

adjuvant lead to the formation of follicular structures and plasma cells within the thymus, and have suggested that Freund's adjuvant may reach the thymus after injection at a distant site.

The present investigation indicates that trypan blue reaches the adult thymus easily, and that 'Thorotrast' and carbon are frequently found in the new-born mouse thymus. The reason that the thymus does not respond to ordinarily administered antigenic stimuli may be more complex than failure of antigen to reach thymic tissue.

The fact that the thymus of the new-born appears more permeable to particulate material than the adult thymus is of interest and deserves further investigation.

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Toxicity and Fluorescence Properties of the Aflatoxins

THE isolation, characterization and some of the properties of four closely related components of aflatoxin, the mixture of toxic metabolites produced by certain strains of *Aspergillus flavus*, have been described previously¹. These components were named aflatoxins B_1 , B_2 , G_1 and G_2 . Structural formulae have been proposed for aflatoxins B_1 and G_1 (ref. 2) and the relationships between these compounds and B_2 and G_2 have been established^{1,3}, the double bond in the terminal dihydrofuran ring in the first two components having become hydrogenated in the others.

This communication is concerned with the relative intensities of fluorescence of the four major components and their acute toxicities when administered to day-old ducklings.

The peak fluorescence intensities of the aflatoxins in methanol solution were measured in arbitrary units on an Aminco-Bowman spectrofluorimeter. Freshly re-crystallized material and freshly prepared solutions (0.2 $\mu\text{g}/\text{ml}$.) were used. This concentration was in the range where a linear relationship existed between concentration and fluorescence intensity. The relative fluorescence intensities⁴ KQ of the four components were obtained by comparing the intensity of the solutions (calculated to 1 $\mu\text{g}/\text{ml}$.) with that of a solution of quinine sulphate of the same concentration in 0.1 N sulphuric acid when excited at 350 m μ and measured at 450 m μ . The KQ values are recorded in Table 1. In the case of aflatoxin B_1 KQ was found to rise to 1.5 after 3 h and then fall again to 0.5 after a further 17 h.

Table 1. FLUORESCENCE PROPERTIES OF THE AFLATOXINS

Aflatoxin	$\lambda_{\text{excitation max.}}$ (m μ)	$\lambda_{\text{emission max.}}$ (m μ)	KQ^*
B_1	365	425	0.5
B_2	365	425	4
G_1	365	450	2.5
G_2	365	450	6.5

$$* KQ = \frac{\text{fluorescence intensity of aflatoxin solution}}{\text{fluorescence intensity of standard quinine sulphate solution} \times \text{concentration of aflatoxin solution } (\mu\text{g}/\text{ml}.)}$$

The acute toxicity of each compound was determined by biological assay using day-old Khaki Campbell ducklings (average weight 38 g). Various dose-levels of each compound dissolved in dimethylformamide were administered to groups of 8 fasting ducklings. Each bird received 0.1 ml. by tube into the gizzard and mortality was recorded for 6 days after administration. Histopathological examinations of the livers were carried out to determine whether death was due to aflatoxin poisoning. The four compounds were found to produce similar histopathological hepatic changes which were indistinguishable from the lesions seen in field cases of aflatoxicosis in ducklings⁵. The LD_{50} value for each compound was calculated on the basis of 50 g body-weight day-old duckling. Under these conditions the LD_{50} for B_1 (3 assays) was 18.2 μg (5 per cent fiduciary limits 14.0-23.8 μg), for B_2 (single assay) 84.8 μg (5 per cent limits 65-110 μg), for G_1 (2 assays) 39.2 μg (5 per cent limits 27.1-56.7 μg) and for G_2 (2 assays) 172.5 μg (5 per cent limits 158-188 μg). No deaths occurred in control groups dosed with 0.1 ml. dimethylformamide alone nor were any hepatic changes observed on histopathological examination.

From these results it can be seen that reduction of the isolated double bond in the terminal dihydrofuran ring of B_1 and G_1 to give B_2 and G_2 , respectively, lowers the toxicity. The reduction in activity is very similar in both series, the ratio of the LD_{50} values of $B_1 : B_2$ being 1 : 4.66 and for $G_1 : G_2$ being 1 : 4.40. Expansion of the pentenone ring in the B series to give a second lactone ring in the G series also reduces the toxicity; the ratio of the LD_{50} values of $B_1 : G_1$ is 1 : 2.15 and of $B_2 : G_2$ is 1 : 2.03.

The pronounced difference between the fluorescence intensities of the four aflatoxins together with the difference in their biological activities makes it difficult to relate the toxicity of a mixture of these compounds to its fluorescence intensity.

Accordingly, if fluorescence is to be used as a precise indication of toxicity it is desirable that each constituent should be separated and individually determined. Moreover, the measurements of fluorescence should be made under carefully controlled conditions.

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2-(α -Hydroxybenzyl)-benzimidazolium Salts and their Influence on Cultured Cells infected with Poliovirus

1-ALKYL-2-(α -HYDROXYBENZYL)-BENZIMIDAZOLIUM (I) give a high degree of protection against the cell degeneration which follows infection of tissue cultures with poliovirus (types 1, 2 and 3)¹. The action of corresponding quaternary salts (II) is of considerable interest in view of: (a) the much greater solubility of these compounds in water; (b) the introduction of the positive charge; (c) the removal of the type of hydrogen-bonding (III) or metal-chelating (IV) propensities that might (for example, by allowing attachment to protein or nucleic acid) play some