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ON THE ACCUMULATION OF MERCURY IN THE BLOOD, LIVER, SPLEEN AND KIDNEY OF *HALOBATRACHUS DIDACTYLUS* SCHNEIDER AND RESULTING HAEMATOLOGIC, CYTOHAEMATOLOGIC AND HISTOPATHOLOGIC ALTERATIONS

O AKUMULACIJI ŽIVE U KRVI, JETRI, SLEZENI I BUBREGU KOD *HALOBATRACHUS DIDACTYLUS* SCHNEIDER I REZULTIRAJUĆIM HEMATOLOŠKIM, CITOHEMATOLOŠKIM I HISTOPATOLOŠKIM PROMJENAMA

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The effects of mercury on the blood, liver, spleen and kidney of *Halobatrachus didactylus* Schneider were studied to contribute to the knowledge of the influence of mercury on fish.

Fish were exposed to 0.1 ppm of mercury ($HgCl_2$) in sea water during 10, 20, 35 and 45 days.

Accumulation of mercury in blood, liver, spleen and kidney was followed. The haematologic, cytohaematologic and histopathologic effects were analyzed.

INTRODUCTION

Fish species can generally accumulate mercury compounds either directly from the sea water or indirectly through throphic chains. Kečkeš and Miettinen (1972) published an extensive revision on the contamination of marine organisms by mercury from the external environment. It is also necessary to emphasize the studies of Pentreath (1976a and b) on the accumulation of inorganic mercury directly from the sea water by plaice, *Pleuronectes platessa* L. This author noticed that plaice rapidly accumulated both forms of mercury. Bouquegeau (1973), and Miettinen, Heyraud and Kečkeš (1972) dealt with this problem, as well.

Histopathologic effects of mercury on the intestine of *Halobatrachus didactylus* Schneider have recently been studied by Gutiérrez, Establier and Arias (1978). Establier, Gutiérrez and Arias (1978) also carried out extensive observations on the accumulation and

histopathologic effects of mercury in golden grey mullet *(Liza aurata* /Risso/).

The present paper deals with mercury accumulation in the blood, liver, spleen and kidney, as well as with the effects of mercury on haematologic characteristics and its histopathologic effects on the blood cells, liver and kindey.

These studies were realized at the Institute for Fishery Researches, Cadiz (Spain), within the Pilot Project »On the effects of pollutants on marine organisms and their populations« of the Joint FAO (GFCM) / UNEP Coordinated Project on Pollution of the Mediterranean.

MATERIAL AND METHODS

The individuals of *Halobatrachus didactylus* used in these observations were caught in the Cadiz Bay (España) and acclimated during 10 days. The experiment was carried out in June and July 1977, at temperatures ranging from 19.5 to 23.6°C, salinities from 36.01 to 36.55‰ and pH from 7.86 to 8.01.

The total lengths of six individuals, one male and five females, treated ranged from 26 to 31 cm and the weights from 310 to 530 g. Two control individuals, one male and one female, showed total lengths of 22 and 23 cm, and weights of 170 and 190 g respectively. Most of the individuals exposed were spent (one was ready to spawn).

Two additional individuals showing total lengths of 17 and 18 cm, and weights of 100 and 130 g were used for cytohaematologic and histopathologic studies, but only at the end of the experiment.

The experiment was realized in the perspex tanks containing 300 1 of sea water sufficiently aerated (oxygen saturation exceeding 80%). Two thirds of the sea water volume were exchanged every 48 hours by natural sea water containing an adequate quantity of $HgCl_2$ to obtain the 0.1 ppm concentration of Hg in the accumulation experiment and by natural sea water in the control tanks. During the experiment fish were fed on crustacean *Carcinus maenas* and flesh of the of hake, *Merluccius* sp. After 10, 20, 35 and 45 days of exposure, the fish were removed from the water and immediately stunned with a blow on the head.

The standardised sampling procedures for blood, as well as for liver, spleen and kidney have been used.

Mercury was analyzed by flameless atomic absorption spectrophotometry (cold vapour techniques) after previous wet digestion of samples at temperature controlled as described in proceeding papers (Establier, 1972; Gutiérrez, Establier and Arias, 1978).

The following haematologic properties were observed: number of erythrocytes, haematocrit and quantity of haemoglobin. Standard techniques were applied.

Very fine extensions of blood were done on the chemically clean and dry slides. The dried extensions were fixed and coloured by methods of May-Grünwald-Giemsa, although Gutiérrez Citopancrom (1962, 1967) was generally applied. For histopathologic studies small pieces

of liver and kidney were fixed in alcoholized Bouin, dehydrated and included in parafin of M. p. at 56°C to obtain sections of 5—7 μ. Colouring was performed by haematoxylin and Harris' eosin according to the usual techniques. The preparations were included in the synthetic resin.

ANALYSIS OF THE RESULTS

a) Accumulation

The accumulation of total mercury in the blood, liver, spleen and kidney of only two specimens were analysed after 10, 20 and 35 days' treatment. Fish were analysed individually.

The mean values of mercury accumulation are shown in Table 1. The data show a progressive accumulation of total mercury in the blood, as well as in the liver, spleen and kidney. The maximum accumulation took place in the liver. However, mercury content is higher in the kidney than in the liver with reference to dry weight (higher content of water in the kidney).

Table 1. Total mercury content (mean values) expressed in mg/kg (ppm), in control and treated individuals of *Halobatrachus didactylus* Schneider

Days of treatment	Total length cm	Blood		Liver		Spleen		Kidney	
		wet	dry	wet	dry	wet	dry	wet	dry
				Control					
35	22 and 23	0.05	0.37	0.55	1.67	0.33	1.19	0.48	2.67
				Treated					
10	29 and 31	1.59	11.82	8.79	26.06	12.62	48.35	10.11	55.08
20	28 and 31	2.73	20.49	42.31	133.97	24.23	91.09	24.18	140.99
35	26 and 29	3.69	26.47	70.86	209.64	37.50	139.88	39.84	227.90

b) Effects on haematologic characteristics

The data were collected after 10,20 and 35 days (Table 2).

The lowest value of erythrocyte number and haematocrit was recorded after 10 days' exposure and the highest after 35 days' exposure. However, the specimen which showed the highest values was smallest in total length and it was male. This male excluded, the erythrocyte numbers ranged from 0.37 to 0.52 $\times$ 10^6/mm^3, the haematocrit from 18.0 to 25.8 % and the haemoglobin from 3.5 to 5.6 g.

The haemoglobin values recorded after 20 days' exposure were somewhat lower than those recorded after 10 days of exposure.

Numbers of erythrocytes found in control individuals were rather high and, to some extent, also haemoglobin quantity. Conversely, haematocrit values were relatively low. However, control fish were smaller. The data on them were not consistent with those on the shortest exposure time (10 days).

Table 2. Data on haematologic characteristics in control and treated individuals of *Halobatrachus didactylus* Schneider

Days of treatment	Total length cm	Weight g	Sex	Erythrocyte number $10^6/mm^3$	Haema-tocrit %	Haemoglobin quantity g
			Control			
35	23	190	♂	0.60	22.4	4.8
	22	170	♀	0.72	22.3	6.4
			Treated			
10	29	400	♀	0.37	18.0	4.8
	31	530	♀	0.50	23.2	5.6
20	31	460	♀	0.50	22.7	4.1
	28	310	♀	0.42	25.8	3.7
35	29	370	♀	0.52	23.7	3.5
	26	330	♂	0.71	28.8	7.2

c) Histopathologic and cytohaematologic effects

The observations were carried out after 10, 20, 35 and 45 days.

The cyto-histopathologic details for liver are shown in Figures 1 and 2, those for kidney in Figures 3 and 4, and those for blood in Figures 5 and 6.

The histopathologic effects were generally most obvious in the kidney which showed the most important alterations. It may be seen that there was a progressive vacuolization with tubular degeneration and depolarization of the nuclei, which caused a disorder in the normal uniform pattern of nuclei and cytoplasm appearance in the renal tube cells.

In the liver there were recorded the signs of tumefaction, vacuolization with an increase in the number of nuclei, marked reticulation and irregular disposition of nuclei.

Cytohaematologic observations limited to the morphology of the erythrocytes showed a slight erythroanisocytosis with isolated erythrohypochromia and a tendency to fragmentation with formation of erythroplastids.

CONCLUSIONS

A progressive accumulation of mercury was recorded in the blood, liver, spleen and kidney of *Halobatrachus didactylus* Schneider exposed for 10, 20 and 35 days.

The values recorded pointed to individual differences.

The values expressed in ppm which refer to dry weight were the highest for kidney (55.1, 141.0 and 227.9; control 2.7) and then for liver (26.1, 134.0 and 209.6; control 1.7); the values for spleen (12.6, 24.2 and 37.5; control 0.3) and blood (11.8, 20.5 and 26.5; control 0.4) were much lower.

The data on haematologic characteristics seem rather inconsistent. This inconsistency might be due to small numbers of exposed and control individuals.

The most important cytohaematologic alterations included slight erythroanisocytosis, isolated erythrohypocromia and tendency to fragmentation.

A tumefaction, vacuolization and an increase in the number of nuclei with their irregular disposition took place in the liver.

A progressive vacuolization with depolarization of nuclei and accumulation of eosinophilic detritus obstructing the lumen of renal tubes was recorded in kidney.

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KRATAK SADRŽAJ

Izvršena su opažanja o akumulaciji žive u krvi, jetri, slezeni i bubregu kod ribe *Halobatrachus didactylus* Schneider, kao i o njezinim hematološkim, citohematološkim i histopatološkim efektima.

Opažanja su izvršena u lipnju i srpnju 1977, pri temperaturi od 19,5 do 23,6°C, salinitetu od 36,0 do 36,6‰ i pH od 7,9 do 8,1.

Upotrebljeno je ukupno 6 odraslih primjeraka, jedan mužjak i pet ženki a dijelom i dodatna dva juvenilna primjerka. Njihova totalna dužina kretala se od 26 do 31 cm, a težina od 310 do 530 g.

Volumen morske vode iznosio je 300 l, od čega je 2/3 zamjenjivano svježom vodom svakih 48 sati. Koncentracija žive održavana je na 0,1 ppm upotrebom koncentrirane otopine živina klorida.

Opažanja o akumulaciji žive kao i ona o hematološkim i histopatološkim promjenama izvršena su nakon ekspozicije ribe u trajanju od 10, 20 i 35 dana. Citohematološka i histopatološka izučavanja provedena su i nakon 45 dana ekspozicije na dodatna dva juvenilna primjerka totalne dužine od 17 i 18 cm i težine od 100 i 130 g.

Krv i promatrani organi pokazali su slijedeće vrijednosti akumulirane žive: 0,37—26,47 ppm u krvi; 21,67—209,64 ppm u jetri; 1,19—139,88 ppm u slezeni te 2,67—227,90 ppm u bubregu. Vrijednosti se odnose na suhu težinu.

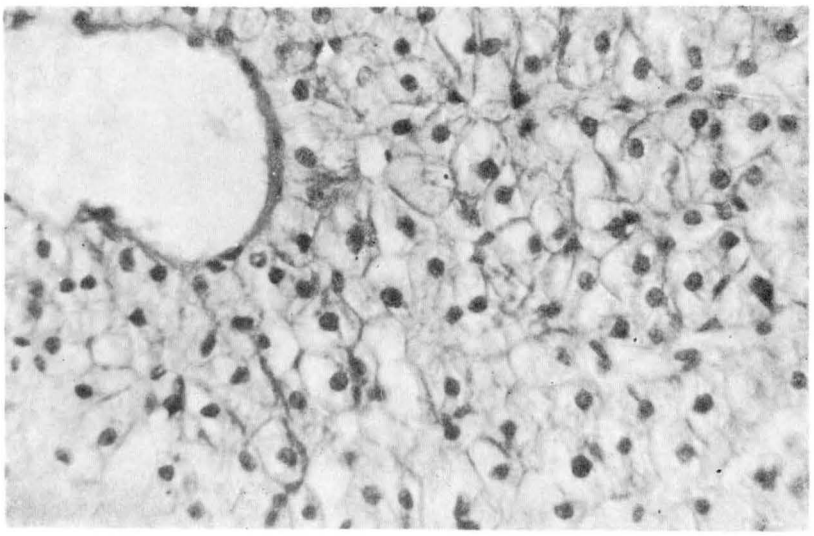
Od hematoloških karakteristika razmatrani su broj eritrocita, hematokrit i količina hemoglobina. Upotrebljene su standardne hematološke metode.

Broj eritrocita kretao se od 0,37 do 0,71 $\times$ 10^6/mm^3, vrijednosti hematokrita od 18,0 do 28,8%, a količina hemoglobina od 3,5 do 7,2 g.

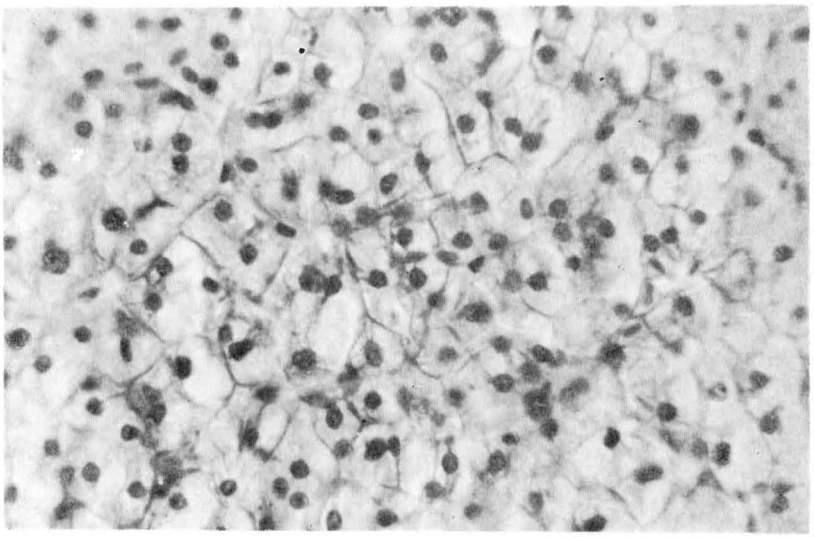
Ustanovljene su lagane degenerativne promjene u obliku eritrocita, dijelom smanjena količina krvne boje (hipohromija), a zabilježena je i tendencija njihova raspadanja (stvaranje eritroplastida) nakon 45 dana izloženosti ribe živinu kloridu.

Zapaženi su znakovi oticanja jetre, vakuolizacija uz povećanje broja jezgara i njihov nepravilni raspored.

U bubregu opažena je progresivna vakuolizacija, nepravilni raspored jezgara, zatvaranje bubrežnih kanala i njihovo propadanje.

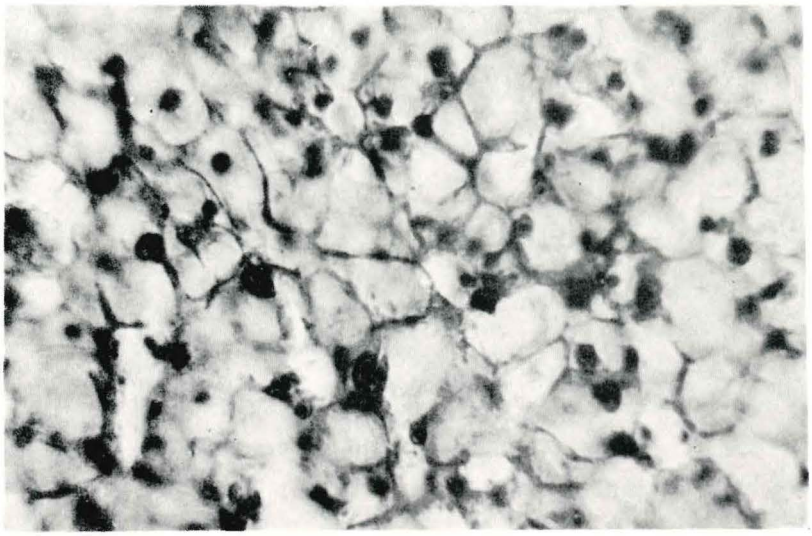


a



b

Fig. 1 a and b. Liver of control individuals. Normal disposition of the hepatocytes with more or less granular cytoplasm and sharp limits. Reticulate tissue slight. X 250



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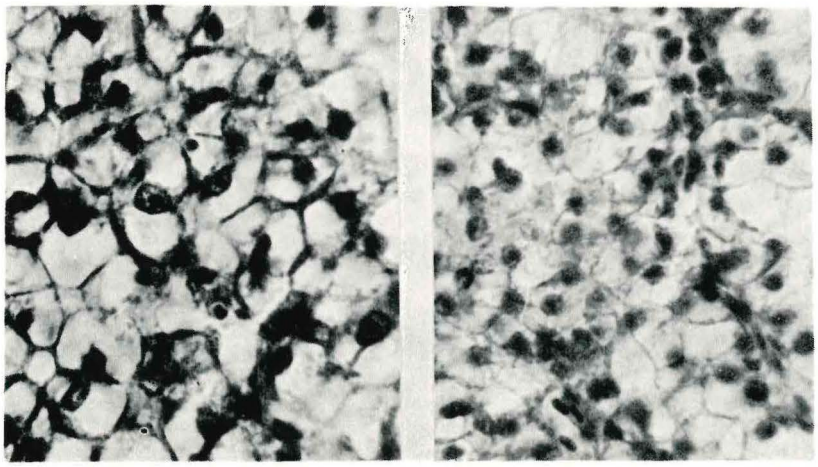
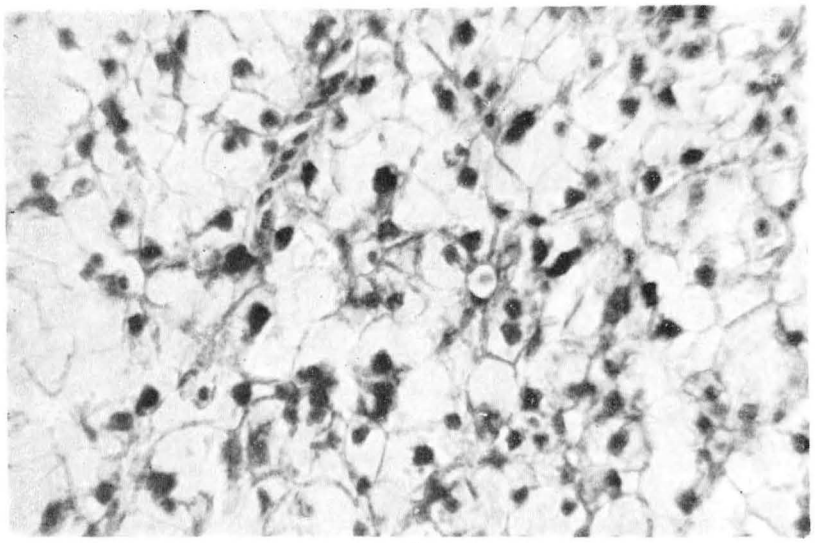
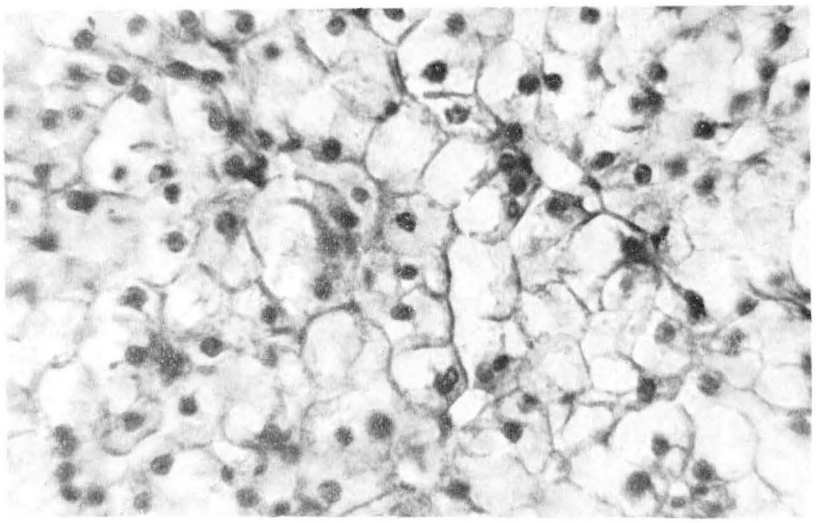


b *c*

Fig. 2 a—c. Liver of individuals treated by mercury
a) during 10 days. An increase in the number of the nuclein, strengthening of the cellular limits and some degree of disorganization.
b) and c) during 20 and 22 days respectively. The anomalous characters more marked. X 250

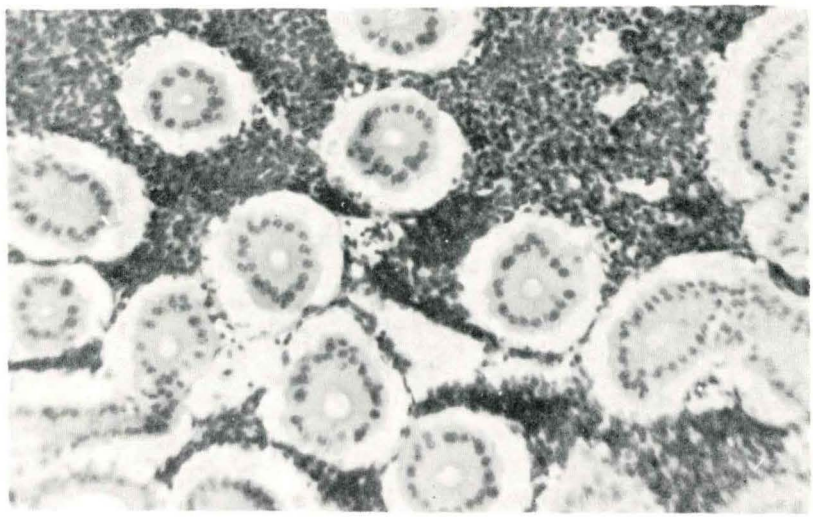


d



e

Fig. 2 d and e. Liver of individuals treated by mercury
d) and e) during 35 and 45 days respectively. The alterations well marked, especially an increase in number of nuclei and a disorganization. X 250



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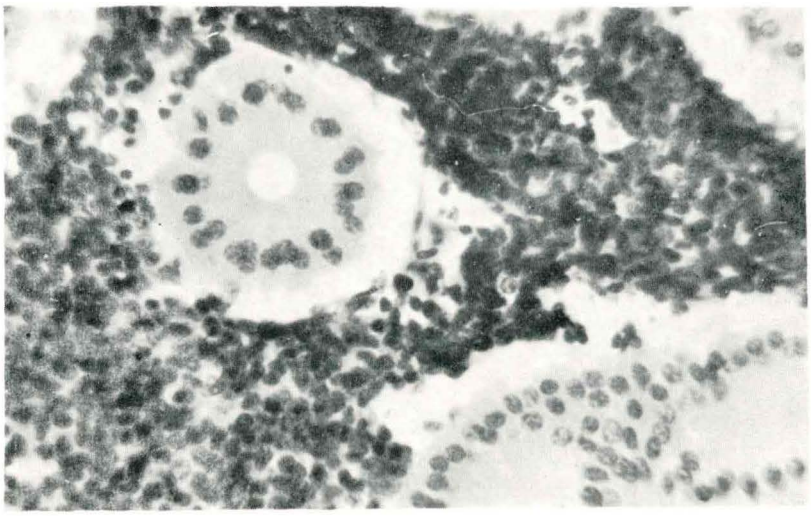
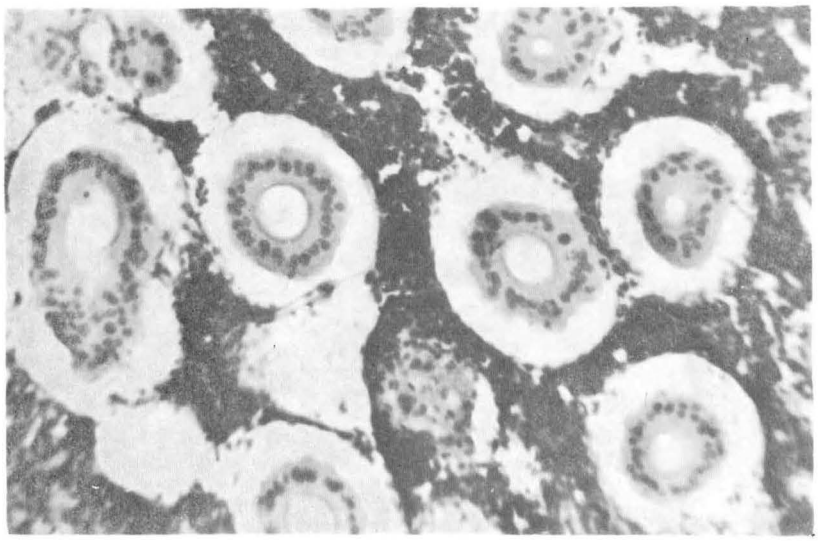


b

Fig. 3 a and b. Kidney of control individuals. Normal disposition of hemocytopoetic tissue between the tubes, with lumens, uniform cytoplasmas and good polarization of nuclei. X 125 and X 250 respectively



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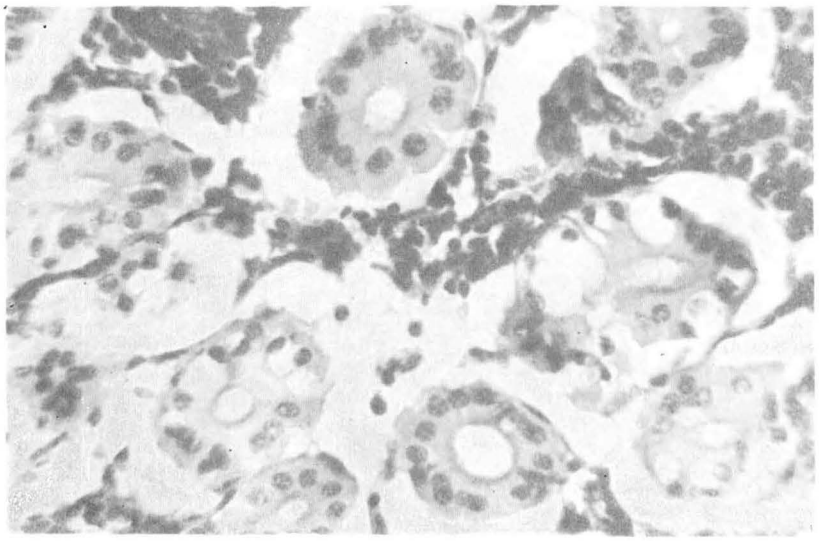
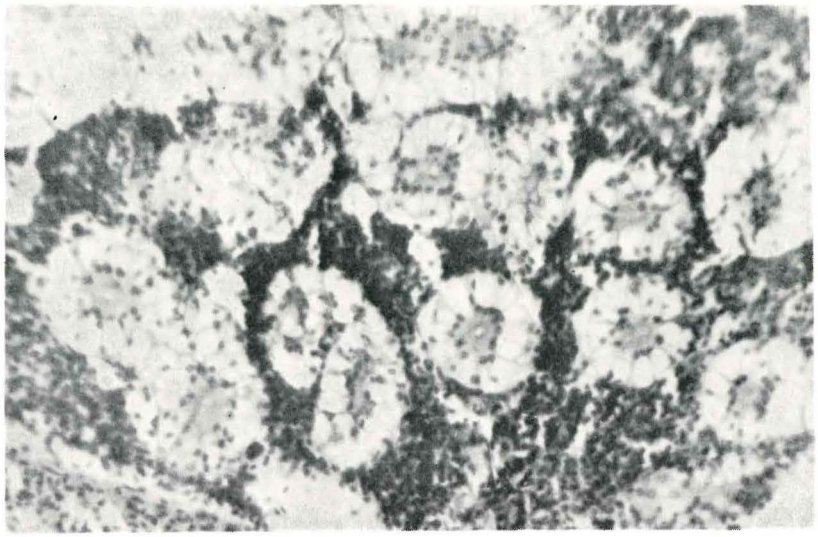
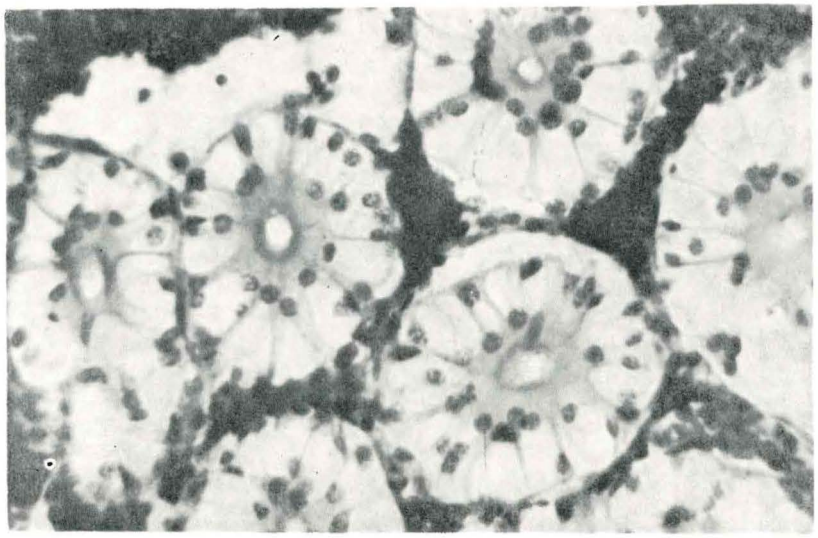


b

Fig. 4 a and b. Kidney of individuals treated by mercury
a) during 10 days. Slight depolarization of nuclei in some tubes.
b) during 20 days. Among normal tubes some with signs of vacuolization and lumens with eosinophilous material. X 125 and X 250 respectively

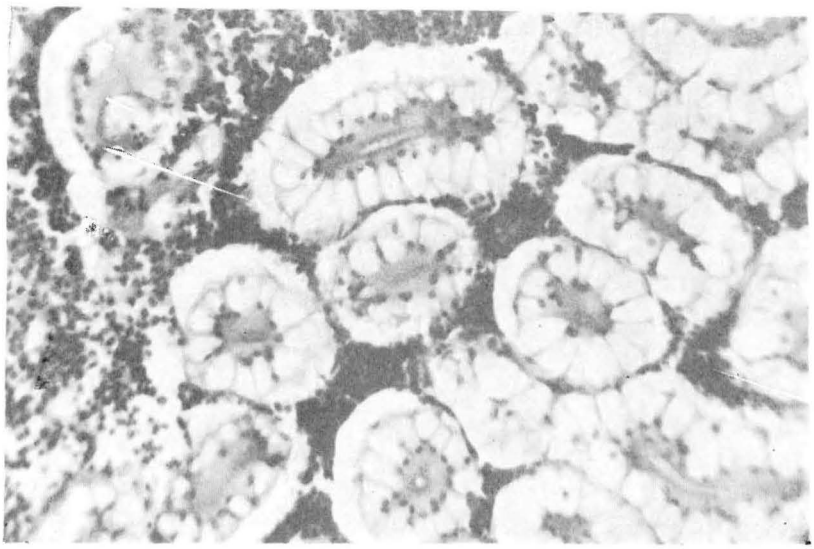


c



d

Fig. 4 c and d. Kidney of individuals treated by mercury
c) and d) during 22 days. Large depolarization of nuclei, marked vacuolization and lumens with abundant eosinophilous material in all the tubes. X 125 and X 250 respectively



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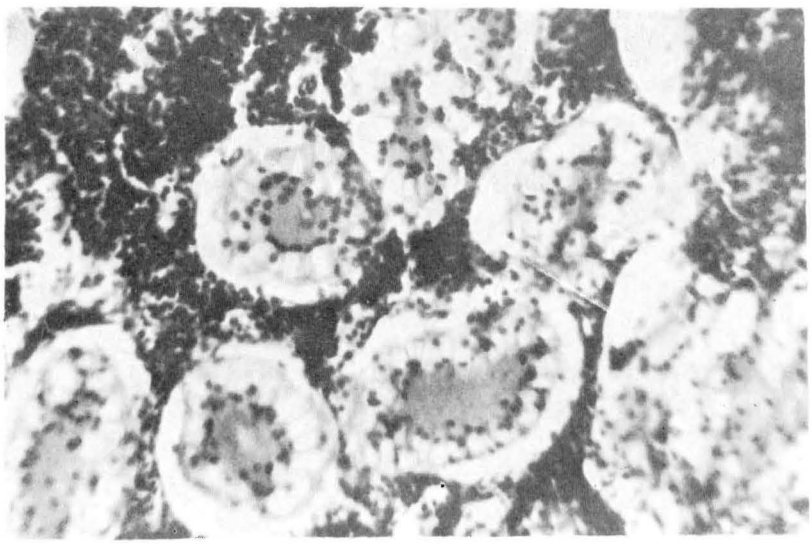
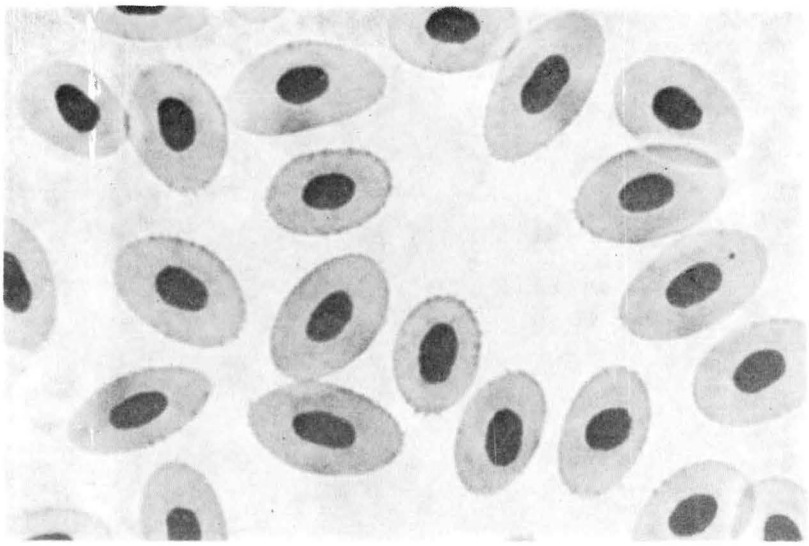
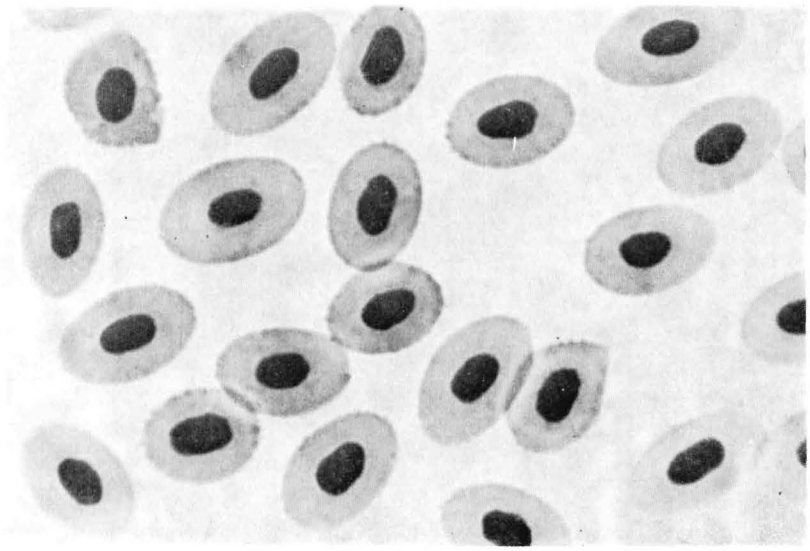


f

Fig. 4 e and f. Kidney of individuals treated by mercury
e) and f) during 35 and 45 days respectively. The alterations more marked, especially after 45 days, obstruction of tubes and destruction of cullular limits, this resulting in the disorientated nuclei. The abundant hemocytopoetic tissue between the tubes. X 125

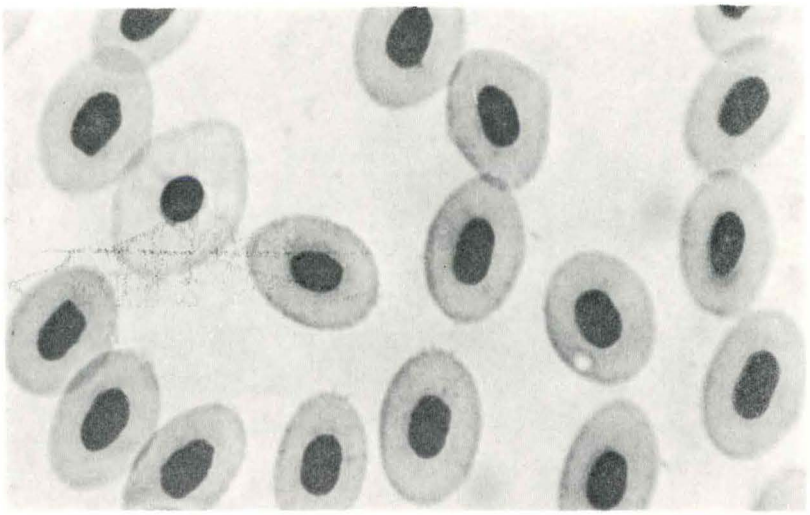


a

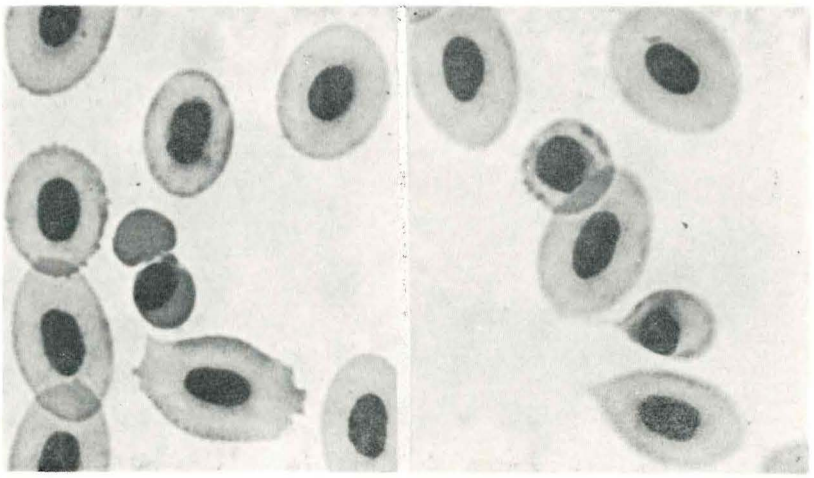


b

Fig. 5 a and b. Blood of control individuals. Isolated erytrocytes with normal characters. Generally isoerythrocytosis with isoerythrocromia. X 625



a



b *c*

Fig. 6 a—c. Blood of individuals treated by mercury.
a) b) and c) during 45 days. Hypoerythrocromia, anisoerythrocytosis and in isolated areas a tendency to vacuolization and fragmentation with formation of erythrocytes without nuclei (erythroplastids). X 625