that it was considered to be negligible. Average nonprotein sulfhydryl content in homogenates was 0.019 μmol of SH/mg of nitrogen.

Analysis of variance showed that sulfhydryl concentration did not change significantly with time of aging within a preparation method ($P = 0.05$). Duncan's Multiple Range test showed HP-II to have significantly higher values than HP-I or HP-III for all aging periods, while HP-I and HP-III were not significantly different from each other ($P = 0.05$).

The greater range of values observed for HP-I and HP-III at each aging time, as shown in Figure 1, indicates that homogenization using a Sorvall blender or sonic oscillator may cause variability in masceration of the tissue and therefore affect the efficiency of exposure of sulfhydryl groups in some way to give a wider range of readings than when tissue is digested with acidified pepsin (HP-II). In triplicate analyses of a particular homogenate, the experimental values were nearly identical indicating the homogenate itself was uniform. In addition, recovery of internal standards was essentially 100%, indicating the method of analyzing for sulfhydryl groups is reproducible and accurate.

The total SH concentration in chicken breast muscle agreed closely with those values reported by Chajuss and Spencer (1962) and was 0.200–0.300 μmol of SH/mg of nitrogen lower than those reported by Gawronski *et al.* (1967). Total sulfhydryl concentration reported by Caldwell and Lineweaver (1969) was about 2 to 3 times higher than that reported here, and nonprotein sulfhydryl concentration was about 30 times higher than the results reported in this study. This difference may be due to the method of analysis used to measure thiol concentration. Those workers used pH values of 6.8 and 7.6 to measure nonprotein and total sulfhydryl content, respectively, by the procedure of Jocelyn (1962), while a pH of 8.1–8.2 was used in this work. Sedlak and Lindsay (1968) have found Jocelyn's method to have poor reproducibility and recovery and reported that color production between protein sulfhydryls and DTNB at a pH of 6.8 or lower would seriously affect the accuracy of total and nonprotein sulfhydryl determination by Jocelyn's method. Another source of variation in results may be due to the use of mature White Leghorn hens as experimental material by these workers and the use of broilers by Caldwell and Lineweaver. Caldwell and Lineweaver used electric stun in dispatch of the birds, while these workers restrained the birds during bleeding.

CONCLUSION

The results of measuring sulfhydryl groups during post-mortem aging reported here show no statistically significant change in sulfhydryl concentration with time. No attempt was made to follow the time course of rigor during post-mortem aging, and it is possible that any meaningful changes in sulfhydryl concentration may have been canceled by bird differences attributable to muscles entering rigor at different times. The influence of variation in the time pattern of rigor could be minimized by measuring pH of the excised muscle sample to follow the post-mortem muscular changes (Marsh, 1954) and adjusting sampling times accordingly. This precaution could be recommended for subsequent studies.

It appears that the method of tissue homogenate preparation does not fully account for the differences in results reported by Chajuss and Spencer (1962) and Gawronski *et al.* (1967) and those reported by Caldwell and Lineweaver (1969). It is possible that the decrease in sulfhydryl concentration during the first 2 hr of post-mortem aging reported by Chajuss and Spencer (1962) and Gawronski *et al.* (1967) was due to the methods of assaying for sulfhydryl content. A comparison of the nitroprusside method, amperometric titration, and the Ellman's reagent method of Sedlak and Lindsay (1968) of assaying sulfhydryl content in muscle tissue at defined stages of rigor would further contribute to determining the reason for conflicting results.

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Phytosterols of *Cannabis* Smoke

The 3-β-hydroxysterols present in American-grown (MS-13) *Cannabis* were identified and quantitated in its smoke. The free sterol fraction of the smoke contained campesterol, stigmasterol, and β-sitosterol in essentially the same ratio as that found in the plant material.

The 3-β-hydroxysterols campesterol, stigmasterol, and β-sitosterol have recently been identified (Doorenbos *et al.*, 1971) and quantitated (Foote and Jones, 1974) in extracts of *Cannabis*. The phytosterols have also been isolated and quantitated in tobacco and its smoke (Stedman, 1968). It was therefore of interest to determine their presence in marihuana smoke. Previously it has been demonstrated that the phytosterols are precursors of carcinogenic hydrocarbons in smoke (Wynder *et al.*, 1959).

Quantitative analyses of sterol content were performed on the smoke condensate of *Cannabis* cultivated at the University of Mississippi (MS-13 Mexican male) by a standard method (Doorenbos *et al.*, 1971). Seventy-millimeter cigarettes were hand rolled and smoked on a smoking machine taking a 40-ml puff of 2-sec duration every minute (Adams and Jones, 1973). The average weight per cigarette was 0.78 g. The smoke condensate was trapped in Celite at Dry Ice–isopropyl alcohol temperature and removed from the trap by washing with methylene chloride. The smoke condensate obtained from smoking 390 g of

Table I. Phytosterols of *Cannabis* Smoke

	%	mg of sterol/g of *Cannabis* smoked
Campesterol	16.1	0.0092
Stigmasterol	14.2	0.0081
β-Sitosterol	69.6	0.0806

marihuana was concentrated to a viscous oil weighing 6.24 g. The entire sample was taken up in 80% ethanol, stirred, and allowed to stand under refrigeration for several hours before being filtered. The precipitate was washed with 80% ethanol, and 5 ml of hot 2% digitonide in 80% ethanol added. The solution was concentrated under reduced pressure to an oil that was then allowed to cool gradually and to stand overnight. The digitonide was filtered onto a weighed sintered glass filter funnel and washed with 80% ethanol, and subsequent washing with ether gave a white solid that was satisfactory for successive treatment. The sintered funnel and sample were dried by heating at 90° for 2 hr and placed into a desiccator upon removal from the oven. After reaching ambient temperature the weights of the funnel and sample were taken and the sample weight determined: 0.1589 g of digitonide or 40.2 mg of sterols (Wall and Kelley, 1974). The sterols were isolated for gas chromatography by decomposing the digitonide with 20 ml of hot Me_2SO and extracting the cooled mixture with three 25-ml portions of hexane. The combined hexane fraction was dried ($MgSO_4$) and concentrated. The resulting solid was then made to volume in tetrahydrofuran and aliquots were subjected to gas chromatographic analysis (Grunwald, 1970; Foote and Jones, 1974). Samples were injected into a gas chromatograph (Beckman GC-45) using a flame ionization detector. The sterols were separated in a 10 ft × 4 mm glass column packed with 5% OV-101 on Gas Chrom-Q, 80–100 mesh, with a column temperature of 275° and helium carrier gas flow of 75 cm^3/min at 34 psi. Samples were injected on-column and quantitative analysis was performed by electronically integrating the peaks. The relative weight response was determined from synthetic mixtures. Only campesterol, stigmasterol, and β-sitosterol were found in the sterol fraction of the smoke from MS-13 plant material. The calculated levels of individual sterols presented in Table I are essentially of the same ratio as those in the free phytosterol fraction of the plant material (Foote and Jones, 1974) smoked in this study.

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Extraction Rate Equations for Paprika and Turmeric with Certain Organic Solvents

In order to describe concentration–time data obtained from spice extractions, it was necessary to derive extraction rate equations using a kinetic, rather than an equilibrium, approach. Three types of rate-limiting steps were tried, simple first order, diffusion controlled, and one in which the rate included a term that increased with time. The last case led to the following equation which appears to fit the data reasonably well, $c/c_0 = (1 + 2\gamma t^*) \exp[-k_1 t^*(1 + \gamma t^*)]$, where t^* is time after the start of elution from the bed, c_0 and c are initial concentration and concentration at t^*, respectively, and k_1 and γ are constants.

During a study of the rates at which various solvent systems were capable of extracting the pigment of paprika (about 46% capsanthin, 20% β-carotene, 20% zeaxanthin, 8% kryptoxanthin, and 6% capsorubin; Andre, 1973), it became apparent that it would be useful to have mathematical expressions with which to represent time–concentration data and from which "rate parameters" could be deduced. However, an examination of the literature failed to reveal expressions suitable for use for extractions of interest. From plots of the time–concentration data it appeared that for a few extractions a simple semilog representation was satisfactory, but for others a more complex representation would be required. Therefore, the objective of this work was to derive the mathematical models necessary to describe the extraction data. Since the concentration of extract eluting from the paprika bed was well below that necessary for saturation, a kinetics (rather than an equilibrium) approach was taken similar to that used for systems in which one of the reactants is a solid, *e.g.* polymer pyrolyses, metal oxidations, catalyst poisonings, etc. Three types of rate-determining steps were considered. (a) The first is a simple first-order process, *i.e.* the rate of extraction is proportional to the mass concentration of extract in the bed. (b) The second is a process controlled by diffusion of solvent into or solute out of the solid particles, which results in the rate being inversely proportional to the extent of reaction, similar to metal oxidations which form nonvolatile oxides. (c) The third is a process in which the measured concentration initially decreases less rapidly (or may possibly increase for a short time) than would be predicted from the treatment in a. Case c was necessary to treat the data from the spice ex-