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Detection and Estimation of Ochratoxin A

THE recent isolation¹ of ochratoxin A from *Aspergillus ochraceus* Wilh., which has a widespread natural distribution, made the development of a rapid method for the detection and estimation of the mycotoxin desirable. The following methods have been used during screening of fungal strains and media for toxin production.

A 20-g sample of mouldy material in a 100-ml. Soxhlet apparatus is extracted with 1:1 chloroform-methanol for 12 h. The extract is diluted with an equal volume of chloroform, extracted with 0.1 M aqueous sodium bicarbonate, and the toxin extracted with chloroform from the aqueous phase after acidification with 2 M hydrochloric acid. After removal of the solvent *in vacuo*, the residue is dissolved in 5 ml. chloroform and a 20- μ l. portion spotted on a silica thin-layer chromatoplate together with a standard sample of pure ochratoxin A. The chromatoplate is developed in 3:1 benzene-acetic acid and inspected under ultra-violet light. Ochratoxin A appears as a green fluorescent spot at R_F 0.5. Blue-green and pale green fluorescent spots at R_F 0.35 and 0.65 indicate the presence of ochratoxins B and C, the dechloro and the ethyl ester derivatives of ochratoxin A (ref. 2). On spraying with 0.1 N aqueous sodium hydroxide, ochratoxins A, B and C exhibit a bright blue fluorescence. The ochratoxin A content of the original material can be estimated by comparing the size of the observed spot with that from a standard solution of ochratoxin A. In this manner 0.1 p.p.m. ochratoxin A added to maize meal could be detected.

In cases where unknown substances interfere with this method, or where additional proof is desirable, the results can be verified by thin-layer chromatography on silica using 12:2:1 benzene-methanol-acetic acid (giving a green fluorescent spot at R_F 0.6) or paper chromatography on Whatman No. 1 paper with the system 3:1 propanol-3 N aqueous ammonium carbonate (giving a blue fluorescent spot at R_F 0.65).

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Elevation of Alkaline Phosphatase Activity in the Intestine of the Chick Embryo by Actinomycin D

DURING the development of the mouse, alkaline phosphatase activity in the duodenum rises sharply before birth, falls back slightly, and then increases twenty-fold or more between 15 and 20 days¹. This second rise is accelerated rather than retarded by inhibitors of RNA

and protein synthesis^{2,3}. Injection of actinomycin D, an inhibitor of DNA-dependent RNA synthesis⁴, into 9-day-old mice more than doubles their phosphatase activity within a 54 h period³, and the enhanced activity shows the normal increased preference for phenylphosphate over beta-glycerophosphate. Because these effects were obtained at a period of development when a considerable amount of phosphatase activity is already present, we thought it would be interesting to investigate the effect of actinomycin at a time when phosphatase activity first appears.

In the duodenum of the chick embryo, alkaline phosphatase activity is first found in the brush border of the epithelial cells at about 17 days^{5,6}. By 21 days the adult level of phosphatase activity is reached—the enzyme activity has undergone a 100-fold increase after its initial appearance⁵. The effect of actinomycin D on this increase has been investigated during the 16–19-day interval, during which the enzyme activity increases about ten-fold. It has been found that phosphatase activity in the chick embryo duodenum in these stages is elevated two to three times above the normal levels by actinomycin injection at 16 days.

Single 5 μ g doses of actinomycin D (obtained from Merck, Sharp and Dohme) dissolved in 0.1 ml. of 0.9 per cent saline were injected into a vein in the chorio-allantoic membranes of 16-day-old chick embryos; controls received an equal volume of saline alone, or were not injected. Embryos were killed after 1 or 3 days (that is, at 17 and 19 days of incubation); the duodenal loop was removed, weighed, and homogenized in iced distilled water with a 'Ten Broeck' grinder. Homogenates were assayed for protein content⁷ and for enzyme activity on phenylphosphate (PhP) and beta-glycerophosphate (BGP) as described by Kato⁸, except that 0.2 M sodium carbonate-sodium bicarbonate buffer was used in the incubation mixture.

Table 1. EFFECT OF ACTINOMYCIN D INJECTED AT 16 DAYS ON ALKALINE PHOSPHATASE ACTIVITY OF THE DUODENUM OF THE CHICK EMBRYO

Injection	Day of killing	No.	Specific activity* (mean and range)	PhP† BGP	Protein concentration (μg/mg fresh weight)
Saline	17	5	45.1 (13.5–84.6)	2.62	97.5
Actinomycin	17	7	159.7† (77.1–266)	6.59	97.5
None	19	7	402.6 (207–543)	7.98	90.7
Saline	19	14	372.0 (141–600)	7.39	89.4
Actinomycin	19	11	947‡ (301–2,203)	8.25	94.8

* μ g phosphorus released from PhP per mg protein per 30 min.

† Ratio of the amount of phosphorus released from PhP to amount of phosphorus released from BGP.

‡ For actinomycin injected versus saline control, $P < 0.001$ as determined by Student's *t* test (ref. 10).

The results of these experiments are given in Table 1. In 17-day-old embryos which received actinomycin 24 h earlier, the duodenal phosphatase activity is more than three and a half times that of saline-injected controls. Even at 19 days the enzyme activity is more than twice the normal level for that stage. The increases in both 17- and 19-day-old embryos are highly significant. Histochemical investigations of duodena from embryos into which actinomycin had been injected show that the elevated phosphatase activity is localized in the brush border, where it is normally found⁶. Actinomycin treatment elevates phosphatase activity on both substrates tested, but a relatively greater enhancement of activity on PhP was observed, as indicated by the increased PhP/BGP ratio in 17-day-old embryos. This ratio normally rises at the time when phosphatase activity first appears in the apical border of the epithelial cells⁶. The protein content of the duodenum was not lowered by treatment with actinomycin; body-weight was not significantly affected, nor did development appear to be retarded.