tional to reaction rate, approximate apparent activation energies were calculated for cocoa butter, sperm oil, refined cottonseed oil, and sesame oil using the Arrhenius equation. The range of temperatures used was 140 to 200C. The activation energies calculated ranged from 21 to 31 kcal per mole.

This preliminary investigation indicates that a study of the phenomenon of oxyluminescence in oils and related products may be of value in the elucidation of the mechanism and kinetics of the autoxidation process. The use of oxyluminescence to investigate antioxidants (1,2) in polymers suggests its similar application to oils and related products. In view of the increasing number and diverse nature of the organic materials in which oxyluminescence has been observed, it is suggested that any material susceptible to oxidation under similar conditions to those outlined above might be worthy of investigation by this technique. Further study of this phenomenon is planned at this laboratory. The construction of a more refined apparatus for accurate measurements on selected samples of natural materials and pure model compounds is necessary to determine the role played in this phenomenon by impurities found in natural fatty substances.

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Fluorodensitometry of Aflatoxin on Thin-Layer Plates

FLUORODENSITOMETRY has been found to be a useful tool for the quantitative estimation of aflatoxin B_1 directly from a developed thin-layer chromatographic plate. Standard concentrations of aflatoxin B_1 may be easily compared with the unknown samples. This objective procedure has a number of distinct advantages over the visual method of comparison now being used (3,4). Poor quantitative values are obtained by the visual methods unless the thin-layer plates are read by a trained observer. The visual technique requires that the sample be directly compared with a standard spot of approximately the same concentration. Accuracy is dependent on visual discrimination and acumen. Finally, the fluorescent spots may be visually quite faint and the relative intensity very difficult to judge.

The fluorodensitometry technique has been used to quantify the final extracts obtained in the determination of aflatoxin as described by Pons and Goldblatt (4) and Engebrecht, Ayres and Sinnhuber (3). The stationary phases used in these determinations, respectively, are Silica Gel G-HR and Silica Gel G (Brinkman Instruments, Inc.). Comparison of the fluorodensitometric and visual results using these two methods on four cottonseed meal samples is shown on Table I.

The instrument employed in this technique is manufactured by the Photovolt Co. (1115 Broadway, New York 10, N.Y.) and consists of a recorder, a multiplier photometer, an ultraviolet light source, a phototube unit, thin-layer chromatography stage equipment and filters. The ultra violet light passes from a mercury vapor lamp (320–390 mμ emmission), through a primary filter (365 mμ band), through a primary slit (1 mm × 25 mm) and to the stationary silica gel phase on the plate. The aflatoxin B_1 absorbs the 365 mμ light and emits fluorescent light at 425 mμ (2). This light passes through the glass plate, the secondary slit (0.1 × 6 mm), the secondary filter (445 mμ band) and into the phototube. The secondary filter permits only the emitted or visible fluorescent light to pass into the phototube; all utlraviolet light from the lamp is screened out.

The developed TLC plate to be read is placed on

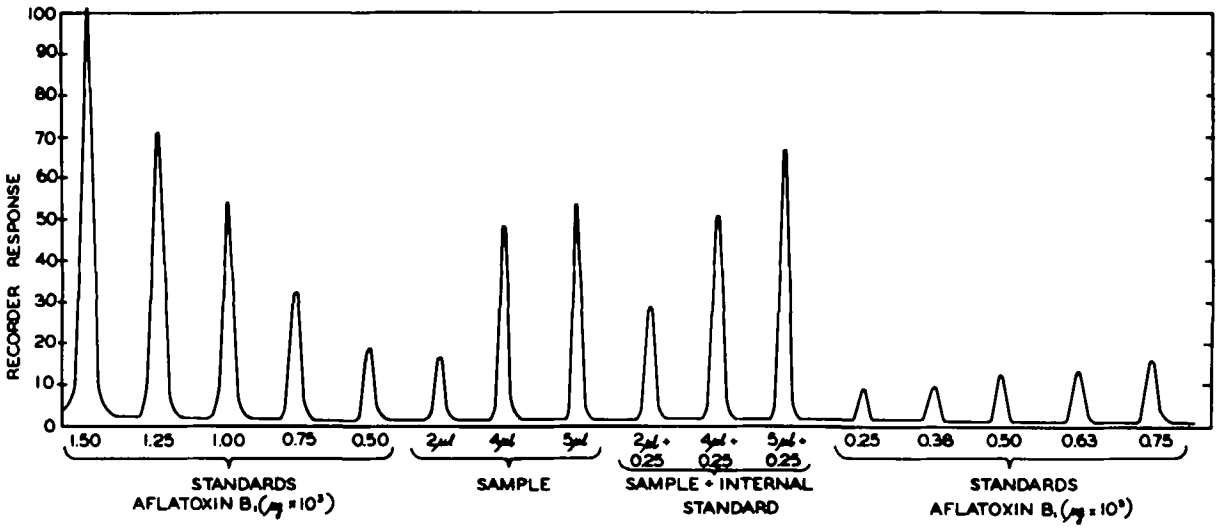

FIG. 1. A typical fluorodensitogram of aflatoxin B_1 on a TLC plate.

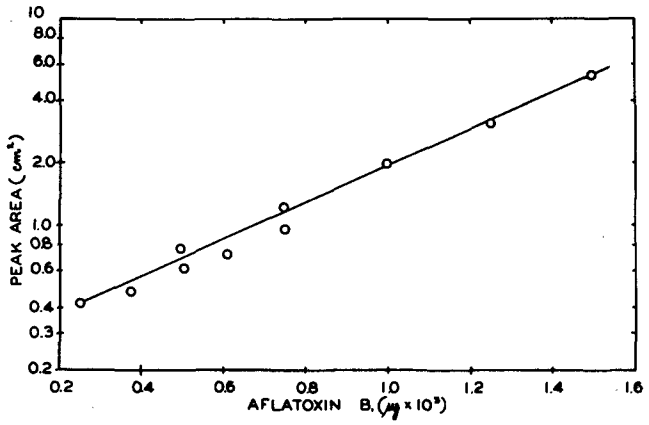

FIG. 2. Relationship of peak area to concentration of aflatoxin B_1.

the stage with the stationary phase down and the response selector switch set at 5. At this setting, the area under the recorded response is a logarithmic function of the light intensity. The zero line is adjusted with the dark point control and sensitivity control on the multiplier photometer unit. To check the sample for interfering material, the sample is spotted and developed by the usual method (3,4). The plate is then positioned so that the aflatoxin B_1 spot is in the light path. The sensitivity is then adjusted using the full light control to give the desired pen travel. The plate is scanned from the origin to the solvent front for each sample and for an aflatoxin B_1 standard.

For quantitative analysis several standard solutions were prepared using pure aflatoxin B_1 and the ultraviolet spectrophotometric extinction coefficients reported by Asao et al. (1). These standards along with 1, 2 and 4 μl portions of sample extract are spotted and the plate developed by the usual solvent system (3,4). After development the plate is placed so that the direction of scan is perpendicular to the direction of development. The plate is positioned over the aflatoxin B_1 standard of highest concentration and the recorder response adjusted with the full light control. The plate is then scanned by means of the motor drive on the TLC stage and the recorder (Fig. 1). The peak areas are determined by $A = \frac{1}{2}$ Base $\times$ Height and areas for standards are plotted against aflatoxin B_1 concentration on semilog paper (Fig. 2). The concentration of the sample is then determined by interpolation from this plot.

Standard deviation may be calculated from the three sample aflatoxin B_1 areas. An internal standard may also be applied to the sample spots before development as an additional check on the method. After development and fluorodensitometry the added aflatoxin may be subtracted from the interpolated sample value.

TABLE I

Comparison of Visual and Fluorodensitometry Methods for the Determination of Aflatoxin B_1

Cottonseed No.	Engebrech, Ayres and Sinnhuber procedure (3)		Pons and Goldblatt procedure (4)	
	Visual	Fluorodensitometry	Visual	Fluorodensitometry
1	190 ppb	182 ± 12 ppb	150 ppb	178 ± 15 ppb
2	80	82 ± 6	110	90 ± 9
3	150	146 ± 19	150	153 ± 14
4	19	22 ± 3	20	23 ± 5

The results obtained from four aflatoxin-containing cottonseed meal samples are shown in Table I.

For both the visual and fluorodensitometry techniques, 10 standards, 3 sample and 3 sample plus internal standard spots were compared. The standard concentrations ranged from 0.25 to 1.5×10^{-3} (μg aflatoxin B_1) per spot and the internal standard used was 0.25×10^{-3} (μg aflatoxin B_1). This scan is illustrated in Figure 1.

The sensitivity of the method is limited by the presence of interfering materials and their separation from aflatoxins on the TLC plate. Pure aflatoxin in amounts as low as 8×10^{-5} μg on a TLC may be quantified. With lesser amounts, instrument noise becomes a problem and the fluorescence background on the plates interferes with the symmetry of the peaks on the scan.

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• *Addendum*

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FIG. 1. Absorption spectra determined for 0.4 mg sterol/s per tube; color development at 6 ml total volume for 30 minutes, 25C; 1 cm light-path.