implies that the uptake process is driven by the concentration gradient of Na^+ across the plasma membrane of the hepatocyte. It appears reasonable to assume that such a secondary active process reacts sensitively to cell damage caused by the isolation procedure and that, as a consequence, bile acid uptake is diminished in isolated hepatocytes. The demonstration that ICG uptake does not depend on extracellular Na^+ concentration suggests a different mechanism and is compatible with facilitated transfer across the membrane[8] followed by binding to ligandin[19]. Such a mechanism may be relatively insensitive to cell damage. An increase in cell surface and possibly an increased exposure of cell proteins which bind ICG could then lead to enhanced ICG uptake. This view is supported by the observation that unspecific damage increases the uptake of the anionic dye sulfobromophthalein by isolated hepatocytes[20] and by liver surface membranes[21]. Thus, the basic differences in the postulated mechanisms responsible for hepatocellular uptake of bile acids and anionic dyes could explain why the uptake capacity for taurocholate is larger in intact liver than in isolated hepatocytes, and why the reverse is true for ICG.

1 This work was supported by the Swiss National Science Foundation.
2 The authors thank Mr Th. Weber for technical assistance.
3 V.C. Glasinovic, M. Duval and S. Erlinger, J. clin. Invest. *55*, 419 (1975).
4 J. Reichen and G. Paumgartner, Am. J. Physiol. *231*, 734 (1976).
5 G. Paumgartner, Schweiz. med. Wschr. *105*, suppl. (1975).
6 G. Paumgartner and J. Reichen, Experientia *31*, 306 (1975).
7 C.A. Goresky, Am. J. Physiol. *207*, 13 (1964).
8 B.F. Scharschmidt, J.G. Waggoner and P.D. Berk, J. clin. Invest. *56*, 1280 (1975).
9 G. Paumgartner and J. Reichen, Clin. Sci. mol. Med. *51*, 169 (1976).
10 L.R. Schwarz, R. Burr, M. Schwenk, E. Pfaff and H. Greim, Eur. J. Biochem. *55*, 617 (1975).
11 M. Schwenk, R. Burr, L. Schwarz and E. Pfaff, Eur. J. Biochem. *64*, 189 (1976).
12 P.O. Seglen, in: Methods in Cell Biology, vol. 13, p. 29. Ed. D.M. Prescott. Academic Press, New York 1976.
13 R. Herz, B. Cueni, J. Bircher and G. Paumgartner, Naunyn-Schmiedebergs Arch. Pharmac. *277*, 297 (1973).
14 G.N. Wilkinson, Biochem. J. *80*, 824 (1961).
15 M.S. Anwer, R. Kroker and D. Hegner, Biochem. biophys. Res. Commun. *73*, 63 (1976).
16 G. Paumgartner and J. Reichen, in: The hepatobiliary system, p. 287. Ed. W. Taylor. Plenum Press, New York 1976.
17 F.A. Wilson and L.L. Treanor, Biochim. biophys. Acta *406*, 280 (1975).
18 S.G. Schultz and P.F. Curran, Physiol. Rev. *50*, 637 (1970).
19 I.M. Arias and P. Jansen, in: Jaundice, p. 175. Ed. C.A. Goresky and M.M. Fisher. Plenum Press, New York 1975.
20 M. Schwenk, R. Burr and E. Pfaff, Naunyn-Schmiedebergs Arch. Pharmac. *295*, 99 (1976).
21 J. Reichen, B.L. Blitzer and P.D. Berk, Clin. Res. *25*, 468 A (1977).

Effects of low doses of ochratoxin A after intratesticular injection in the rat

J. Moré and R. Camguilhem

Laboratoire de Pharmacologie et Toxicologie, INRA, 180, chemin de Tournefeuille, F-31300 Toulouse (France), 6 November 1978

Summary. The toxic effect of various doses of ochratoxin A on the rat testis was investigated after a single intratesticular injection. At time of sacrifice (day 10) degenerating changes occur in the testicular tissues: seminiferous tubules dilatation, cytolysis of the seminiferous epithelium, hyperplasia of the interstitial tissue, vascular thrombosis. The relations between the blood supply disturbances and the observed lesions are discussed.

The testis of many mammals reacts to various natural or synthetic chemical compounds[1]. This action varies, for a definite substance, according with the route of injection (general and/or topical in the testis). For ochratoxin A, the different toxicological trials made p.o., i.p. or i.v. on swine[2], dog[3], rat and mice[4] do not mention the least damage on the male gonad. We have established[5] that administration of this toxin by gastric tubing (16 mg/kg i.e. MTD p.o.) does not modify libido, fecundity or the morphology of testicular tissues in the rat. Intratesticular injection gives similar results to i.v. injection and in the rat, the LD 50 of ochratoxin A injected i.v., is 12.75 mg/kg[4].

The aim of this work is to define the testicular effects of low doses (about $\frac{1}{3}$ of the LD 50 i.v.) of ochratoxin A on the rat testis receiving a single injection of this mycotoxin.

Material and methods. Male rats (Sprague-Dawley) weighing 282.18±5.33 g were housed 3 to a cage and received food (pellets UAR - A 304) and tap water ad libitum. Experimental animals were randomly divided into 4 groups. Each group received ochratoxin at different dose level (4.0, 4.2, 4.6 and 5.0 mg/kg) for each rat. Injections (0.1 ml/testis) were made into both testes under light ethyl ether anaesthesia. The control animals (20 rats) each received the same volume of the solvent of ochratoxin A (CO_3HNa, 14 g/l). All the animals were euthanized the 10th day following injection (day 0). The animals are weighed at day 0 and at time of sacrifice. The testis were weighed. For each animal, the percentage of testes weight (mg/per 100 g b.wt) was calculated. Testes and epididymis were fixed in Orth fluid and paraffin imbedded. Tissue sections were then stained with the PAS-hematoxylin of Leblond and Clermont[6] for histological study.

Weight results. Mean values of testes weight per 100 g b.wt are shown in the table. Means for treated animals do not

Testes weight results

Doses (mg/kg)	Mean testis weight (mg/100 g b.wt)
4	987.5 ± 64
4.2	1002.5 ± 42.4
4.6	985.0 ± 20
5	1032.5 ± 88.8
Control	1112.5 ± 70

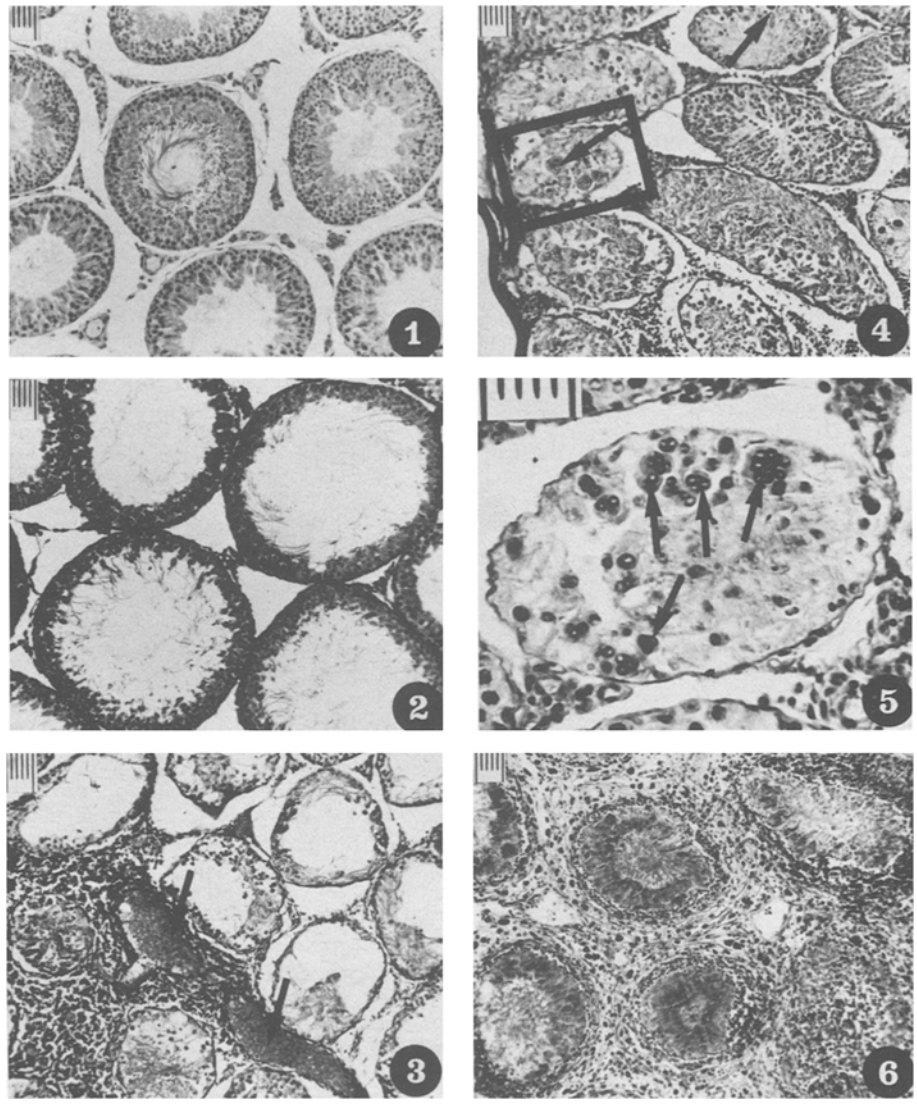

Fig. 1. Control rat testis. Various stages of normal spermatogenesis.
Fig. 2. 4 mg/kg. Tubular dilatation and hyperchromic cytoplasms.
Fig. 3. 4.2 mg/kg. Proliferation of the interstitial tissue (bottom left) and degenerating tubules. Note vascular hyperaemia (arrows).
Fig. 4. 4.6 mg/kg. Cytolytic tubules with multinucleated giant cells (arrows).
Fig. 5. High-power of the area marked in figure 4. Multinucleated giant cells (arrows).
Fig. 6. 5 mg/kg. Tubular necrosis and interstitial tissue hyperplasia.

The scale (top left) = 50 µm.

differ significantly ($p < 0.05$) from those obtained in control animals.

Histological results. 4 mg/kg (figure 2). The seminiferous epithelium shows a general cytoplasmic hyperchromicity. Tubules are dilated and their wall is considerably thinned. However, successive stages of transformation of seminiferous epithelium into mature spermatozoa are respected, including Sertoli cells. The interstitial tissue appeared normal but blood vessels are strongly dilated and hyperemic. The epididymis of these animals is normal.

4.2 mg/kg (figure 3). Tubular wall hyperchromicity is replaced by cytoplasmic hypochromicity. In the centre of the testis, seminiferous epithelium has degenerated. In some tubules, the only layer of spermatogonia with pycnotic nuclei subsisted. Sertoli cell nuclei are strongly damaged. In some tubules only the basement membrane subsists. In some areas of the centre of the testes, we can observe nests of Leydig cells. The epididymal tube is filled with scattered necrotic debris and practically devoid of spermatozoa.

4.6 mg/kg (figures 4 and 5). Proliferation of the Leydig cells occurs over a greater area than for the preceding animals. The gametogenic elements are destroyed. Seminiferous tubules are filled with necrotic debris among which many multinucleated giant cells appear. The epididymis shows the same characteristics as those previously described.

5 mg/kg (figure 6). At palpation the testis appears hard. The simiferous tubes are completely converted into a mass of cytolytic and necrotic debris. The interstitial zone is invaded by fibrous connective tissue in which we noted a great proliferation of Leydig cells nests. Blood vessels are often obstructed by fibrinous thrombi. The epididymis was necrotic.

Discussion. The results of this study show that a mycotoxin as ochratoxin A, topically injected, induces severe damage to testicular tissues as do alkylating agents, nitrofurans, dinitropyrroles and some mineral salts ($CdCl_2$), etc.

The observed damage due to ochratoxin A varied according to the dose injected. Lower doses (4 and 4.2 mg/kg) only affected the central portion of the testes. By contrast, higher doses (4.6 and 5 mg/kg) involved the whole organ. Vascular injury (described also in i.p., i.v. and oral intoxications) is probably responsible for the tubular defects observed. We suggest that the blood supply and drainage disturbances induced by ochratoxin A are sufficient to cause the previously described lesions. Moreover, the multinucleated giant cells may be compared to those observed in rat testis after temperature elevation[8]. In such injury, the blood flow

is also disturbed and the giant cells are now believed to be formed by the fusion of damaged spermatids.

The lowest dose (4 mg/kg) induces tubular dilatation, showing an increase into seminiferous tubule fluid pressure. This dilatation is a clear indication that there is a disruption in the control mechanisms of the bloodtestis barrier. Baradat[9] showed that ochratoxin A increases ions transfer in the rat colon. So we suggest that in the testis ochratoxin A acts in the same way as does the well-known cadmium chloride.

In our study, the introduction of ochratoxin A into the testis shows 2 kinds of damage. 1., The lowest dose used only modifies seminiferous tubules permeability, inducing their dilatation. 2., Higher doses act on the vascular supply causing the degenerating changes observed in the spermatogenic epithelium. Interstitial tissue hyperplasia (4.6 and 5 mg/kg) involving Leydig cells and connective tissue elements has also been described in other intoxications. Its etiology remains unknown; so, we shall only try to define it.

1 A.D. Johnson, W.R. Gomes and N.L. Vandemark, in: The Testis, vol. III, p. 377. Academic Press, New York 1970.
2 P. Krogh, B. Hald and J. Pedersen, Acta physiol. Scand. *81*, 689 (1973).
3 D.N. Kitchen, W.W. Carlton and J. Tuite, Vet. Path. *14*, 154 (1977).
4 P. Galtier, J. Moré and G. Bodin, Ann. Rech. vét. *5*, 233 (1974).
5 J. Moré and R. Camguilhem, unpublished observations, 1978.
6 C.P. Lebond and Y. Clermont, Am. J. Anat. *90*, 167 (1952).
7 F.J.C. Roe, C.E. Cameron, K.H. Pugh and B.C.Y. Mitchley, Br. J. Cancer *18*, 674 (1964).
8 E. Steinberger and W.J. Dixon, Fert. Steril. *10*, 578 (1959).
9 C. Baradat, unpublished observations, 1977.

Attenuation by carbocromen of cardiac metabolism alterations due to ischemia

J.L. Andrieu, M. Ollagnier, M. Lièvre, I. Hugueny, R. Tédone and G. Faucon[1]

Département de Pharmacologie Médicale, Université Claude Bernard, 8, avenue Rockefeller, F-69373 Lyon Cédex 2 (France), 12 October 1978

Summary. Carbocromen prevents to some extent, particularly in subendocardial layer, carbohydrate cardiac metabolism alterations induced by the ischemia obtained by intermittent occlusion of left coronary artery.

Administered i.v. at relatively high doses, carbocromen elicits a considerable and persistent increase of coronary flow[2,3]. This result is more uncertain in presence of obstructive lesions of the coronary bed, especially of the large vessels. However, after reduction in dogs of the circulatory flow in left ventricular wall by 40–70% by narrowing of the coronary artery, the rise of flow was still observed, to a lesser extent than under normal conditions but nevertheless significant, especially if the preliminary reduction had been moderate[4]. The purpose of this study was to verify these data by investigating whether the cardiac metabolism alterations consecutive to ischemia[5,6] were prevented by carbocromen, a compound devoid of any direct effect of its own on cardiac metabolism[7,8].

The investigations were carried out particularly on subendocardial layer in which the modifications of the main substrate concentrations due to ischemia are more marked[6] and the circulatory activation attributable to carbocromen and the other vasodilator drugs of the same category challenged[9,10].

Methods. The experiments were performed under total cardiopulmonary by-pass, so that sampling of the myocardial wall required for cellular metabolism exploration could be achieved several times in the course of an experiment, carbocromen remaining able to act in this way as normally on coronary circulation.

Dogs weighing 20–32 kg, barbiturate anaesthetized, were divided into 2 groups, 6 being subjected only to ischemia for 45 min and 6 being given 4 mg/kg of carbocromen i.v. 15 min after the beginning of ischemia. Considering the difficulty of initiating an ischemia stable at a given degree, the blood flow was alternately completely interrupted for 2 min and left entirely free for an equal period, by means of a thread around left coronary artery. Thus, the mean reduction of flow did not reach 50% because of the reactive vasodilatation raising the flow beyond its reference values

Compared variations of lactate, glycogen, glucose and free fatty acid concentrations in subepicardial layer in dogs subjected to ischemia only and to ischemaia and carbocromen, 4 mg/kg i.v. Mean values±SEM. 1, 2 and 3 asterisks refer to the significance at the 5%, 1% and 0.1% respectively.

		Reference	Ischemia 5 min	15 min	20 min	30 min	45 min
Lactate	Control	6.26±1.46	16.95±3.52(*)	19.83±4.10(*)	20.27±2.55(***)	15.91±1.52(***)	21.81±3.15(***)
	Treated	6.55±0.26	14.60±1.49(***)	20.16±5.27(*)	21.15±4.29(*)	18.80±2.53(***)	17.13±3.63(*)
Glycogen	Control	5.52±0.65	4.91±0.50	3.55±0.71(*)	3.85±0.24(*)	4.41±0.46	3.13±0.61(*)
	Treated	5.38±0.42	4.54±0.81	4.02±1.34	3.82±0.93	4.46±0.65	4.72±0.86
Glucose	Control	8.16±0.97	7.04±0.82	7.00±0.97	7.67±1.21	7.72±1.17	7.21±1.02
	Treated	11.60±0.84	10.35±1.43	9.91±1.68	8.43±1.21	11.01±2.17	10.92±1.22
FFA	Control	12.85±1.17	11.90±1.19	11.94±1.26	12.36±0.99	13.00±1.19	12.53±1.66
	Treated	13.19±1.91	13.56±1.22	13.00±1.08	12.52±1.06	12.80±1.26	11.90±0.95