the toxin passes into the nucleus. (2) It binds with the DNA and thereby prevents the action of the RNA polymerase. (3) There is a reduced synthesis of RNA, involving in particular the inhibition in messenger RNA (mRNA) as exemplified by the inhibition of the cortisone-induced synthesis of tryptophan pyrrolase. (4) The inhibition of mRNA is reflected after some fifteen minutes in a reduced protein synthesis (as shown in the experiments on normal liver slices to which aflatoxin B_1 has been added *in vitro*).

Such a reduction in mRNA synthesis would appear to continue for at least forty-eight hours as protein synthesis is depressed for this length of time after poisoning. Revel & Hiatt (1964) reported that most of the mRNA of the rat liver cell has a life of around forty hours. With the long interaction of aflatoxin B_1 with the DNA it would be expected that by forty hours in the poisoned liver cell all protein synthesis would become inhibited and result in the death of the cell. Actinomycin D itself does not cause liver necrosis in the livers of control rats. This may be due to the fact that the effect of actinomycin D on the mRNA synthesis has started to decline by sixteen hours after poisoning, and it would not therefore have an inhibitory effect on the synthesis of those proteins that are formed by a long life mRNA. On the other hand, in regenerating liver actinomycin D does produce cytological changes (Schwartz *et al.* 1965). In this situation there is an increase in mRNA turnover and a stimulation of protein synthesis and the effect of the DNA interaction with actinomycin D would now manifest its effects much earlier. The failure to demonstrate *in vivo* an inhibition of amino-acid incorporation into the liver proteins of rats poisoned with aflatoxin B_1 or actinomycin D can be explained if *in vivo* the major part of liver protein synthesis depends on long life mRNA and would therefore be virtually unaffected before forty hours after poisoning.

Finally, in view of our experimental findings on the action of aflatoxin B_1 (Clifford & Rees 1966), it may be that the differences in toxicity shown by other varieties of aflatoxin are determined in part by the extent to which they bind with DNA. Likewise the species specificity may also be a reflection of the particular composition of the DNA of a given species which could then determine the degree of binding with aflatoxin which would take place.

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Collagen and Lathyrism

Lathyrism is a human disease which has been known for the past hundred years; it occurs mainly in India, also in Spain during times of famine, when the population feeds on a diet consisting mainly of the chick pea, *Lathyrus sativus* (Selye 1957). The disease is described as affecting young people, producing a spastic paraplegia which is said to be progressive. Some years ago, American workers attempted to isolate a toxic factor from the chick pea, at that time without success; however, subsequent investigation of other species of pea showed that young rats fed on a diet consisting of 50% of sweet pea seeds, *Lathyrus odoratus*, developed severe skeletal deformities and often rupture of the aorta (Geiger *et al.* 1933, Ponseti & Baird 1952). More recently, the toxic factor responsible was isolated from the sweet pea seed and shown to be β-aminopropionitrile, $NH_2\ CH_2\ CH_2\ CN$, now known as the lathyrus factor, BAPN (Dasler 1954*a*, Schilling & Strong 1954, McKay *et al.* 1954). It should be emphasized that this experimental condition, designated as *osteo*-lathyrism, bears no obvious relationship to the human disease known as *neuro*lathyrism.

The lesions produced by treatment with BAPN have been best described in the rat; the most typical ones consist of marked periosteal new bone formation, exostoses of the long bones, kyphoscoliosis, hernias and aortic dissection (Selye 1957, Ponseti 1957, Dasler 1954*a*). In addition to the skeletal and aortic lesions, a striking and characteristic finding in lathyritic

animals is the marked fragility of tendinous and ligamentous attachments, epiphyseal plates and healing wounds (Stamler 1955, Enzinger & Warner 1957, Dasler 1954*b*, Follis 1956, Kalliomäki *et al.* 1957, Krikos & Orbison 1960). Studies on BAPN-treated chick embryos revealed a dramatic loss of tensile strength of the collagenous tissues, associated with an increase in the salt-solubility of the collagen, although there was no increase in the total amount of collagen synthesized (Levene & Gross 1959). This increased extractability of collagen was also found in croton-oil pouches of rats treated with BAPN (Clemmons 1958) and was confirmed in other systems (Mikkonen *et al.* 1960, Wirtschafter & Bentley 1962). Examination of the purified lathyritic collagen with the methods then available showed it to be normal, and so we concluded that the fragility and increased solubility of collagen in lathyrism indicated that the basic lesion induced by BAPN was a failure of polymerization of the tropocollagen molecules into normal collagen fibrils (Levene & Gross 1959). Subsequent comparison of purified normal and lathyritic collagen by newly developed techniques showed that the component polypeptide chains forming the sub-units of tropocollagen were inadequately bonded in lathyrism. There was therefore an intramolecular as well, probably, as an intermolecular defect in cross-linkage (Martin *et al.* 1961), resulting in a collagen fibre of diminished tensile strength.

At that time various compounds were found to be lathyrogenic but there appeared to be no common rationale for their activity. Investigation of this point showed that these could be classified into four groups – certain nitriles, ureides, hydrazides and hydrazines – which would produce an increase in the fragility, and increased extractability of collagen in the chick embryo, and that these four groups had in common the property of blocking carbonyl groups (Levene 1961). A working hypothesis was therefore proposed that lathyrogenic agents act by blocking hypothetical carbonyl groups in the collagen molecule which are necessary for its normal assembly and subsequent polymerization into fibrillar form. In support of this hypothesis, it was found that whilst purified normal collagen reacted with 2, 4-dinitrophenyl-hydrazine, a carbonyl-blocking agent, purified lathyritic collagen, failed to do so (Levene 1962). Recently, Gallop and his colleagues have isolated an aldehyde-containing moiety from normal fish collagen (Rojkind *et al.* 1964).

One interesting aspect of osteolathyrism is the light it may shed on the pathogenesis of certain so-called congenital deformities. In order to produce the previously described connective tissue fragility and increased collagen extractability, the lathyritic compound was injected into the embryo at 14 days of incubation. If, however, the compound was injected ten days earlier, at 4 days, the most powerful lathyrogens produced bowing of the tibiæ and an S-shaped deformity of the mandible (Chang *et al.* 1955, Rosenberg 1957). The likeliest explanation seems to be the effect of normal muscle tension on a weakened cartilaginous matrix, acting at a vulnerable time in embryonic development.

Our interest in lathyrism, itself an artificially induced condition, lies in the possibility it offers of inhibiting the normal polymerization of collagen in such pathological states as mitral stenosis, cirrhosis of the liver and silicosis. This possibility of a degree of control over the fibrotic process would seem to render a study of the linkage sites in collagen of practical as well as theoretical value.

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