synthetic, steroid or non-steroid that causes vaginal cornification in rats or mice is designated as estrogen. Therefore in the present investigation, the extended cornified stage observed under β-sitosterol treatment suggests the probable estrogenicity of the compound. Similar findings have been corroborated[8] while observing the effect of *Embelia ribes* on oestrous cycle, wherein 70% of the rats exhibited prolonged cornified stage within 12 days of treatment. This has been attributed to the estrogenic nature of the plant principle.

It is generally accepted that sequential changes of vaginal smear in different phases of the oestrous cycle are closely associated with gonadal steroids which in turn are controlled by the secretion of pituitary gonadotrophins[9]. The observed increase in the pituitary, ovarian and uterine weights as studied by bioassay techniques and the uterotrophic response and cornification of vagina all confirm the estrogenic property of this phytosterol.

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HISTOCHEMICAL STUDY OF THE METABOLISM AND TOXICITY OF MERCURY

D. C. SHARMA*, P. K. SHARMA, K. K. SHARMA, J. S. MATHUR and P. P. SINGH*

Departments of Biochemistry and Pathology, J.L.N. Medical College, Ajmer 305 001, India.
** Department of Biochemistry, R.N.T. Medical College, Udaipur 313 001, India.*

MERCURY and its compounds are well known to be toxic but the precise mechanism of their toxic action is not clearly understood. This knowledge is essential in view of the increased environmental contamination of mercury. Since almost all the proteins and enzymes are potential targets of the mercurials[1], we have used a histochemical method for the demonstration of mercury or mercury-protein complexes in the tissues. Our aim was to study the intraorgan localization of injected mercury at different periods of time after the administration of a toxic dose of mercuric chloride. The information obtained was used to draw inferences about the metabolism, the site, and the mechanism of toxicity of mercury.

Male albino rats of Norway strain (*Rattus norvegicus*), weighing 160–200 g, were injected with mercuric chloride (5 mg/kg body weight) by intraperitoneal route. The control rats received 0.2 ml of 0.85% saline. The animals were then sacrificed in pairs under ether anesthesia after 1,2,3,4,5,6 and 24 h of receiving mercuric chloride. The liver and kidneys were removed, fixed, sections cut, stained for mercury by sulphide-silver method of Timm[2], and another section for histological examination was stained with hematoxylin-eosin.

The injected mercury was demonstrable as brownish-black deposits mainly in the kidney and to a lesser extent in the liver of experimental group rats. However, the distribution varied with time after mercury administration. During the first hour, mercury was found in the glomerular tufts, arterioles and interstitially between the cortical tubules. In the second hour, although mercury was diffusely present throughout the epithelia of cortical tubules, it was mainly concentrated in the glomeruli. By the third hour, mercury staining in the glomerulus persisted, and was also present diffusely in the proximal convoluted tubules, where it was more marked near the luminal margins of the tubular cells. This is in contrast to distal convoluted tubules which showed lighter staining and that too at the tubular epithelium margins. By the fourth hour,

mercury got concentrated in the luminal apices of cortical tubular epithelia while the glomerulus was only faintly stained. By the fifth hour, intense diffuse staining of mercury persisted in the proximal convoluted tubules with only mild luminal marginal staining in the epithelia of distal convoluted tubules (figure 1). By the sixth hour, intense staining in the interstitium of medullary part of renal tubules was observed and after 24 hours mercury staining persisted only in the granular cast material found in the collecting tubules of the medulla.

This histochemical study of renal distribution of mercury suggests that Hg^{2+} is filtered through the glomerulus and then reabsorbed mainly by the proximal convoluted tubules. The apical presence of mercury in distal tubules also points to tubular reabsorption of Hg^{2+}. Although tubular secretion has been suggested as one of the mechanism of handling Hg^{2+} by the kidney[1] this could not be substantiated by the present study since terminal localization of mercury by sixth hour in the interstitium of collecting tubules supports reabsorption, rather than secretion. Thus Hg^{+}, after glomerular filtration tends to be reabsorbed throughout the length of the nephron in the rat and more so in its proximal part. The exfoliation of tubular cells may account for the presence of mercury in the distal tubular casts seen by 24th hour of mercury treatment.

There has been some debate about the cellular site of mercury toxicity[3]. According to one view it is the cell membrane[4], while others suggest subcellular organelles as the possible site[5]. In the present study, histological changes like cloudy swelling, nuclear pyknosis, interstitial edema and proximal tubule cell necrosis were observed which tend to support sub-cellular organelles as the possible site of nephrotoxic action of mercury. These changes were also observed by us in rats dosed with colloidal silver, gold or mercury[6] and by Nicholson *et al*[7] in cadmium and mercury nephrotoxicity in mice. Of particular interest is nuclear pyknosis in view of mercury binding to nucleic acids[8] and reported DNA damage induced by mercury[9]. The cloudy (mitochondrial) swelling correlates well with observed increase in volume densities of the lysosomal and mitochondrial compartments[5]. If sub-cellular structures are the actual site of toxic action of mercury it would lend credence to our hypothesis proposed earlier[10,11] that mercury toxicity is at least partly due to its combination with coenzyme A and resultant interference in CoA functions, as the coenzyme A exists in free and ester forms in every tissue in different intracellular compartments[12].

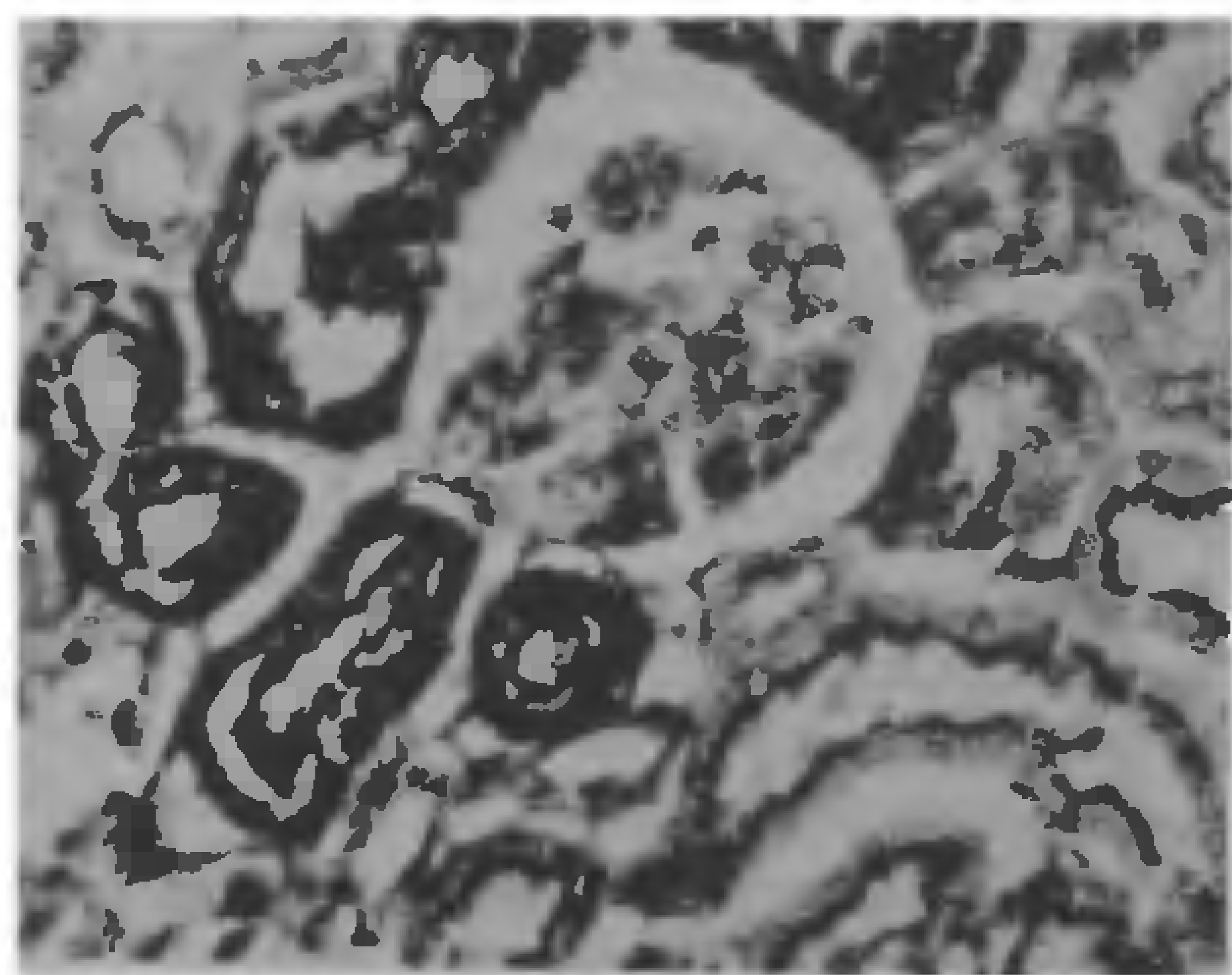

Figure 1. Rat kidney showing intense diffuse brownish-black staining of mercury in proximal convoluted tubules and mild luminal marginal staining in the epithelium of distal convoluted tubules (× 128). Counterstain - safranin.

In the liver of rats with mercury injections, the brownish-black staining due to mercury was present as incomplete branching strands in between hepatocytes representing bile canalicular area which suggests that Hg^{2+} is excreted in bile through the liver. This conclusion is consistent with our recent observations that injected mercury binds with certain amino acids of plasma[13] and cysteine and glutathione of liver[14] and that biliary excretion of Hg^{2+} and methylmercury is important in the elimination of mercury from rats[15].

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EFFECT OF SIMULATED WATER STRESS ON IAA OXIDASE IN GROUNDNUT (*ARACHIS HYPOGEA*) SEEDLINGS

S. T. MATHEWS, V. M. LATHA,
V. N. SATAKOPAN and R. SRINIVASAN
Department of Biochemistry,
P. S. G. College of Arts and Science,
Coimbatore 641 014, India.

A variety of physiological and biochemical changes take place in plants subjected to water stress. Several enzymatic activities are modulated during water stress. These include peroxidase[1] and indoleacetic acid (IAA) oxidase[2]. It is well-known that IAA oxidase activity is responsible for oxidation of IAA, but the function of peroxidase still remains unclear. There are reports that peroxidase may also be involved in the maintenance of satisfactory auxin levels in plants tissues by degrading excess amounts[3]. Much controversy centres round the molecular identities of IAA oxidase and peroxidase due to their close physical association. According to one view the two activities are resident on a single enzyme while another hypothesis suggests that these two are separable and distinct enzymes[4]. Since these enzymes have a regulatory function in auxin metabolism, they have been examined again under simulated water stress conditions.

The G-2 variety of groundnut seeds (*Arachis hypogea*), obtained from the Gujarat Cooperative Oil Seeds Growers' Federation were germinated at room temperature in the normal way. Water stress was created by transferring the one-week-old seedlings to varying osmoticum solutions of polyethylene glycol '6000' for different durations. A range of 4 water potentials (−3, −5, 7.5 and −10 bars) were created by dissolving 11.5, 19.6, 23.5 and 28.9 g of polyethylene glycol '6000' in 100 ml of distilled water respectively[5]. The effect of concentration and temperature on water potential of PEG '6000' solutions differs from those for most salts and sugars and is apparently related to structural changes in the PEG polymer. The weights represent the actual values experimentally verified. The empirical equation given below roughly represents the relationship between water potential, temperature and concentration of PEG.

$$\Psi = -(1.18\times 10^{-2})\, C-(1.18\times 10^{-4})\, C^2 + (2.67\times 10^{-4})\, CT+(8.39\times 10^{-7})\, C^2 T,$$

where Ψ is the water potential in bars, C the concentration of PEG in g/kg of water and T the temperature in degrees celsius. The seedlings were grown under the above mentioned stresses for 24, 72, 120 and 168 h. For control, one-week-old seedlings were grown in distilled water for the same duration. The total peroxidase activity in leaves was measured by the method of Machly[6] and the IAA oxidase activity measured by the method of Darbyshire[7].

The results obtained for the peroxidase activity are shown in figure 1. The peroxidase activity in leaves in the control group increased steadily up to the 7th day. The same pattern was also seen for the test groups as well, but the activity considered for each day was considerably less than that for the control group. The pattern was,

Control > −3 bar > −5 bar > −7.5 bar > −10 bar.

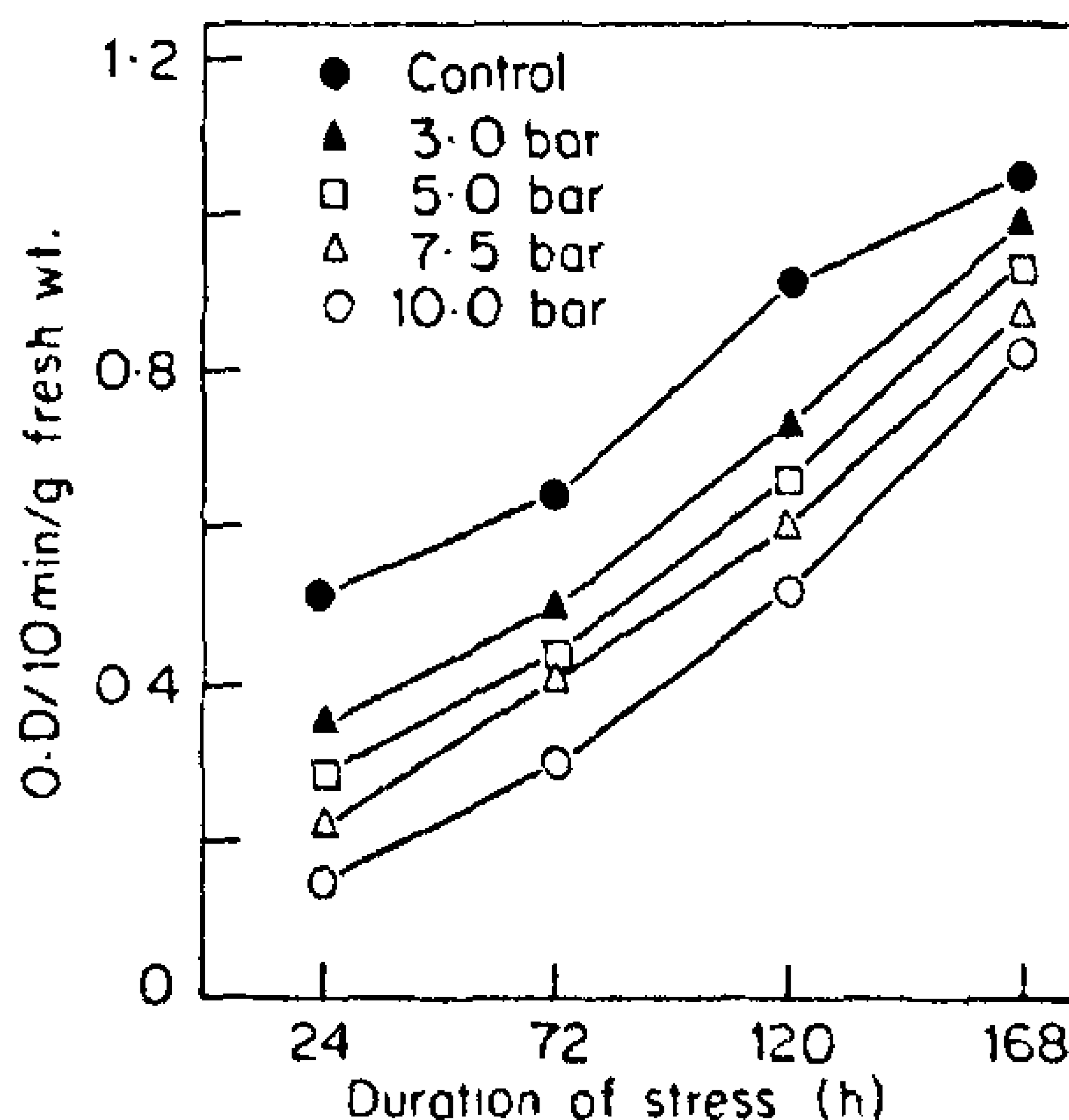

Figure 1. Effect of water stress on peroxidase activity.