) for 30 min. This was to ensure the maximum release of those enzyme systems located in the cell organelles. At the end of this period, the suspension was re-homogenized and then dialyzed for 4 h against ice-cold distilled water. All measurements except proteins and alkaline phosphatase were carried out on the dialyzed homogenate. Protein was estimated on samples removed prior to dialysis in order to avoid loss of protein molecules of low molecular weight. Complete extraction of alkaline phosphatase from the plasma membrane was achieved by shaking the homogenate with n-butanol for 1 h instead of adding the detergent. The resulting emulsion was treated as described previously[3]. The resulting aqueous product containing the enzyme was dialyzed against ice-cold distilled water.

Statistical analysis: Analysis of variance (treatment effect) was done on the data using the Hewlett-Packard HP-67/HP-97 Stat Pac 1, programme for analysis of variance (one way) (Hewlett-Packard 1976).

Cumulative dose given: The overall total dose of chloroquine administered to each treated rat over the study period per kg of body weight.

Results. The mean values for each of the three enzymes measured in terms of serum, kidney, liver and heart tissues showed a significant ($p < 0.01$) effect for all the serum and tissue variables as shown by analysis of variance.

Serum, kidney and liver AcP changes: Serum AcP was elevated from the first day after drug treatment (table), reaching a peak on day 12. Kidney and liver AcP were greatly reduced, particularly during the first few days of chloroquine administration. The fall in kidney enzyme activity was more pronounced than that observed for the liver. The serum enzyme level and the activities of kidney and liver enzymes showed a good correlation in relation to the cumulative concentrations of chloroquine given. The correlation coefficient for serum enzyme in relation to the cumulative dose of the drug administered, up to day 12, was $r = +0.99$, while those for the kidney and liver were $r = -0.65$ and $r = -0.57$ respectively up to day 12.

Serum, kidney and liver AP changes: Serum AP did not increase appreciably during the first day after drug treatment. The activity of the enzyme rose linearly from day 6 to day 12 after the first injection. AP in the kidney and liver dropped during the first 4 days. The activity fluctuated between days 5 and 9, and rose slightly on day 12, although the levels were still below the controls. The correlation between the serum enzyme level ($r = +0.94$) and the cumulative dose of chloroquine given was good, but those found for the kidney ($r = -0.15$) and liver ($r = -0.21$) were not.

Serum, kidney and liver LDH changes: During the first day after chloroquine administration, the increase in the serum LDH level was slight (table). Immediately after, the activity increased rapidly, reaching a very high peak of 317.46 $\pm$ 14.87 U/mg of protein on day 6. The activity fluctuated between day 6 and day 12. Kidney LDH fell throughout the entire period of investigation, with levels of 622.39 $\pm$ 17.42 U/mg of protein on day 1 and 295.32 $\pm$ 9.32 U/mg of protein on day 12, although the decrease was slow between day 1 and day 6. The pattern was similar for the liver enzyme except that there was a continuous and steady decrease between day 4 and day 12. There were good correlations with the cumulative dosages of the drug given in the serum LDH ($r = +0.77$), and also in the activities of kidney LDH ($r = -0.95$) and liver LDH ($r = -0.71$).

Heart AcP and AP changes: There was a continuous but slight decrease in the activity of AcP up to day 12 from the time of the first insult with the drug. The pattern in the AP

Mean values and standard error of the mean of the effect of chloroquine on the activities of AcP, AP and LDH on rat serum, kidney, liver and heart*

Day after first injection	Cumulative dose given (mg/kg)	AcP Serum	AcP Kidney	AcP Liver	AcP Heart	AP Serum	AP Kidney	AP Liver	AP Heart	LDH Serum	LDH Kidney	LDH Liver
Control	0	0.80 (0.03)	142.71 (3.61)	50.68 (0.43)	5.32 (0.12)	0.95 (0.05)	204.96 (3.64)	4.40 (0.38)	6.61 (0.20)	110.37 (7.05)	829.44 (75.13)	1159.22 (15.69)
1	40	1.52 (0.11)	53.03 (2.10)	26.50 (0.68)	3.31 (0.31)	0.82 (0.03)	116.93 (4.12)	2.13 (0.08)	6.08 (0.23)	113.18 (1.90)	622.39 (17.42)	687.02 (30.01)
4	120	2.88 (0.27)	52.86 (3.96)	23.12 (2.00)	3.31 (0.39)	1.00 (0.05)	88.82 (8.72)	2.19 (0.21)	4.86 (0.43)	137.14 (3.84)	552.65 (24.36)	479.88 (39.75)
6	200	3.43 (0.29)	51.79 (3.85)	21.12 (1.25)	4.55 (0.24)	1.91 (0.17)	103.91 (5.34)	2.53 (0.13)	4.66 (0.35)	317.46 (14.87)	540.25 (29.00)	528.87 (37.28)
9	320	5.13 (0.31)	50.70 (3.34)	21.73 (0.83)	4.61 (0.40)	3.18 (0.12)	65.76 (4.99)	1.42 (0.15)	4.35 (0.24)	222.52 (7.80)	299.43 (23.69)	492.08 (18.76)
12	440	5.66 (0.25)	56.86 (2.83)	28.21 (1.87)	3.68 (0.30)	5.18 (0.28)	136.44 (12.00)	2.85 (0.30)	271.84 (0.64)	295.32 (7.57)	500.22 (9.23)	(29.98)
Correlation coefficient		+0.99	−0.65	−0.57	−0.49	+0.94	−0.15	−0.21	−0.53 −0.89**	+0.77	−0.95	−0.71

*Values are means of 13 determinations ±SEM. All means are significant at $p < 0.01$. **Regression analysis was run on all data except data from animals injected with a cumulative total of 440 mg/kg, i.e. animals sacrificed on day 12 from the start of injections. Numbers in brackets indicate ±SEM.

was similar to that observed for AcP, except that heart AP was tending to rise on day 12. The correlations between the heart AcP ($r = -0.49$) and Ap ($r = -0.53$) and the cumulative doses of chloroquine given were less well marked than those observed for serum AcP ($r = +0.99$) and serum AP ($r = +0.94$).

Discussion. The results of the present investigation demonstrate that acute chloroquine administration to rats could result in some damage to the cells of the kidney, liver and heart tissues, resulting in moderate to high increases in the specific activities of serum AcP, AP and LDH enzymes. Corresponding losses were observed in the activities of these enzymes in the tissues of chloroquine-treated animals. The increase in serum AcP, a lysosomal enzyme [23], was markedly noticeable one day after the first injection with the antimalarial agent. AcP activity was still significantly high up to day 12 (table). Kidney AcP, on the other hand, decreased significantly one day after the first injection with the drug. Although AcP in the liver tissue decreased during the same period, the kidney tissue appeared to have responded more adversely to chloroquine in terms of tissue enzyme loss. These results indicate an early renal lysosomal involvement in chloroquine-induced tissue damage.

These observations are strongly supported by earlier reports that chloroquine, a weak base, and a tertiary amine, accumulates in the lysosomes, owing mainly to the low pH within these cellular organelles [7, 14]. This results in the formation of autophagic vacuoles containing numerous acid hydrolases, which seemingly represent early stages in the degradation of cytoplasmic organelles within 8 hours after chloroquine administration [6]. Other reports indicate chloroquine-mediated alterations of lysosomal acid hydrolases, as shown by increases in free enzyme activities [10, 19]. Previous reports have indicated that chloroquine could be inhibitory to some lysosomal enzymes, in vivo and in vitro [8, 20], but in the present studies, this drug did not appear to be inhibiting AcP, as has been demonstrated by the linear and progressive increase of this enzyme in the serum of drug-treated animals.

The correlation between the cumulative drug concentrations administered and the tissue AP appeared to be rather poor compared to that observed in the serum of treated animals. The correlations were also poor compared to those found for the kidney and liver AcP (table). Although the tissue enzyme activities were decreased one day after the drug was administered, these activities tended to fluctuate very slightly and in fact were found to be increasing towards the end of the studies, presumably resulting from de novo synthesis of enzyme molecules. The increasing serum AP found to occur during the period of the investigation could partly be expected to be related to damage done to other source tissues. With regard to serum and tissue LDH (table) and relating the activities to the cumulative drug concentrations, good correlations were found for the kidney ($r = -0.95$) and liver ($r = -0.71$) enzymes, and for the serum LDH ($r = +0.77$). Both the kidney and liver enzymes decreased while serum LDH increased.

Histopathological examination of tissues of the rats treated with chloroquine and sacrificed on day 12, although not illustrated in this text, revealed very slight focal changes in the cardiac muscle cells. The liver showed some focal degenerative changes and fibrosis. The changes in the kidney consisted mainly of progressive proximal tubular degeneration and glomerular congestion. The changes in the kidney were the most prominent of those in the three tissues examined. These observations are in agreement with recent morphological investigations including histochemical and electron microscopical studies in leukocytes and other cell-types, which revealed that both chronic and acute chloroquine administration produced cytotoxic changes related to lysosomal permeability alterations accompanying autophagy [6, 9, 10]. These changes have also been shown to occur in areas of the cytoplasm with early degenerative alterations, which subsequently become aggregated within vacuoles and undergo digestion [6]. LDH is found in the soluble fraction of the cell cytosol [5, 15].

The changes observed in the present studies may represent the effects of the numerous acid hydrolases arising from chloroquine-induced lysosomal damage. The changes observed in the cardiac tissues in relation to AcP reduction were not very marked (table) and could not be said to be comparable to those changes found in the kidney, and therefore our observations do not completely support some earlier reports of a primary chloroquine-induced activation of the myocardial lysosomal system in the chicken [11]. This is presumably due to species-related differences in the animals studied. The reasons for the poor response of tissue AP to chloroquine treatment in the present studies are not completely understood, particularly when the changes in the serum appeared to be appreciably consistent. Chloroquine is known to affect various plasma membrane systems by binding quantitatively to them, reducing plasma membrane binding capacities, and displacing plasma protein and enzyme molecules, including

the membrane-bound AP molecules, through derangement of the re-cycling of receptors[6,9,18,24]. The results of the present investigation may therefore suggest that the plasma membrane enzyme determination may not be as sensitive an indicator of chloroquine-related tissue damage as the determination of the lysosomal acid hydrolases, particularly with regard to renal damage.

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Chronic cadmium intake results in dose-related excretion of metallothionein in urine

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Summary. Urinary excretion of metallothionein was measured by radioimmunoassay in rats given drinking water containing 5 or 50 mg cadmium/l for up to 2 years. The metallothionein levels corresponded to the concentration of cadmium in the drinking water and increased linearly over the course of the study. These results demonstrate that urinary metallothionein is a sensitive biological indicator of oral cadmium exposure.
Key words. Cadmium; metallothionein; urinalysis; chronic exposure.

Cadmium (Cd) is an important occupational toxicant and environmental pollutant. Chronic exposure to this metal results in its progressive accumulation, mainly in liver and kidney and can lead to renal tubular dysfunction characterized by proteinuria, glucosuria and aminoaciduria in experimental animals and man[1,2]. For this reason, it is important to develop a sensitive and reliable biological indicator of exposure to this metal. Several studies have examined the usefulness of urinary metallothionein (MT) as a measure of Cd body burden and also in the determination of Cd-induced renal dysfunction. Elevated levels of urinary MT have been demonstrated in human populations exposed to Cd in the environment[3-5], in occupationally exposed Cd workers[5,7-11], and in rats injected s.c. with cadmium chloride ($CdCl_2$)[5,12-14].

In the present study, the urinary excretion of MT was investigated in rats treated for up to 2 years with Cd-containing drinking water. The purpose of this long-term study was to mimic the human environmental exposure and evaluate whether urinary MT is a biological marker of Cd exposure.

Methods. Male Wistar rats, aged 27 days, were obtained from Charles River Breeding Laboratories, Inc. The animals were divided into three groups of 68 each and given distilled drinking water containing 0 (group 1), 5 (group 2) or 50 (group 3) mg Cd as $CdCl_2$ per liter (Mallinkrodt, Inc., Paris, KY) and commercial laboratory chow (Charles River, RMH 1000) ad libitum. The animals were housed two per cage in large plastic shoebox cages for up to 2 years and cared for in accordance with institutional guidelines. The animals were moved to metabolic cages for urine collection. From 8 rats in each treatment group, 24-h urine specimens were collected over ice, once every 6 to 8 weeks, during the course of the study. Every 3 months, 8 additional rats from each group were sacrificed. Their urines were collected one week before the sacrifice. All urine specimens were stored at −20 °C until analyses.

MT in urine was analyzed by a modification of our radioimmunoassay method described earlier[12]. The modification consisted of substituting Pansorbin (Calbiochem LaJolla, CA), in place of ammonium sulfate, as the precipitating agent for bound antigen.

Linear regression analysis was performed on the data relating urinary MT levels to the duration of exposure. Where group means were calculated, the reported values are arithmetic means and standard errors.

Results and discussion. The water consumption of all animals on various treatment regimens was measured on a weekly basis. The water consumption of rats in group 3 was significantly lower than that of group 2 or control rats. This phenomenon may be ascribed to adversive gustatory or olfactory stimuli associated with Cd-containing drinking solutions[15,16]. The resulting mean weekly Cd intakes of groups 2 and 3 were calculated to be 1.1 ± 0.04 mg/rat and 8.5 ± 0.2 mg/rat, respectively (fig. 1). Therefore, the actual