ALLOXAN INDUCED MULTIPLE BENIGN HEPATOMA IN A FRESH WATER TELEOST, *CLARIAS BATRACHUS*

ALLOXAN, a widely used diabetogen, has been found by the authors[1] causing leukemoid condition in the fish *clarias*. Consequently, present study was designed to test the carcinogenicity of alloxan in the liver of the fish, *clarias batrachus*.

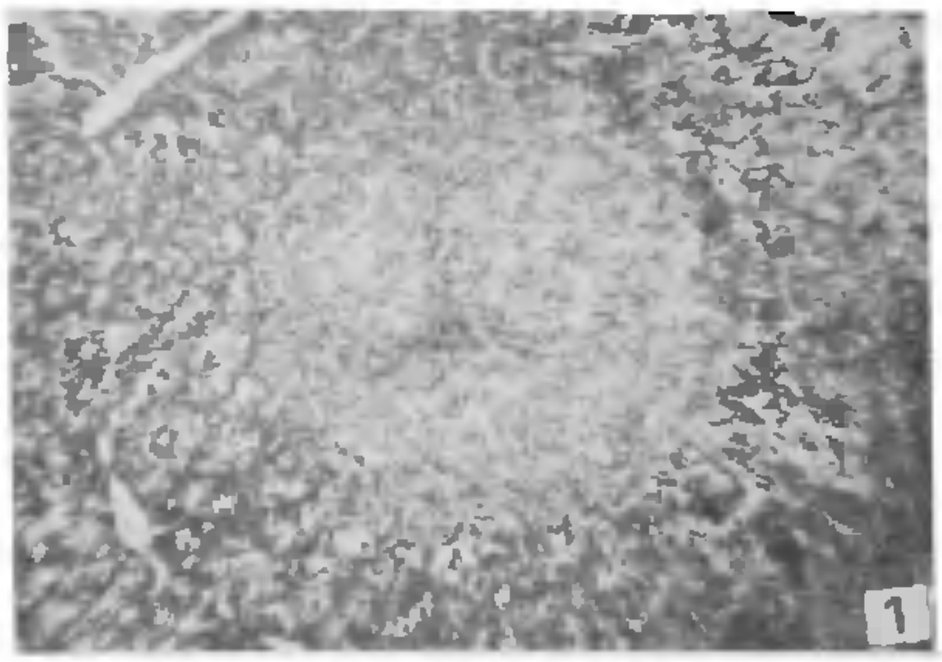
Material and Methods

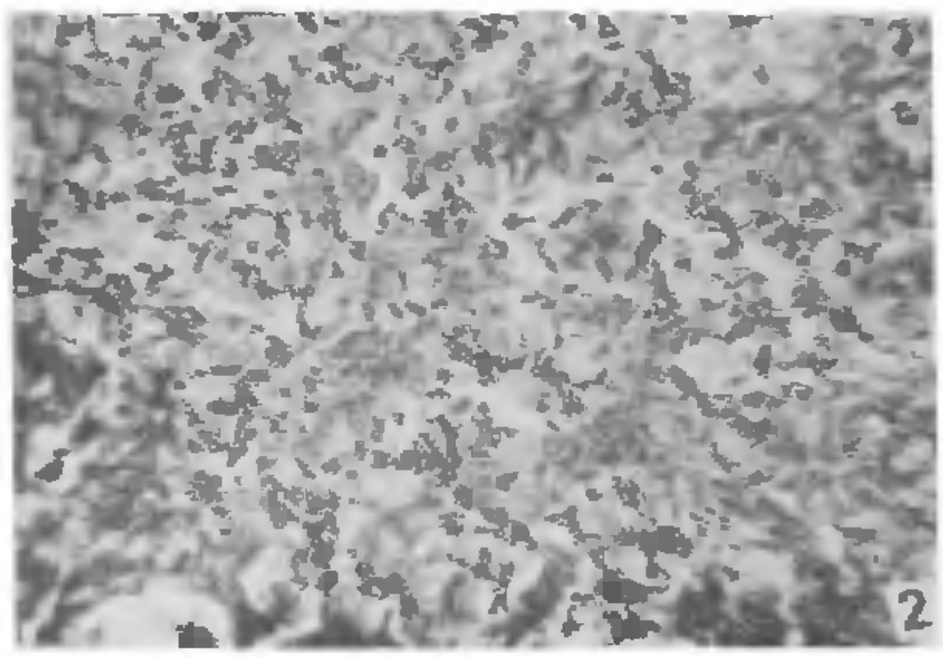
One hundred fishes, weighing 70–100 g each, collected from local fresh water reservoirs, were first acclimated to the laboratory conditions for 7 days. These fishes were divided into two groups of 50 each. All the fishes of one group were injected intramuscularly at the base of caudal peduncle with a freshly prepared 1% (W/V) alloxan (Sigma) solution in citrate-phosphate buffer, pH 4 0, at the dose of 50 mg/kg body weight. The injections were repeated every third day. The other group received equal amounts of citrate-phosphate buffer and served as control. Six to ten fishes were killed and autopsied after every 10 days. Their entire livers were fixed in Helly's, neutral buffered 10% formalin and Rossman's fixatives and were stained with hematoxylin-eosin, Best's carmine and PAS.

Results

After 30 days of alloxan treatment (15 doses of 50 mg/kg body weight each), abrupt loss of eosinophilia in H and E stained sections was observed at a few sites (Fig. 1). Such areas were found to be strongly PAS positive (Figs. 3, 4) but negative to Best's carmine. These sites, though different in staining behaviour from the rest of the tissue, were in continuation with adjacent hepatic tissue. Hepatocytes in the affected area were necrosed and contained proliferating spindle-shaped cells. The nuclei of these cells were seen undergoing division and fragmentation (Fig. 2).

After 40 days, the peculiar structures described above, took a definite circular shape and differentiated from the adjacent tissue by an encapsulating covering. However, such strucutres without encapsulating covering were also seen, even in the same section (Fig. 3). These structures resemble benign hepatoma in anatomical details. The encapsulated tumors contained spindle cells with indistinct cytoplasmic outline and enlarged nuclei (Fig. 2). The spindle cells were compact and evenly distributed giving the tumor a pseudoglandular appearance (Fig. 4). Most of the tumors were seen projecting out of the surface of liver (Fig. 3), which correspond with a few tiny pale yellow spots on the surface of comparatively enlarged and hardened liver.





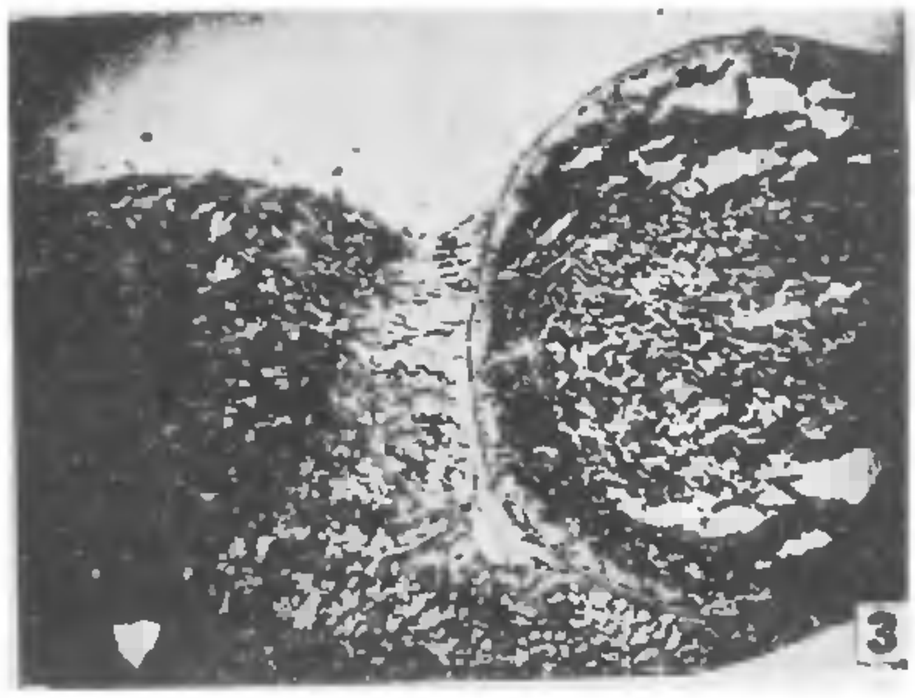
FIGS. 1–2. Fig. 1. Developing hepatoma showing loss of eosinophilia. H and E, × 40. Flg. 2. Developing tumor showing spindle cells with enlarged dividing nuclei. H and E, × 400.

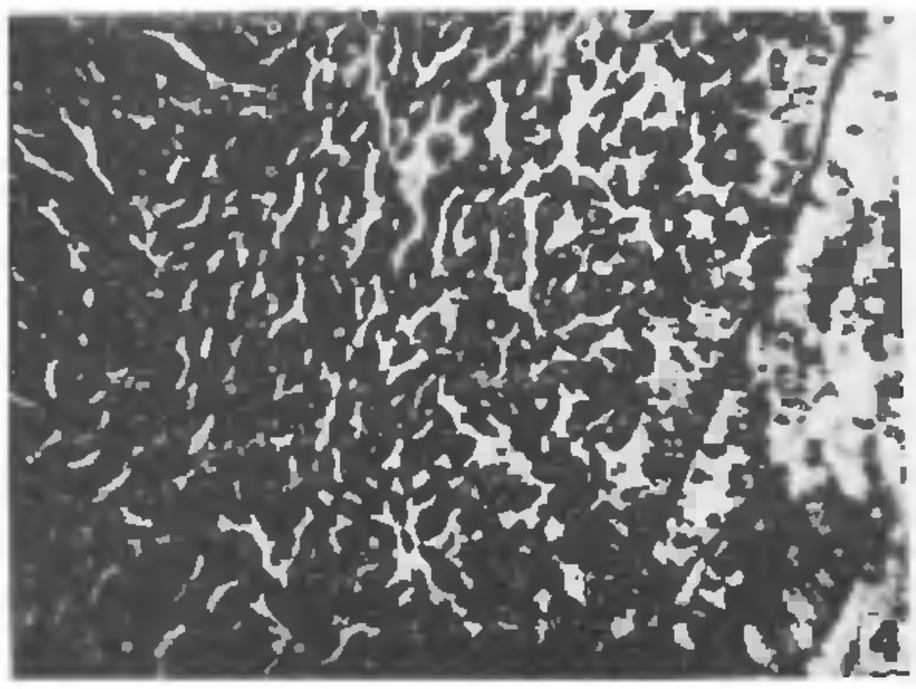
No such tumor was found in any of the control fish while it developed in 80% of the treated fishes killed after 30 and 40 days.

Discussion

Spontaneous tumors have been reported to be rather exceptional among cold blooded animals excepting the cod fish and infectious Lucke tumor in the leopard frogs (Guerin *et al.*[4]). Schlumberger and Lucke[6] and Thomas[7] observed renal tumors in fishes but these were of rare occurrence.

The histogenesis of the tumors induced with alloxan is indisputable because of their histologic appearance and staining affinities. Loss of eosinophilia in H and E preparations, strongly PAS positive and Best's carmine negative reactions of tumor cells have also been reported by Kirkman and Bacon[5] and Elias[3]. It can be concluded on the basis of the present observations that these tumors developed at the expense of differentiated hepatocytes and in the context of nomenclature used here, they fall into the category of multiple benign hepatoma. The spindle cells provide the tumor, sarcoma like appearance. The benign nature of the tumors is confirmed as these were localised, encapsulated and did not invade the





FIGS. 3–4. Fig. 3. Encapsulated and developing tumors. PAS, × 40. Fig. 4. Encapsulated tumor showing spindle cells PAS, × 600.

adjacent tissue. These findings are also supported by Duijn[2].

The present findings are of utmost importance since these indicate the carcinogenicity of alloxan which is till now regarded as diabetogen.

The authors are thankful to Dr. S. S. Khanna for his interest in this investigation.

Thanks are also due to U.G.C., New Delhi, for providing financial assistance during part of the investigation.

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May 15, 1979.

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DOMINANT LETHALS INDUCED BY CADMIUM CHLORIDE IN *DROSOPHILA MELANOGASTER*

CADMIUM pollution is a serious environmental problem due to its extensive use in industries. It has been reported that cadmium is teratogenic in mouse[1,2], rat[3], and hamster[4]. In *Drosophila* Vasudev and Krishnamurthy[5] and Sorsa and Pfeifer[6] have shown that at a concentration of 50 ppm it could cause delay in the rate of development. Cadmium chloride is also found to induce infertility in male mice[7]; in consistent with this, Kar *et al.*[8] and Parizek[9] have reported the "cytotoxic" effects on spermatogenic cells. The effect on the chromosomes of *Poekilocerus pictus* has also been investigated[10]. Thus there is some evidence to support the possible drastic effects of cadmium on cell and hence the present studies were undertaken to study this chemical in relation to its ability to induce dominant lethals in *D. melanogaster*.

Male flies emerged out of media mixed with 5, 10 and 20 ppm of cadmium chloride, were aged for five days and used in the present experiments. The tests for the induction of dominant lethals were carried out according to the methods described by Sankaranarayanan[11]. The results are presented in Table I.

TABLE I

Induction of dominant lethals by cadmium chloride in Drosophila melanogaster

Concentration	No. of eggs counted	No. of eggs unhatched	% dominant lethals
Control	1076	52	4·83
5 ppm	1244	147	11·8*
10 ppm	1375	196	14·3*
20 ppm	1390	199	14·3*

* Significant at 5% level.

A dominant lethal mutation indicates a major genetic damage, which kills an individual heterozygous to it. Perusal of Table I indicates that all the concentrations of cadmium chloride tested induced significant percentage of dominant lethals. There is also a dose dependent relationship in the production of dominant lethals. These results are similar to those of Shiraishi *et al.*[12], Shiraishi and Yoshida[13] and Bauchinger *et al.*[14] where they have shown the chromosome breaking ability in human lympocytes. In