

comparative studies are being conducted on the toxin production of *Aspergillus flavus* and other moulds.

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<sup>1</sup> Sargeant, K., Sheridan, Ann, O'Kelly, J., and Carnaghan, R. B. A., *Nature*, **192**, 1096 (1961).

<sup>2</sup> Toxicity Associated with Certain Batches of Groundnuts. *Rep. Interdepartmental Working Party on Groundnut Toxicity Research* (1962).

<sup>3</sup> Platt, B. S., Stewart, R. J. C., and Gupta, R., *Proc. Nutr. Soc.*, **30**, 21 (1962).

<sup>4</sup> Sargeant, K., O'Kelly, J., Carnaghan, R. B. A., and Allcroft, R., *Vet. Rec.*, **73**, 1219 (1961).

### Determination of the Aflatoxins

WE report a new chemical method for determining the aflatoxins and, in particular, aflatoxin  $B_1$ . Previous methods have depended on the measurement of fluorescence<sup>1,2</sup> and are subject to certain errors, for example, quenching by impurities. Moreover, it is known that the fluorescence of solutions of aflatoxin  $B_1$  in methanol varies with time<sup>3</sup>.

All four aflatoxins have an ultra-violet absorption peak of high molar extinction at 363  $m\mu$  (ref. 4). The measurement of the optical density of this peak was chosen as the basis of an alternative assay procedure. This method is less sensitive than that depending on dilution to extinction of fluorescence, but it provides a more reliable means of assaying meals containing 1 p.p.m. or more of aflatoxin.

A brief outline of the procedure follows.

Groundnut meal (40 g) was extracted for 6 h with light petroleum (40°–60° C) in a Soxhlet extractor, dried and re-extracted for 6 h with methanol. The methanol solution was diluted with half its volume of distilled water, and continuously extracted with chloroform for 6 h. The residue from evaporation of the chloroform was made up to 10 ml. in chloroform, and 0.2 ml. portions of this solution were applied in a line across the bottom of a 'Kieselgel G' chromatoplate (thickness 750  $\mu$ ). This was developed in diethyl ether, dried and developed twice more in chloroform (2 per cent methanol). The chromatoplate was viewed under ultra-violet light (principal wave-length 365  $m\mu$ ) and rejected if the bright blue fluorescent band of  $B_1$  was not reasonably straight, clear of other fluorescent bands and running ahead of brown material near the origin.

The  $B_1$  fluorescent band was then scraped from the chromatoplate treated for 5 min with cold methanol, the solution filtered and the silica washed with methanol. The methanol filtrate was made up to 5 ml. and the amount of  $B_1$  calculated from the optical density of the solution at 363  $m\mu$  ( $\epsilon$  363  $m\mu$  = 22,000)<sup>4</sup>.

In practice the ultra-violet spectrum was measured from 210 to 400  $m\mu$  on a recording spectrophotometer. A criterion of purity was obtained from the resemblance of the spectrum to that of pure  $B_1$ .

A number of extractions were made of a homogenized batch of groundnut meal which is being used as a standard for chemical and biological work. Several determinations were carried out on each extract, and the aflatoxin  $B_1$  content of the meal was found to be  $10.2 \pm 0.19$  p.p.m. (95 per cent confidence limits). Aflatoxin  $B_2$  was detected as a second blue fluorescent band running behind  $B_1$ <sup>4,5</sup>. The amount present was determined by pooling the extract of this band obtained from several plates and was estimated as 0.2 p.p.m. in the original meal. The completeness of the removal of aflatoxin by 6 h Soxhlet extraction was checked by extraction of the meal for a further 6 h with methanol; this yielded no more  $B_1$  or  $B_2$ . The possibility of loss of  $B_1$  in the stages after the methanol extraction was checked by the addition of known quantities of pure  $B_1$  to methanol extracts of the standard toxic meal and

of a non-toxic meal and assay of the resultant products. There was good agreement between the found and calculated values for these samples.

The foregoing method has also been used for the determination of aflatoxins  $B_1$  and  $G_1$  in crude crystalline aflatoxin isolated from both synthetic media and sterilized groundnuts inoculated with *Aspergillus flavus*.

We thank Mr. A. B. Wood for the measurement of the ultra-violet spectra.

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### An Ion-exchange Method for the Estimation of Strontium-90 in Milk

THE classical method of Bryant *et al.*<sup>1</sup> for the estimation of strontium-90 in milk is both tedious and hazardous. Davis<sup>2</sup>, Farabee<sup>3</sup> and other workers<sup>4,5</sup> have all reported separations of strontium from calcium using ion-exchange methods, and such techniques would appear to offer promise for the estimation of strontium-90 in milk.

In the investigation recorded here, ion-exchange columns 22 cm long and 1 cm in diameter of the sodium form of 'Zeo-karb 225' (52–100 mesh) were set up and the resin conditioned by the passage of three bed volumes of a 2 per cent solution of the disodium salt of EDTA at pH 5. The flow rate through the column was limited throughout the investigation to three bed volumes per hour.

Samples of milk ash (10 g) were dissolved in 10 M nitric acid, inert strontium carrier (50 mg) added and the mixture heated until evolution of brown fumes ceased. 5 M sodium hydroxide was added until alkaline, the paste dissolved in 10 per cent EDTA solution (100 ml. disodium salt) and the pH adjusted to 5.00 with 5 N hydrochloric acid.

The solution of ash was passed down the column, which was washed with a 2 per cent solution of EDTA (disodium salt, 200 ml.) at pH 5.25 and, finally, with distilled water (200 ml.). The strontium was eluted from the column with 5 N hydrochloric acid (70 ml.), followed by distilled water (100 ml.). The total eluate volume was reduced to less than 100 ml. by evaporation and the pH adjusted to 5 by the dropwise addition of 5 M sodium hydroxide. After heating to 80° C, '880' ammonium hydroxide (3 ml.) was added, followed by an excess of ammonium carbonate solution. After cooling to room temperature the suspension was centrifuged, the supernate discarded and the precipitate washed several times with distilled water. The precipitate was dissolved in the minimum volume of 6 M nitric acid and inert yttrium carrier (10 mg) added. The solution was left to stand for 14 days<sup>6</sup> to allow the yttrium-90 daughter to grow in.

The solution was made alkaline with carbonate free ammonium hydroxide, the time noted and the precipitate coagulated by heating on a water bath. The suspension was centrifuged, both precipitate and supernate being retained. The precipitate was dissolved in the minimum volume of 6 M nitric acid, diluted to 10–15 ml. and the yttrium reprecipitated with ammonium hydroxide. After centrifuging (the supernatant liquid being added to that obtained earlier), the precipitate was again dissolved in the minimum volume of 6 M nitric acid. The solution was heated to 80° C and the yttrium reprecipitated by the addition of an 8 per cent aqueous solution of oxalic acid (20 ml.). The precipitate was rendered granular by