THE HISTOPATHOLOGICAL EFFECTS OF AFLATOXIN B_1 AND THE PALMOTOXINS B_0 AND G_0 ON THE LIVER OF THE DEVELOPING CHICK EMBRYO

O.BASSIR and A.A.ADEKUNLE
Department of Biochemistry, University of Ibadan, Ibadan, Nigeria

Received 11 August 1970

1. Introduction

A considerable amount of investigation has been made on the effects of aflatoxin on the liver. It has been shown [1] that a single dose of aflatoxin given after the sixteenth day of pregnancy could cause liver damage in the rat. Though turkey poults have been found to be resistant to the acute toxic effects of aflatoxin [2], other reports [3] have indicated that the tolerance did not prevent pathological changes in the liver. In an earlier investigation [4], feeding aflatoxin-contaminated peanut meals to rats was shown to produce in them multiple liver tumours. Bassir [5] and Newberne [6] have also reported good correlation between liver tumour incidence and dietary aflatoxin content.

In this study, an attempt is made to define the hepatic embryotoxic characteristics of palmotoxins B_0 and G_0, previously identified as metabolites of *Aspergillus flavus* [7], and to compare the histopathological effects of these toxins with those of aflatoxin B_1.

2. Materials and methods

Medium-size fertile eggs obtained from the White rock strain of chicks were supplied by the University Farm, University of Ibadan, Nigeria. Toxins used were obtained from palmsap cultures of a strain of *A. flavus* (Link ex Fries) obtained from mouldy local legumes. They were isolated and purified by the method of Levi and Birker [8], Wiseman, Jacobson and Harmeyer [9] and Bassir and Adekunle [7].

Thirty-three six-day old chick embryos were used for assaying each of these toxins. Before inoculation, each egg was carefully cleaned with methanol. The dosage of 0.3 μg/egg was delivered with a microsyringe in 0.05 ml of propylene glycol while the same volume of propylene glycol was injected into each of the controls made up of the same number of eggs.

They were incubated at 37° in an automatic clockwork incubator which also turned the eggs every 24 hr.

Livers were obtained from the animals at the end of 21 days incubation under strict aseptic conditions, and fixed in 10% formol saline. Paraffin sections (of approximately 3 μ) were prepared by the method of Butler and Wigglesworth [1] and stained with Ehrlich's haematoxylin and eosin.

3. Results

3.1. *Light microscope findings*

In the control liver (fig. 1), it was found that the lobular architecture was well preserved. The hepatocytes appeared healthy as judged by granulation and vacuolization. Focal aggregates of lymphocytes in the region of the central veins were observed while the portal tracts appeared normal.

In the liver of the chick embryo injected with aflatoxin B_1 (fig. 2), the hepatocytes seemed to be undamaged, the Kupfer cells appeared slightly prominent. There was a focal area of fatty change. Lymphocytic infiltration was observed. The portal tracts, like those of the control birds, exhibited no damage.

North-Holland Publishing Company – Amsterdam

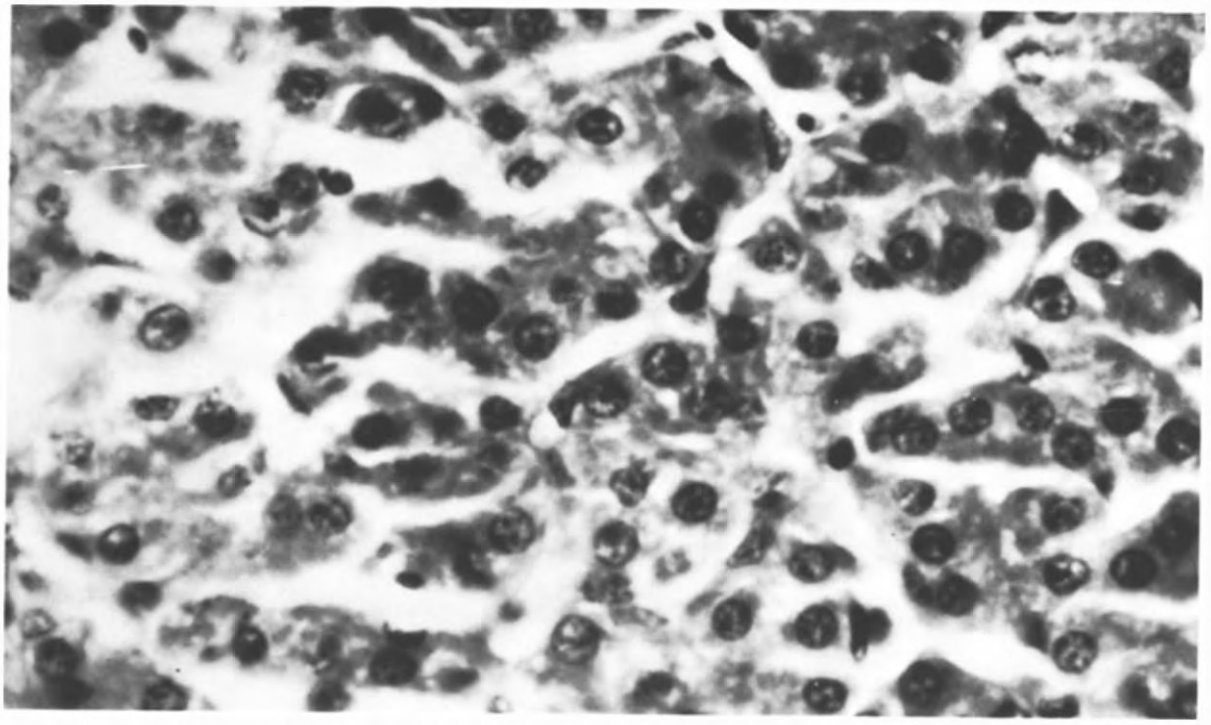

Fig. 3. A liver section from palmotoxin G_0-treated chick embryo (X 1320).

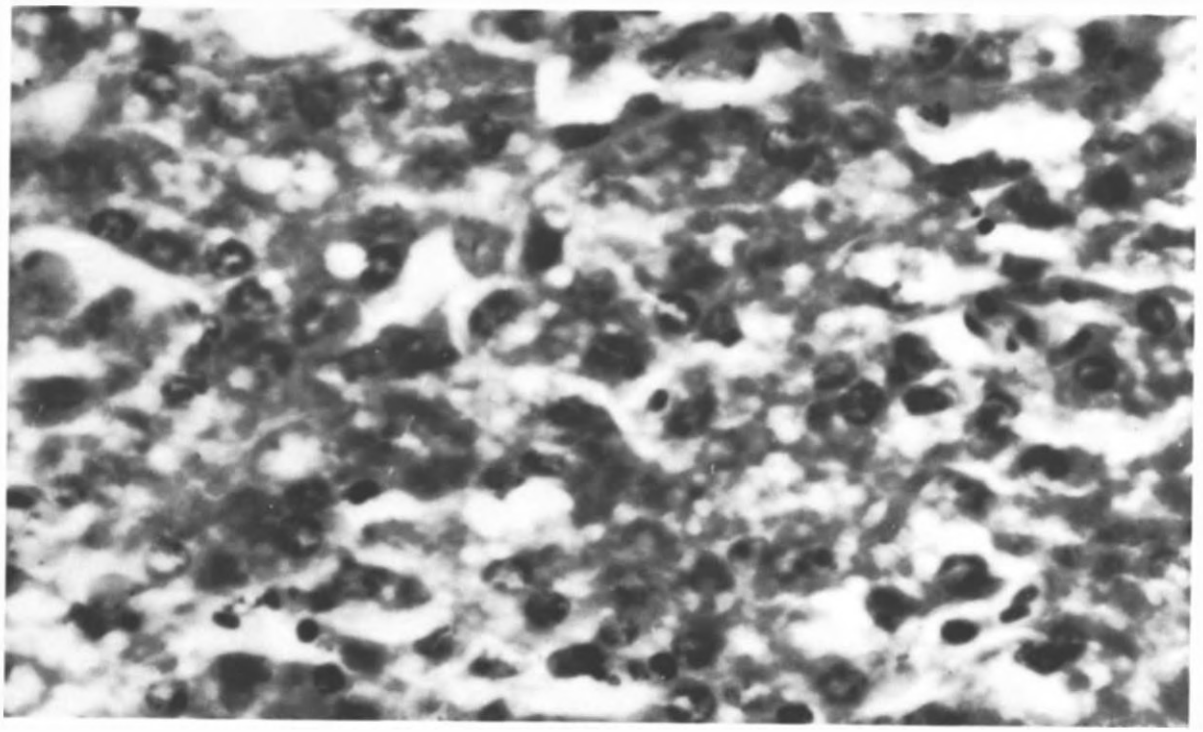

Fig. 4. A liver section from palmotoxin B_0-treated chick embryo (X1320).

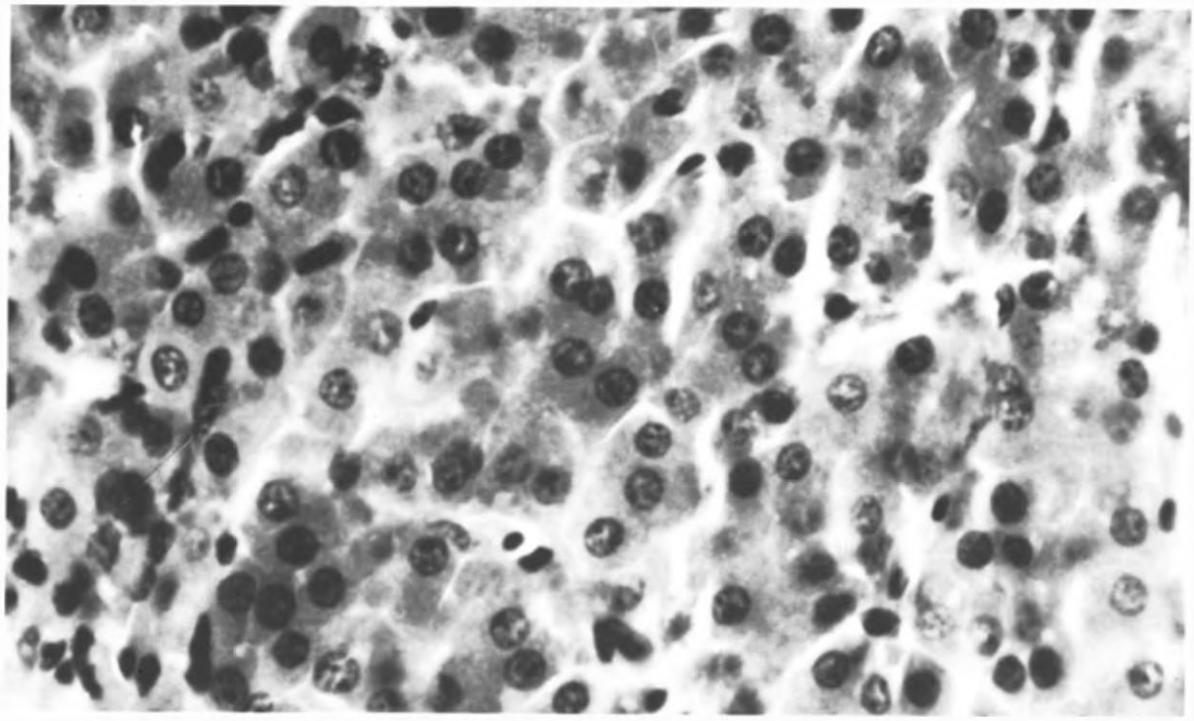

Fig. 1. A liver section from propylene glycoltreated chick embryo (X 1320).

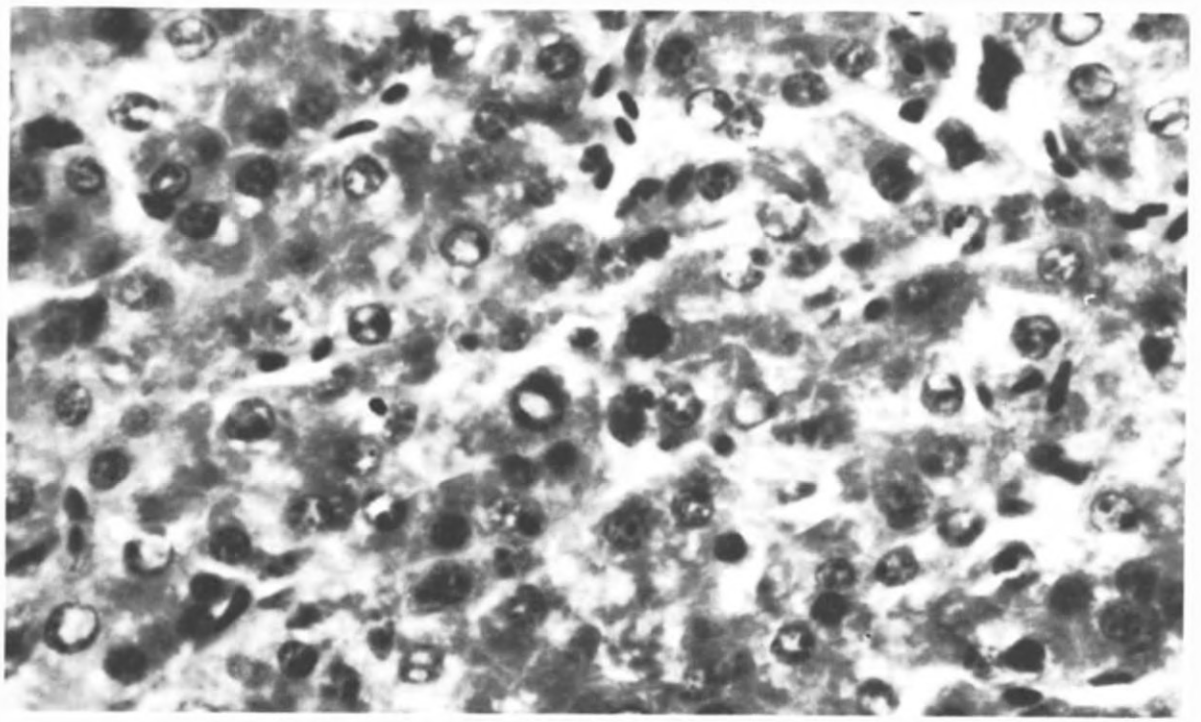

Fig. 2. A liver section from aflatoxin B_1-treated chick embryo (X 1320).

In the liver of animals injected with palmotoxin G_0 (fig. 3), there were also peripheral focal areas of fine fatty vacuolization of hepatocytes. Focal necroses with mononuclear cellular infiltrations were observed.

In the liver of animals inoculated with palmotoxin B_0 (fig. 4), focal areas of marked periportal necroses were observed. The tissue also elaborated a severe fatty change. These results were confirmed with replicated experiments.

4. Discussion

The common lesion observed in this investigation is fatty infiltration. It appeared from the severity of cell damage that palmotoxin B_0 and aflatoxin B_1 are more potent toxins than palmotoxin G_0. This may signify that the chick embryos are resistant to palmotoxin G_0 or that this toxin, *per se*, possesses a structural chemical configuration quite dissimilar to that of aflatoxin B_1 or palmotoxin B_0. These severe fatty changes are not found in normal animals. Other workers [10, 11] have reported that aflatoxin poisoning was accompanied by liver fat increase. The biochemical mechanism relating hepatic fat increase with aflatoxin and palmotoxin poisoning is now being investigated.

The presence of foci of necrosis in the livers of embryos treated with palmotoxin B_0 and palmotoxin G_0, respectively, was another consistent finding which exhibited greater severity in the palmotoxin B_0-treated animals.

Acknowledgement

We wish to acknowledge the cooperation of Professor A.O.Williams of the University College Hospital, Ibadan, on the histopathological aspect of this work. We also wish to thank Messrs. A.Enyongai, A.A.Dina, H.Chimbo and T.A.Asojo for technical assistance.

References

[1] W.H.Butler and J.S.Wigglesworth, Brit. J. Exptl. Pathol. 47 (1966) 242.
[2] J.M.M.Brown, S.African Med. J. 39 (1965) 778.
[3] S.E.Magwood, K.Jericho and A.H.Corner, Can. J. Comp. Med. Vet. Sci. 30 (1966) 17.
[4] M.D.Lancaster, F.P.Jenkins and J.McPhilp, Nature 192 (1961) 1095.
[5] O.Bassir, W.African Med. J. 14 (1964) 54.
[6] P.M.Newberne, in: Mycotoxins in Foodstuffs, ed. G.N. Wogan (M.I.T.Press, Mass., 1965) p.151.
[7] O.Bassir and A.A.Adekunle, FEBS Letters 2 (1968) 23.
[8] C.P.Levi and E.Birker, J. Assoc. Offic. Agr. Chemists 51 (1968) 600.
[9] H.G.Wiseman, W.C.Jacobson and W.C.Harmeyer, J. Assoc. Offic. Agr. Chemists 50 (1967) 982.
[10] J.D.J.Harding, J.T.Done and R.Allcroft, Brit. Vet. Sci. 4 (1963) 217.
[11] R.B.A.Carnaghan, G.Lewis, D.S.P.Patterson and R.Allcroft, Pathol. Vet. 3 (1966) 601.