polyriboT), on the other hand, primes the synthesis of polyU or polyA, respectively.

If the replicative form of poliovirus RNA were double-stranded, the observation (*19*) that in HeLa cells infected with this virus, the base ratios of the RNA newly synthesized in the presence of actinomycin D are similar to those of poliovirus RNA, and therefore do not reflect an equal synthesis of complementary RNA, might be explained by the assumption that only "plus" strands are produced on the double-stranded template. However, these results as well as those on the detection in vivo of only one kind of messenger RNA strand might also be interpreted as meaning that, while both strands are produced, one of them is subsequently eliminated. There is no evidence so far, although it is not unlikely, that newly formed viral RNA is exclusively of the "plus" type.

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1. Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; ATP, GTP, UTP, CTP, 5′-triphosphates of adenosine, guanosine, uridine, cytidine; polyA, polyadenylic acid; polyU, polyuridylic acid; polyriboT, polyribothymidylic acid; tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediamine-tetraacetate.
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21. We thank Professor S. Ochoa for his advice and encouragement, and Dr. J. S. Krakow for many stimulating discussions. One of us (C.W.) is a fellow of the Stiftung fuer Stipendien auf dem Gebiete der Chemie, Switzerland, and the American-Swiss Foundation for Scientific Exchange; the other (P.B.) is a Rockefeller Foundation fellow.

25 September 1963

Aflatoxin B_2: Chemical Identity and Biological Activity

Abstract. *Aflatoxin B_2, a blue-fluorescent metabolite of* Aspergillus flavus, *was isolated from cultures grown on crushed wheat. Chemical structure of the compound was elucidated as dihydroaflatoxin B_1. Biological activity was determined in day-old male white Pekin ducklings. The criteria of activity were reduction in growth and liver size and the extent of bile-duct hyperplasia.*

Aflatoxins B and G, two metabolites of the fungus *Aspergillus flavus*, which were associated with the toxicity of animal feeds (*1*), have recently been isolated and their structures elucidated (*2*). When cultured under laboratory conditions, this fungus produces a complex mixture of blue and yellow-green fluorescent compounds which are separable by chromatography. A system of nomenclature has been suggested in which the two major components (B and G above) would become B_1 and G_1 (*3*).

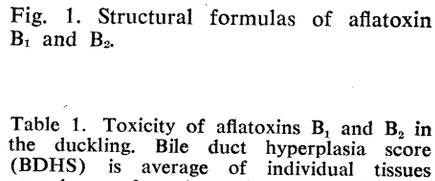
We have identified and evaluated the toxicity of a second blue-fluorescent compound, aflatoxin B_2, produced in relatively small quantities by the fungus. The compound was isolated from cultures of *A. flavus* grown for 7 days at 30°C on sterilized crushed wheat. Cultures were thoroughly extracted with chloroform and the crude mixture of toxic fluorescent substances was precipitated by addition to 20 volumes of petroleum ether. Crude extracts were thus obtained in yields of approximately 750 mg per kilogram of wheat.

Fractionation of the crude mixtures was accomplished by thin-layer chromatography on silica gel G (Merck) with a mixture of chloroform and methanol (97:3) as developing solvent. The blue-fluorescent substance migrating with a slightly smaller R_F value than aflatoxin B_1 was isolated by extraction of the adsorbant with methanol. This compound, aflatoxin B_2, was purified by recrystallization from chloroform-pentane.

Its molecular weight (m.w.) was 314 (mass spectrograph); m.p. 286° to 289°C (decomposed); $\lambda_{max}^{ethanol}$ 222, 265, 362 mμ (ε19,600, 9,200, 14,700); $\nu_{max}^{CHCl_3}$ 1760, 1685, 1625, 1600 cm^{-1}; $[\alpha]_D^{CHCl_3}$ −429°. These data are very similar to those of aflatoxin B_1 (*2*): $C_{17}H_{12}O_6$, m.w. 312; m.p. 268° to 269°C (dec.); $\lambda_{max}^{CHCl_3}$ 223, 265, 362 mμ (ε 25,600, 13,400, 21,800); $\nu_{max}^{CHCl_3}$ 1760, 1665, 1630, 1600 cm^{-1}; $[\alpha]_D^{CHCl_3}$ −558°. The data strongly suggest that aflatoxin B_2 is dihydroaflatoxin B_1 (Fig. 1).

In order to test this hypothesis, dihydroaflatoxin B_1 was prepared by catalytic hydrogenation of aflatoxin B_1 in ethanol over 1 percent palladized calcium carbonate. The reduction was interrupted after one mole of hydrogen had been absorbed. The resulting product had m.w. 314 (mass spectrograph); m.p. 287 to 289°C (dec.); $\lambda_{max}^{ethanol}$ 222, 265, 362 mμ (ε17,600, 11,000, 20,800); $\nu_{max}^{CHCl_3}$ 1760, 1685, 1625, 1600 cm^{-1}; $[\alpha]_D^{CHCl_3}$ −430°. On thin-layer chromatograms, its R_F value was identical with that of aflatoxin B_2. These physical data agree well with those reported by other investigators (*4*, *5*). Thus aflatoxin B_2 is dihydroaflatoxin B_1 and has the structure shown in Fig. 1.

The toxic properties of aflatoxin B_2 isolated from culture extracts were compared with those of aflatoxin B_1



$C_{17}H_{12}O_6$ MOL. WT. 312 $C_{17}H_{14}O_6$ MOL. WT. 314

Fig. 1. Structural formulas of aflatoxin B_1 and B_2.

Table 1. Toxicity of aflatoxins B_1 and B_2 in the duckling. Bile duct hyperplasia score (BDHS) is average of individual tissues scored on a 0 to 4+ scale (× 10). Six animals at each dose.

Dose (μg)	Body wt. 8-day (g)	Liver weight		BDHS
		Actual (g)	Relative to body wt. (%)	
		Aflatoxin B_1		
0.0	133	7.1	5.3	0
2.0	129	6.0	5.0	10.0
3.9	125	6.6	5.3	16.0
7.8	101	4.6	4.6	30.0
15.7	112	4.8	4.3	30.0
		Aflatoxin B_2		
0.0	142	8.2	5.9	0
50.0	147	7.1	4.9	16.0
80.0	127	6.2	4.9	20.0
125.0	116	6.3	4.7	20.0
200.0	100	4.5	4.5	30.0

by a biological assay procedure. The appropriate test compound was dissolved in propylene glycol in a series of concentrations which were administered orally to day-old male white Pekin ducklings. Each animal received 0.1 ml of solution daily for 5 consecutive days. Surviving animals were killed by decapitation on the 8th day and their livers were removed, weighed, and fixed in formalin. Frozen sections stained with hematoxylin and eosin were subjected to histologic evaluation. The criteria of toxicity were reduction in growth and liver size and the extent of bile duct hyperplasia characteristic of the field syndrome (*1*), which has proved to be a consistent and reproducible response to these toxic compounds.

The doses were selected to provide a range in intensity of the response from minimal to severe bile-duct hyperplasia, without extensive mortality. The range of 5-day total doses was 2.0 to 15.7 μg for aflatoxin B_1 and 50 to 200 μg for the derivative. The results of the assays are shown in Table 1.

These data indicate that both compounds have toxic properties manifested by depression in growth, reduction of liver weight, and histopathologic lesions in the liver. The intensity of the toxic response is related to dose in all instances. Clearly, however, the biological potency of aflatoxin B_2 is markedly reduced by comparison with aflatoxin B_1. It may be presumed, therefore, that the point of unsaturation in the B_1 compound,—absent in the B_2—is an important contributing factor to the potency of aflatoxin B_1 (*6*).

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26 August 1963

Behavior of Adult Rats Is Modified by the Experiences Their Mothers Had as Infants

Abstract. *Some rat pups were handled for 20 days in infancy, while others were not. When the rats reached adulthood the females were bred. Some of the offspring were left with their natural mothers, others were fostered to mothers of the same background (handled/nonhandled) as that of their natural mothers, while still others were fostered to mothers with a different background from that of their natural mothers. The offspring were weaned and weighed at 21 days; at 50 days, activity and defecation scores were obtained in the open field. The weights at weaning and the defecation scores at 50 days were significantly influenced by the experience in infancy of the "postnatal" mother, whether she was the natural mother or a foster mother. The natural mother and the foster mother jointly affected the open-field activity of the offspring.*

Handling rats in infancy has marked effects upon their subsequent behavioral and physiological processes (*1*). To date no one has investigated whether the handling of female rats in infancy affects their offspring. Modifications of the offspring's characteristics could occur during their fetal period, as a result of physiological changes induced in the mother by the handling she had received in infancy, or they could occur after birth as a result of either physiological changes (which could, for example, modify milk supply) or behavioral changes induced in the mother by the handling she had received in infancy. We now report results of such an investigation (*2*).

About 45 litters of Purdue-Wistar rats were handled in infancy. Handling consisted of removing a complete litter from the home cage (leaving the mother in the cage), placing the pups on shavings in a can for 3 minutes, and then returning the pups to their home cage. This was done once a day from day 1 through day 20 of life. About 45 other litters were not disturbed during this time. Once these litters were born the shavings in their cages were never changed; food and water were supplied without opening the cages.

At 21 days the handled and nonhandled litters were weaned, and the females were placed in specially designated cages. When mature, the females were bred to a random sample of colony males; the males were systematically moved from one cage to another and were exposed equally often to handled and nonhandled females.

When pregnant, the females were placed in stainless-steel maternity cages. The day after birth all litters were sexed, and those containing more than eight pups were reduced to four of each sex when possible, but never to less than two of one sex. No litter containing less than seven pups was used. The litters were then returned to their natural mothers. At this time (i) some litters were left with their natural mothers, (ii) other litters were fostered to mothers that had had the same experience (handled or not handled) in infancy as the natural mothers, and (iii) still other litters were fostered to mothers that in infancy had received the treatment opposite to that of the natural mothers. Fostering was done by moving the mothers from one cage to another, leaving the pups in the cage in which they had been born. In most instances fostering took place between litters born on the same day; in six cases the foster mother had given birth 1 day earlier than the natural mother, and in one case the foster mother had given birth 3 days earlier than the natural mother. Except for fostering, the litters were not disturbed. At 21 days the pups from 55 litters were weaned, weighed, sexed, earpunched, and placed in laboratory cages with littermates of the same sex.

Starting at 50 days of age, the animals were given 4 days of open-field testing. The field was 45 inches (115 cm) square, painted flat black, with walls 18 inches (46 cm) high. The floor was marked off in 9-inch (23-cm) squares by thin white lines. A rat was placed in one corner of the field, and its behavior was observed for 3 minutes. Total numbers of squares entered and boluses defecated were recorded. Two males and two females from each of 47 litters were tested in the open field. Testing was completed at 53 days, at