curve *b*). No recognizable band appeared in the vicinity of 3650 cm^{-1} when the sample film was scanned at a rotation of 45°. However, a shoulder appeared between 3600 cm^{-1} and 3700 cm^{-1} which may indicate the presence of a small amount of hydroxyl perpendicular to the cleavage plane of the oxidized biotite. The decrease in intensity of the band near 3380 cm^{-1} on rotating the spectrum 45° may suggest that the water molecules at the immediate surface of the mineral are well oriented (*7*). It is difficult to recognize the presence of the band at 3584 cm^{-1} in the spectrum of the oxidized biotite (Fig. 1, curve *a*); this appeared to be the strongest band in the untreated biotite spectrum (Fig. 1, curve *A*). This band may still be present in the oxidized biotite but may be overlapped by the increase in intensity of the band at 3540 cm^{-1} as a result of oxidation, or it may have disappeared during oxidation; presumably, it is also associated with the ferrous iron in the structure.

Farmer *et al.* (*3*, *4*) assigned the band of a weathered biotite flake at 3550 cm^{-1} to the OH groups associated with the ferric iron; the principal band of the unoxidized biotite at 3658 cm^{-1} was ascribed to OH associated with ferrous iron. Farmer *et al.* assigned this band (3658 cm^{-1}) to $(Mg_2Fe)^{2+}$, $(MgFe_2)^{2+}$, and $(Fe_3)^{2+}$ groupings, but they were unable to resolve individual bands. With the aid of thinner oriented films recorded at × 5 scale expansion, we obtained more detailed spectra of these biotite samples in the region of the OH stretching vibrations.

On the basis of the results reported here and by others, it may be concluded that the OH bands of the untreated biotite at 3675 cm^{-1}, 3655 cm^{-1}, and 3640 cm^{-1} may be assigned to OH associated with the groupings $(Mg_2Fe)^{2+}$, $(MgFe_2)^{2+}$, and $(Fe_3)^{2+}$, respectively, in the octahedral layer. These OH groups are oriented perpendicular to the cleavage plane of the biotite crystal. The OH band of the untreated biotite at 3550 cm^{-1} indicates the presence of ferric iron. The band of the untreated biotite at 3587 cm^{-1} may be due to the OH groups associated with other cations such as Al^{3+} (*3*). Oxidation of ferrous to ferric iron in biotite by sodium hypochlorite results in the disappearance of the OH bands associated with the ferrous iron; the band at 3540 cm^{-1} is the only significant lattice OH stretching vibration observed in the spectrum of the oxidized biotite.

Only partial oxidation of ferrous to ferric iron occurred when the biotite powder sample was treated repeatedly at 80°C with 30 percent H_2O_2 containing 0.2*M* KCl. The bands at 3655 cm^{-1} and 3675 cm^{-1} persisted after H_2O_2 treatment (Fig. 2, curve *a*). However, their intensities relative to that of the band at 3550 cm^{-1} were reduced considerably (Fig. 2, curve *b*) as compared with those of the untreated biotite (Fig. 1, curve *B*). This suggests that the presence of an excess amount of K^+ in the solution during the oxidation process would prevent layer expansion of the biotite. Thus, only the Fe^{2+} ions present at the broken edges of the octahedral layer or in other easily accessible sites have been oxidized to Fe^{3+} under this condition, whereas, in the case of oxidation with sodium hypochlorite, the Na^+ ions may replace the interlayer K^+ and cause the layers to expand. Consequently, the Fe^{2+} ions inside the octahedral lattice were made more available for oxidation.

A. S. R. Juo, J. L. White
Department of Agronomy, Purdue University, Lafayette, Indiana 47907

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Oral Contraceptives: Long-Term Use Produces Fine Structural Changes in Liver Mitochondria

Abstract. *Liver biopsies from two groups of women receiving oral contraceptives from 1 to 6 months and from 12 to 30 months, respectively, were examined by electron microscopy. The fine structure of the liver cells from the first group appeared normal, except for an increase in the amount of smooth endoplasmic reticulum. In the second group, however, there were many cases with striking changes—altered shape, increase in size, and presence of paracrystalline inclusions—in the mitochondria. No significant correlation between mitochondrial changes and alterations in liver function was detected.*

Chronic administration of oral contraceptives can occasionally interfere with various aspects of hepatic function, resulting in a decreased excretory capacity for bromosulfonphthalein (BSP), cholestatic jaundice, and a transient increase in serum aminotransferases (*1*). Little data is available, however, on the fine structure of the hepatic cell during long-term treatment with these hormonal agents. We studied two groups of women who regularly had used oral contraceptives (*2*). In group 1 (15 patients) the treatment lasted from 1 to 6 months, while in group 2 (13 patients) treatment was from 12 to 30 months. All the women (ages 19 to 40 years) were clinically normal. None had a history of chronic drug ingestion, heavy alcohol intake, or malnutrition.

To evaluate the liver function the following standard clinical tests were performed: serum bilirubin, serum aminotransferases, alkaline phosphatase concentrations and BSP excretion (45-minute retention and the clearance constant K_1). A liver biopsy was obtained from each patient with the Menghini needle. No patients were biopsied during menstruation, but otherwise the samples represented all stages of the cycle. The tissue was fixed in 4 percent formaldehyde for light microscopy, and in 2 percent osmium tetroxide buffered with Veronal acetate for electron microscopy (*3*). Sections embedded in Epon (*4*) were stained with uranyl acetate and lead citrate (*5*).

Liver-function tests in both groups were normal except for BSP excretion, which, in a few cases, was prolonged. No significant difference was found when both groups were compared statistically. By light microscopy, sections of the hepatic tissue appeared normal in all cases. By electron microscopy,

the hepatic parenchymal cells in most of the cases in group 1 showed a moderate increase in the development of the smooth endoplasmic reticulum. We also observed an increase in the number of peribiliary dense bodies and lipofucsine droplets. The biliary canaliculi appeared normal and the mitochondria were regular in size and shape; in rare instances, however, some mitochondria showed a moderate polymorphism and disarrangement of cristae. The moderate increase in smooth endoplasmic reticulum observed in this group may be related to the induction of detoxifying enzymatic mechanisms which is known to occur with the long-term administration of many drugs (*6*).

In 8 of the 13 patients in group 2, who had received contraceptives for more than a year, the mitochondria showed striking changes (Figs. 1 and 2). The size and shape of these organelles was highly variable, with some mitochondria measuring up to 12 μ in length. Most of them had an increase in matrix density and a reduced number of cristae. Almost all of these mitochondria had numerous intramatrical paracrystalline inclusions. The inclusions consisted of closely packed parallel sheets, tubules, and bundles of filaments. In six of the eight cases showing mitochondrial changes, most of the liver cells were affected. In the other two, the extent of the mitochondrial changes varied from cell to cell. No marked changes were noted in the smooth endoplasmic reticulum from liver cells in group 2.

The remaining five cases from group 2—persons whose liver cells had normal mitochondria—appeared similar to those of group 1, in regard to proliferation of the smooth endoplasmic reticulum and increase in number of peribiliary lysosomes.

Mitochondrial changes in group 2, as well as the previously noted changes in the smooth endoplasmic reticulum in group 1, were found in patients receiving either sequential or combined treatment. In addition, these changes were found in biopsies taken at different times in the menstrual cycle. We did not observe more pronounced mitochondrial changes in patients who had received the contraceptives for longer times. For example, one case after 30 months showed no mitochondrial changes, while in two other cases the changes were striking after 1 year of treatment.

The mitochondrial changes reported here cannot be considered specific. Similar changes have been seen in liver mitochondria from patients with acute alcohol intoxication (*7*), viral hepatitis (*8*), and various other types of liver damage (*9*). Experimental alcohol intoxication in rats (*10*) leads to similar mitochondrial changes which are reversible if alcohol administration is stopped (*11*). Rare and mild forms of mitochondrial alterations have been reported in cells from apparently normal human liver (*12*). The presence of numerous mitochondria with paracrystalline inclusions has been considered (*13*) to be an expression of liver damage. In this respect, it should be pointed out that the extent of the changes observed in group 2 far exceeded the proportion of cells and mitochondria affected in most of the previous reports of induced mitochondrial changes. In our limited series of patients, the striking mitochondrial changes could not be correlated with an alteration in liver function as assessed by the clinical tests performed. However, considering the large reserve capacity of the liver, and the limited sensitivity and functional specificity of currently used clinical liver tests, one should not rule out the existence of biochemical changes at the cellular level. The mechanism leading to these mitochondrial changes is unknown. Steroids like the synthetic hormones contained in contraceptive pills are metabolized by the liver (*14*), but systemic administration undoubtedly involves extensive endocrine changes and it is possible that the liver effect may be only an indirect result of drug administration.

These findings leave several questions open to investigation. They concern not only the mechanism of production of the mitochondrial changes but also their reversibility, the existence of fine structural changes in other tissues, and the possibility of producing these mitochondrial changes experimentally. It should be also determined if similar changes are present in other circumstances where steroid concentrations are increased, such as pregnancy or endocrine tumors.

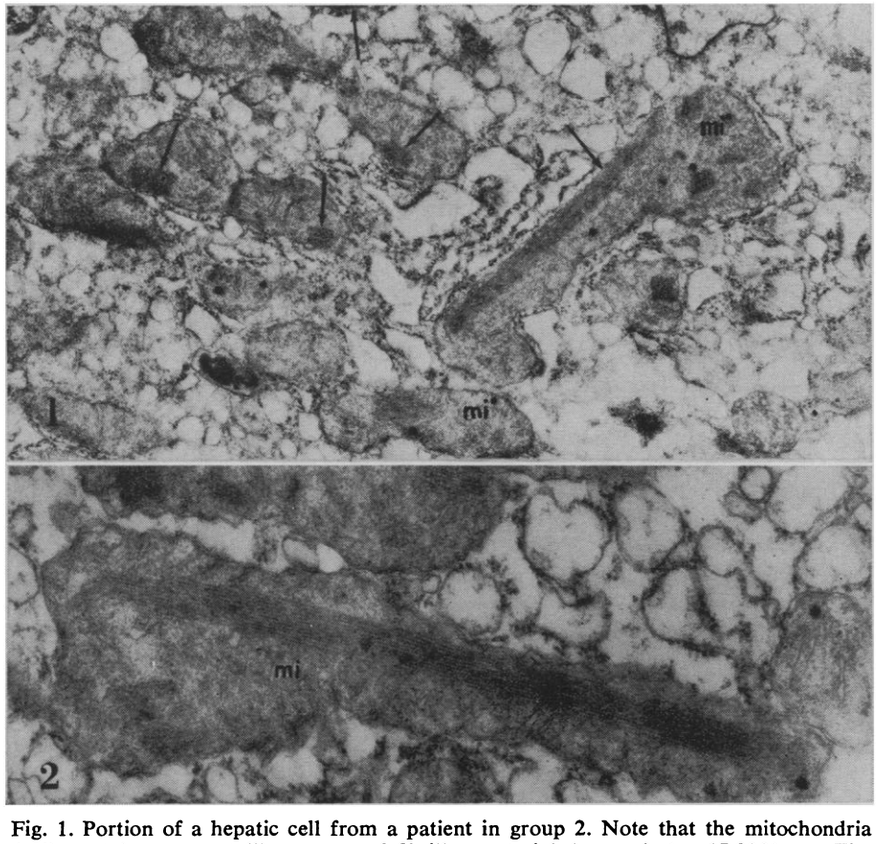

Fig. 1. Portion of a hepatic cell from a patient in group 2. Note that the mitochondria (*mi*) contain paracrystalline arrays of fibrillar material (arrows) (× 17,330). Fig. 2. Higher power of a mitochondrion (*mi*) from material similar to that in Fig. 1, showing in more detail the fibrillar inclusions (× 34,000).

VICTOR PEREZ, S. GOROSDISCH
Instituto Nacional de la Salud Haedo, Buenos Aires, Argentina
J. DE MARTIRE, R. NICHOLSON
G. DI PAOLA
Facultad de Medicina, Universidad Nacional de Buenos Aires, Buenos Aires

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Renal Concentrating Mechanism: Possible Role for Sodium-Potassium Activated Adenosine Triphosphatase

Abstract. *Sodium-potassium activated adenosine triphosphatase activity was found to be almost twice as high in renal medulla as in cortex. Infusion of digoxin, a specific inhibitor of the enzyme, into one renal artery of the dog resulted in unilateral natriuresis, impaired concentrating capacity, and reduction of the enzyme activity in both cortex and medulla. It is suggested that the sodium-potassium adenosine triphosphatase plays an important role in urine concentration mechanisms.*

It is well established that the sodium-potassium activated adenosine triphosphatase (Na^+,K^+-adenosine triphosphatase) is intimately related to the transport of sodium across most biological membranes (*1, 2*). The kidney tubular epithelium is no exception. When cardiac glycosides, specific inhibitors of this enzyme, are injected into the renal circulation of the dog, inhibition of net sodium reabsorption occurs (*3*). The sodium diuresis that results from intrarenal administration of these preparations has been attributed to inhibition of sodium reabsorption in portions of the nephron localized in the cortex (proximal and distal convolutions) (*3*). We have recently demonstrated (*4*), however, that in the dog digoxin inhibits sodium transport in the ascending limb of Henle's loop, since it diminished both free water clearance (C_{H_2O}) and free water reabsorption ($T^c_{H_2O}$). Since the loop of Henle is anatomically located in the medulla, our results suggested that if the Na^+,K^+-adenosine triphosphatase is a receptor for cardiac glycosides, the enzyme may be of primary importance in the mechanism of sodium transport in the medulla. Therefore, this enzyme system may contribute significantly to renal concentrating and diluting functions.

Mongrel dogs of either sex were utilized for all the experiments. Under pentobarbital anesthesia, each ureter was cannulated through a suprapubic incision. Through a flank incision, a curved needle was introduced into the left renal artery, into which normal saline or saline containing digoxin in a concentration of 0.01 mg/ml was infused at 1 ml/min. In four control dogs the saline infused into the renal artery did not contain digoxin. A comparison between the results in these control animals and the experimental animals demonstrated that the infusion of digoxin into one kidney did not significantly affect the contralateral organ either physiologically or biochemically; therefore, the latter is adequate as an internal control. All animals received 5 units of Pitressin in oil intramuscularly 1 hour prior to the experiment in addition to aqueous Pitressin (60 milliunits per kilogram of body weight per hour) intravenously, throughout the duration of the experiment. The clearance of iothalamate iodine-125, present in the sustaining infusion, was utilized as an index of glomerular filtration rate (*5*). All animals were undergoing a modest hypertonic (10 percent) mannitol diuresis; infusion rate was 1.0 ml/min. Control urine samples were obtained from each kidney, after which the infusion of digoxin was begun. An increase in urine flow in the experimental kidney was maximum between 60 and 105 minutes after the infusion of digoxin. In control animals, a waiting period of at least 90 minutes was observed before terminating the experiment. When the maximum digoxin effect was obtained, the kidneys were removed and separated into cortex and medulla without any attempt to separate the latter into an inner and outer portion. The tissue was processed for Na^+,K^+-adenosine triphosphatase activity as previously described (*2, 6*). The assay was carried out by a spectrophotometric procedure (*7*).

In the control dogs, the Na^+,K^+-adenosine triphosphatase specific activity in medullary tissue was approximately twice [72 ± 5 (S.E.M.) μmole of P_i per milligram of protein per hour] that found in the cortex [41 ± 4 (S.E.M.) μmole of P_i per milligram of protein per hour]. Similar values were observed for the internal control renal tissues (uninfused kidney) (Table 1). Furthermore, in all instances the administration of digoxin decreased the enzyme activity in both cortex (proximal and distal convolution) and medulla (loops of Henle). The inhibition appeared to be associated with a sharp rise in the fraction of filtered sodium excreted [C_{Na}/100 ml glomerular filtration rate (GFR)] and an increase in osmolar clearance (C_{osm}/100 ml GFR), three to four times that of control (Table 1). Under these circumstances, free water reabsorption rose only slightly. This failure of $T^c_{H_2O}$ to rise

Table 1. Effect of digoxin on the kidney: functional and biochemical correlates. C_{Na} is clearance of sodium; C_{osm}, osmolar clearance; and $T^c_{H_2O}$, free water reabsorption. All renal function terms were corrected for 100 ml GFR and expressed as percentages. Each figure represents the mean values ± S.E.M. of five experiments. The enzyme activity is expressed in micromoles of inorganic phosphate per milligram of protein per hour.

Test	Renal function			Na^+,K^+-adenosine triphosphatase activity			
	Control		Digoxin	Cortex		Medulla	
	Right	Left	Left	Control	Digoxin	Control	Digoxin
C_{Na}	1.8 ± 0.1	2.0 ± 0.2	9.3 ± 0.7*				
C_{osm}	4.2 ± 0.4	4.3 ± 0.5	14.3 ± 2.0*				
$T^c_{H_2O}$	2.6 ± 0.2	2.5 ± 0.3	3.5 ± 1.0	35 ± 5	20 ± 5*	91 ± 27	44 ± 27*

* The mean of the differences is statistically significant at $.001 < P < .01$.