Gut, 1966, 7, 205

Aflatoxin

In the summer of 1960 there was an outbreak of an apparently new disease in turkeys in farms within a radius of 100 miles from London. Because the aetiology of the disease was obscure it was called turkey 'X' disease.[1–5] The loss of poults was estimated to be over 100,000 and death was due to acute hepatic necrosis.[6] Investigations showed that the disease was nutritional in origin. Suddenly there was an outbreak of the disease in Cheshire and the only common factor between this outbreak and the major one in the London region lay in the presence of Brazilian ground nut meal in the food. A similar disease was found to have occurred in poultry in Brazil and outbreaks of the disease were reported in young poultry in Africa. In all these cases the disease could be traced to the presence of ground nuts in the food.[7–8] It was observed that the most toxic samples of ground nuts were heavily contaminated with fungi and pure cultures of the fungus were grown and identified as a strain of *Aspergillus flavus*. Toxic material was obtained from preparations of heat-sterilized, non-toxic nuts on which the fungus had been grown for some days.[9–10]

The total toxic material that was extracted from fungal-infected ground nuts was designated aflatoxin.[11] Aflatoxin was finally separated by chromatographic procedures into four substances, B_1, B_2, G_1, and G_2.[12–13] Day-old ducklings were particularly susceptible to aflatoxin and in this species B_1 proved to be ten times more toxic than B_2, with G_1 occupying an intermediate position.[13] By the application of spectrophotometric analysis and nuclear magnetic resonance the structural formulae of the aflatoxins was obtained. The aflatoxins are closely related in structure and are pentacyclic structures with one or two six-membered lactone rings.[14–15]

The isolation and purification of the aflatoxins has now permitted investigation into their mode of action and in the main the most toxic member, aflatoxin B_1, has been employed. In all species so far studied administration of the LD/50 dose results in an acute hepatic periportal necrosis.[16] In the rat the lesion develops slowly (three days) and is followed by a long period of bile duct proliferation.[17] Administration to rats during the regenerating phase following partial hepatectomy has been found to inhibit mitosis[18–19] and electron microscopy studies have revealed that the nucleus, and in particular the nucleolus, is the primary site of attack.[20] Small doses of aflatoxin B_1 fed for a short period to duckling,[21] rats,[22] and trout[23] produce hepatomas. It also produces local tumours when injected into the flanks of rats.[24] The addition of aflatoxin to embryonic lung cell cultures inhibits mitosis and increases giant cell formation[25] and inhibits mitosis in the roots of the germinating seedling of *Vicia faba*.[26]

All these changes induced by aflatoxin would point to the desoxynucleic acid (DNA) molecule being closely involved in the site of action of aflatoxin B_1. Studies in regenerating liver showed that aflatoxin inhibited DNA synthesis. However, it did not inhibit a number of enzymes concerned in DNA synthesis in regenerating liver and it was concluded that the failure in synthesis probably lay in the inability of DNA polymerase to transcribe the DNA molecule itself.[27] That this was the most likely explanation for the inhibition in DNA synthesis has come from experiments on the livers of rats poisoned with a necrotizing dose of aflatoxin B_1.

A study was made of the effect of aflatoxin B_1 on protein synthesis *in vivo*, in particular on the synthesis of tryptophan pyrrolase. Tryptophan pyrrolase is normally present in the liver at low concentrations but if the rat is given either an injection of cortisone or tryptophan within a few hours an increased level of the enzyme is induced.[28–29] It was found that in the liver of the poisoned rat there was an inhibition of the cortisone-induced enzyme whilst the substrate induction was unaffected.[30] The cortisone induction of the enzyme is the result of an increased messenger ribose-nucleic acid (mRNA) production by the nucleus.[29] The mechanism of the substrate induction is at present in dispute but it does not operate through an increase in mRNA.[31] The addition of aflatoxin B_1 to liver slices produces an immediate inhibition of RNA synthesis but the inhibition in protein

synthesis takes some 15 minutes to develop. These results indicate that the inhibition of protein synthesis in aflatoxin poisoning is secondary to an inhibition in nuclear RNA synthesis, in particular mRNA. Studies *in vivo* in the livers of poisoned rats confirm that there is an inhibition of nuclear RNA synthesis. These experiments show that there is an inhibition of the RNA polymerase which could be due to the aflatoxin preventing the RNA polymerase transcribing the DNA molecule.[30] Such a situation would be analogous to that demonstrated for actinomycin D[32] and would account for the finding that this agent and aflatoxin B_1 produce very similar cytological changes in regenerating liver cells.[20] Finally it was demonstrated that aflatoxin B_1 interacted with DNA in a similar manner to actinomycin D.[30]

Thus aflatoxin B_1 interacts with the DNA molecule and it is this interaction which prevents the DNA polymerase transcribing the DNA and thereby inhibits DNA synthesis and likewise inhibits the RNA polymerase. It is this latter effect which results in a reduced output of mRNA from the nucleus and thereby inhibits protein synthesis. It is probably this step which ultimately culminates in the death of the cell. As yet it is not possible to decide to what extent the DNA interaction is associated with the carcinogenic action of aflatoxin but in poisoned cells where there is an inhibition of mitosis, chromosome breakage has also been observed.[26] Chromosomal breakage can be produced by carcinogens acting on DNA and is considered to play a role in the process of carcinogenesis.

The most important question to be answered about aflatoxin is whether it is an interesting biochemical tool or a hazard to man? Following the outbreak of turkey 'X' disease it was realized that cattle[33] and pigs[34–35] had been subjected to a similar disease and further that milk from cows receiving toxic foodstuffs contained a toxic metabolite of aflatoxin.[36–37] An examination of the public health aspect of this problem indicated that human beings remained safe throughout the time that toxic groundnuts were being given to farm animals.[38] Nevertheless groundnuts are used as valuable protein to feed the undernourished people of the world and it has been felt by many people working on aflatoxin that it might possibly account for primary carcinoma of the liver in these countries. It must be remembered that the mould producing aflatoxin can grow on cereals and rice. With the demonstration that aflatoxin B_1 produces cytological changes in human embryonic lung cell cultures[25] and inhibits the growth of human liver cells,[39] human cells are at risk if exposed to aflatoxin and their susceptibility is as great as those of experimental animals.

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