ing acutely decentralized muscle are in a state of visible contraction, evidence of their considerable myogenic tone. In fact, during contraction of the muscle with a frequency of 4 Hz the diameter of its arteries may be doubled. We found no differences in the latent period of responses of arteries of different caliber during muscle contraction, as has been observed for vessels of *m. cremaster* of the hamster [6]. Differences in the time course of responses of different vessels, or even different parts of the same vessel, may depend on their state immediately before contraction, which varies because of the presence of vasomotion.

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EFFECT OF CYSTEINE HYDROCHLORIDE AND SULFATE IONS ON MORPHOLOGICAL CHANGES IN THE LIVER IN CHRONIC YELLOW PHOSPHORUS POISONING

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Yellow phosphorus, which is widely used in the national economy, is a highly toxic substance which may cause poisoning. In phosphorus poisoning the liver is particularly severely damaged. Hence the need for effective measures of treatment of phosphorus poisoning, but despite much research, no pathogenic treatment for this serious disease has yet been devised. The search for effective methods of treatment and prevention of phosphorus poisoning that are safe for long-term use is thus a very important task. Cysteine hydrochloride and sodium sulfate have proved promising in this direction [6, 10].

This paper describes a study of the effect of cysteine hydrochloride and sodium sulfate on morphological changes arising in the liver in experimental chronic yellow phosphorus poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on 163 albino rats of both sexes weighing 150-180 g, of which 30 rats served as the control. Animals of group 1 received yellow phosphorus daily by the intragastric route in the form of a solution in sunflower oil in a dose of 1 mg/kg body weight (1/3 LD_{50}), animals of group 2 received phosphorus in the same dose and cysteine hydrochloride in the form of an aqueous solution in a dose of 50 mg/kg body weight, and animals of group 3 received phosphorus in the same dose and an aqueous solution of sodium sulfate in a dose of 25 mg/kg body weight, calculated as sulfate ion. This quantity of sulfate was taken with the drinking water; the concentration of sulfate ions did not exceed the MAC level (500 mg/liter, State Standard 2874-73 for drinking water), and the quantity of cysteine and of sulfate ions was equivalent in sulfur content. For each group there was a corresponding control,

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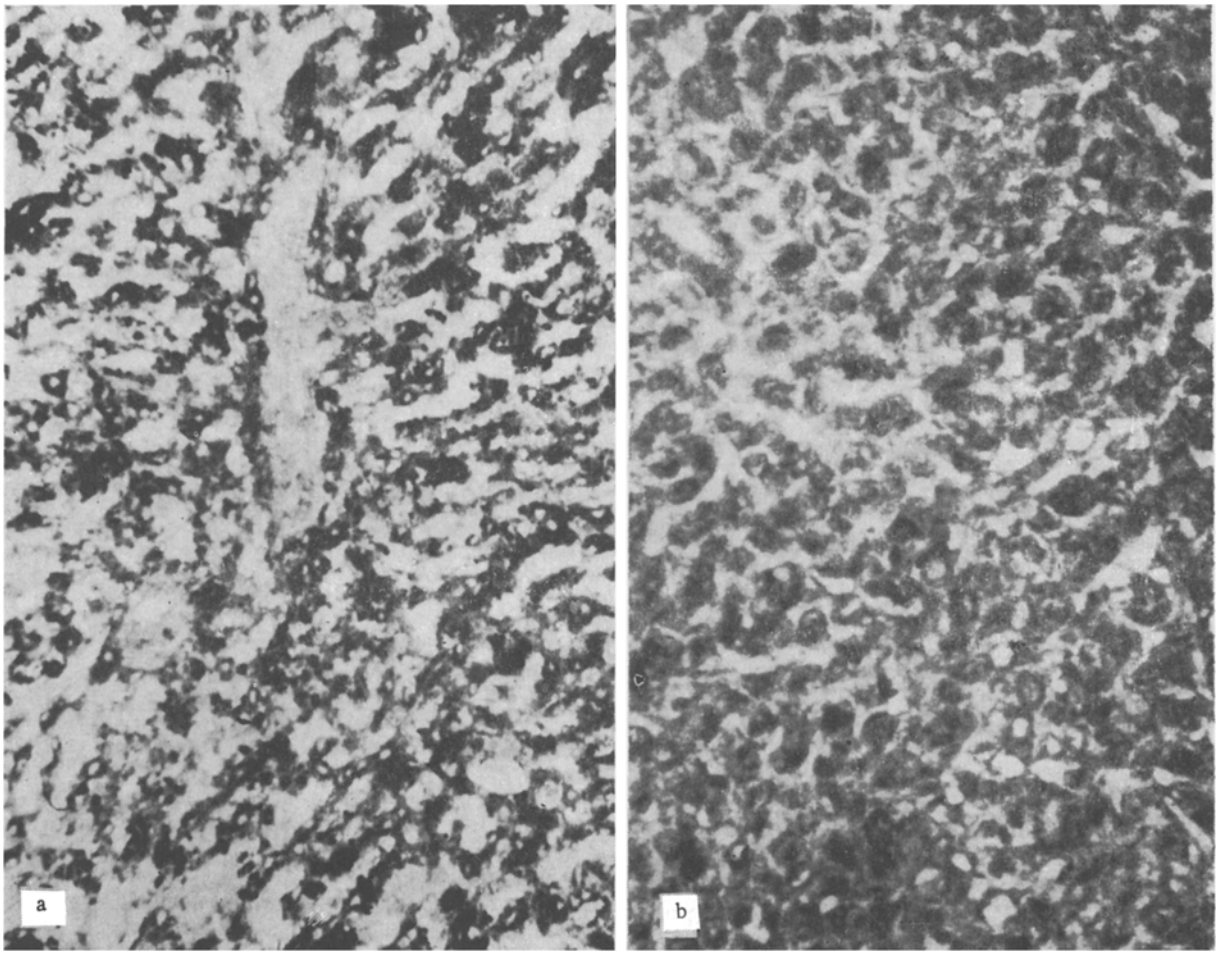

Fig. 1. Chronic yellow phosphorus poisoning, 15th day of experiment. a) Liver of untreated rat: decrease in α-GPDH activity; b) liver of rat treated with sodium sulfate: very slight decrease in α-GPDH activity. Stained by Scarpelli's, Hess's, and Pearse's methods. 200 ×.

namely rats receiving sunflower oil alone by the same mode of administration. The experimental animals and controls were decapitated simultaneously 15, 30, and 45 days and 2, 3, and 4 months after the beginning of the experiment.

Histological sections through the liver were stained with hematoxylin and eosin, by Van Gieson's method, for glycogen by Shabadash's method, and for neutral fat with a mixture of Sudan III and Sudan IV. The following enzymes were studied histochemically: succinate dehydrogenase (SDH), malate dehydrogenase (MDH), NADPH dehydrogenase, lipo-amide dehydrogenase (LADH), β-hydroxybutyrate (β-HBDH), α-glycerophosphate dehydrogenase (α-GDPH), cytochrome oxidate (CCO), ATPase, nonspecific esterases, lipase, acid phosphatase, and phosphorylase. Histochemical detection of the enzymes, cytophotometric determination of the total protein content, and electron-microscopic investigation of the liver were carried out by methods described previously [2, 9].

EXPERIMENTAL RESULTS

The most constant changes demonstrable microscopically in the liver of the rats of group 1 were congestion, punctate fatty degeneration of the hepatocytes of different degrees of severity, and toxic hepatitis, which often was active in character. In some rats 3-4 months after the beginning of the experiment fibrosis and internodular cirrhosis of the liver were either present or in course of formation. Circulatory disturbances were less marked in the later stages of the experiment (3-4 months) than earlier (after 1-2 months), and degenerative and sclerotic changes were predominant.

TABLE 1. Effect of Yellow Phosphorus, Yellow Phosphorus and Cysteine, and Yellow Phosphorus and Sulfate Ions on Total Protein Concentration in Rat Liver (in relative units; M ± m)

Experimental conditions	Time of experiment					
	15 days	30 days	45 days	2 months	3 months	4 months
1. Control (n = 5)	52,556±1,2	52,557±1,1	52,492±1,1	51,998±0,8	52,148±0,7	49,355±0,8
2. Phosphorus (n = 5)	44,280±0,7	48,078±0,6	47,193±0.8	46,929±0,7	61,695±0,7	52,600±0,9
3. Phosphorus + cysteine (n = 5)	53,075±1,0	50,600±0,6	50,077±0,7	49,607±0,7	49,847±0,5	58,241±0.9
4. Phosphorus + sulfate ions (n = 5)	49,741±0,6	51,755±1,1	53,231±1,1	60,556±0,8	52,120±0,5	61,251±1,0
P_{1-2}	<0,001	<0,01	<0,01	<0,01	<0,001	<0,05
P_{2-3}	<0,001	<0,01	<0,05	<0,05	<0,001	<0,01
P_{2-4}	<0,001	<0,02	<0,01	<0,001	<0,001	<0,001
P_{1-3}	>0,10	>0,10	>0,10	>0,05	<0,05	<0,001
P_{1-4}	>0,05	>0,10	>0,10	<0,001	>0,10	<0,001

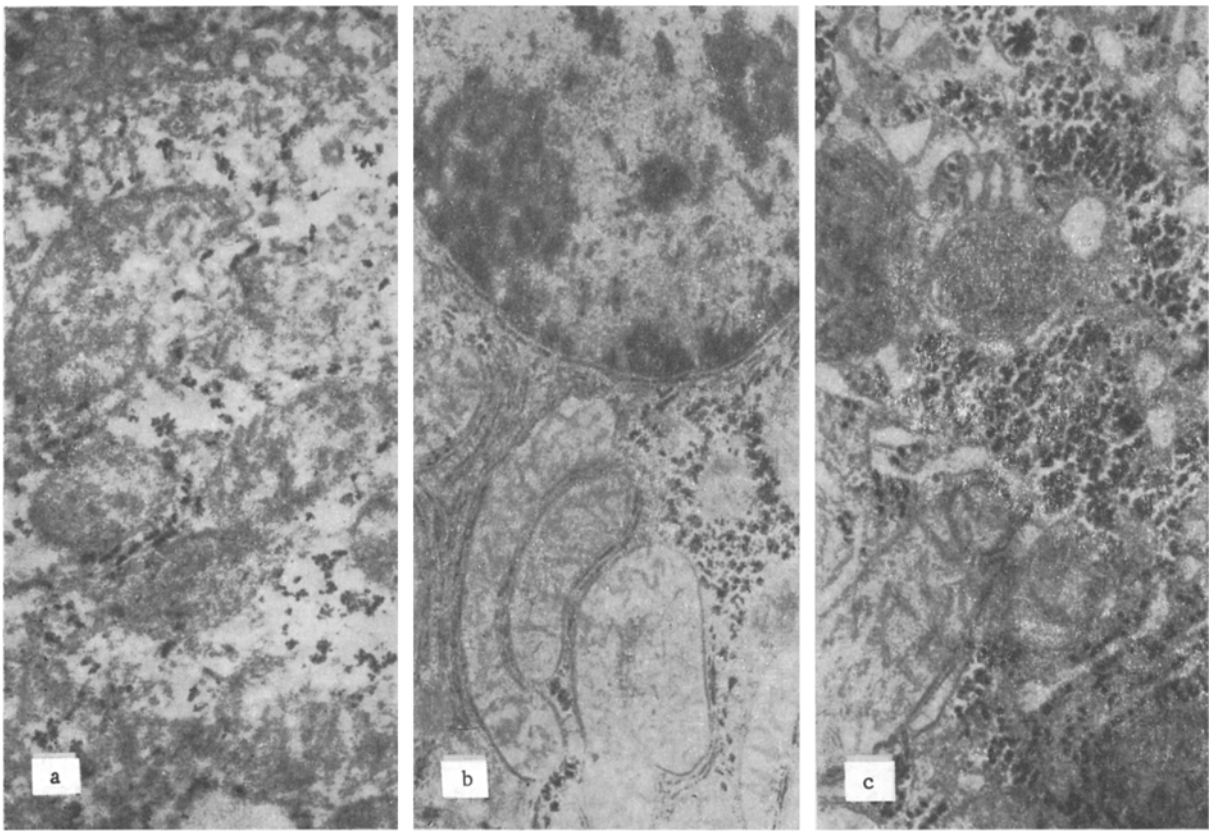

Fig. 2. Chronic yellow phosphorus poisoning, 30th day of experiment. a) Liver of untreated rat: area of destruction of endoplasmic reticulum, with "ghosts" of mitochondria; b) liver of rat receiving cysteine hydrochloride: hypertrophied mitochondria; c) liver of rat receiving sodium sulfate: accumulation of glycogen between mitochondria and structures of endoplasmic reticulum. Magnification: a, b) 26,000, c) 32,000.

Enzyme activity changed in phases in the course of chronic phosphorus poisoning. For instance, activity of SDH, CCO, lipase, NADPH dehydrogenase, MDH, β-HBDH, α-GPDH, and acid phosphatase initially fell, then rose, and fell again (Fig. 1a), with the exception of MDH, whose activity became high again after 4 months. The increase in enzyme activity was probably compensatory and it occurred after 1-2 months. Phosphorylase activity rose initially (after 15 days to 1 month), fell after 45 days, showed another small increase after 2 months, which was followed by a fall. These fluctuations in enzyme activity reflect the phasic course of chronic phosphorus poisoning. Only activity of LADH, nonspecific esterases, and ATPase was inhibited at all times of the experiment. The glycogen concentration also was low at all times of the experiment. Total protein was low from 15 days to 2 months after the beginning of the experiment, but it increased after 3-4 months (Table 1).

Electron-microscopic investigation revealed droplets of fat in the cytoplasm of the hepatocytes at all times of the experiment, accompanied by disturbance of the membrane system of the endoplasmic reticulum and of the structure of the mitochondria of the hepatocytes (Fig. 2a). Later (after 3-4 months) the degenerative changes increased. Elements of the endoplasmic reticulum and mitochondria in dying cells were destroyed and were visible in the form of ghosts; the nuclei were pycnotic. Collagen fibrils could be found in the intercellular space.

At all times of the experiment foci of micronecrosis were found less frequently in the rats of groups 2 and 3 than in the animals of group 1; degenerative changes and circulatory disorders were less marked, whereas regenerative changes were more marked. The toxic hepatitis was less diffuse in character, active hepatitis was less frequently observed, and the sclerotic changes discovered 3-4 months after the beginning of the experiment also were less marked. Both cysteine and sulfate ions prevented the development of cirrhosis of the liver.

Changes in enzyme activity were phasic in character in the animals of groups 2 and 3, just as in the rats of group 1.

In the earlier stages of the experiment (15 days to 2 months) cysteine and sulfate ions sometimes increased, sometimes inhibited enzyme activity, but in the later stages (after 3-4 months) they had a marked activating effect (Fig. 1b). Both cysteine and sulfate ions also had a normalizing action: If enzyme activity was increased, they lowered it. Both substances increased the glycogen and total protein concentrations in the liver in chronic phosphorus poisoning (Table 1), and protein synthesis, as we know, is the principal parameter of the intensity of repair [5, 7, 8]; potentiation of protein synthesis and activation of processes providing the cell with energy are manifestations of compensatory and adaptive reactions of the body [3].

Electron-microscopic studies on animals of groups 2 and 3, just as on the rats of group 1, revealed fat droplets in the cytoplasm of the hepatocytes and collagen fibrils in the intercellular spaces, but these changes were less marked than in the rats receiving phosphorus alone.

Both cysteine and sulfate ions increased the powers of adaptation of the hepatocytes in chronic phosphorus poisoning, but the mechanisms of their action differed. Cysteine protected the mitochondria of the hepatocytes against the action of phosphorus, and potentiated their function. Many swollen and large, twisted mitochondria were found in the hepatocytes under these circumstances (Fig. 2b). Swelling of the mitochondria was accompanied by high functional activity, and the appearance of large, twisted mitochondria is evidence of the increased functional strain on these organelles [1].

The ability of cysteine to potentiate mitochondrial function is probably linked with the fact that it contains a sulfhydryl (SH) group which is easily oxidized and reduced, and protein sulfhydryl groups are found in the composition of the mitochondrial thiol enzymes [4].

Sulfate ions, as electron-microscopic investigations showed, stimulated glycogen accumulation in the cytoplasm of the hepatocytes, and thereby alleviated the course of phosphorus poisoning (Fig. 2c). This was probably connected with the fact that sulfur metabolism is a special function of the Golgi complex, irrespective of the type of cell and class of sulfated substances [12]; polysaccharides also are formed in the Golgi complex and, in particular, the Golgi complex and the smooth part of the endoplasmic reticulum are directly linked with the process of glycogen accumulation [11].

These differences in the mechanism of action of cysteine and sulfate ions were manifested particularly clearly in the early stages of the experiment (15 days to 2 months).

The results thus showed that cysteine and sulfate ions have a favorable action on morphological changes in the liver in chronic phosphorus poisoning: They diminish the circulatory disorders and degenerative and sclerotic changes, they prevent the development of cirrhosis, stimulate processes of regeneration, and increase the powers of adaptation of the hepatocytes.

Cysteine hydrochloride and sulfate ions also acted positively on metabolism in the liver, increased the concentrations of glycogen and total protein, and had an activating and normalizing action on enzymes involved in lipid, carbohydrate, and energy metabolism.

On the basis of these results showing the beneficial action of cysteine and sodium sulfate on experimental phosphorus poisoning, clinical trials of their effectiveness in chronic phosphorus poisoning in man are indicated.

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