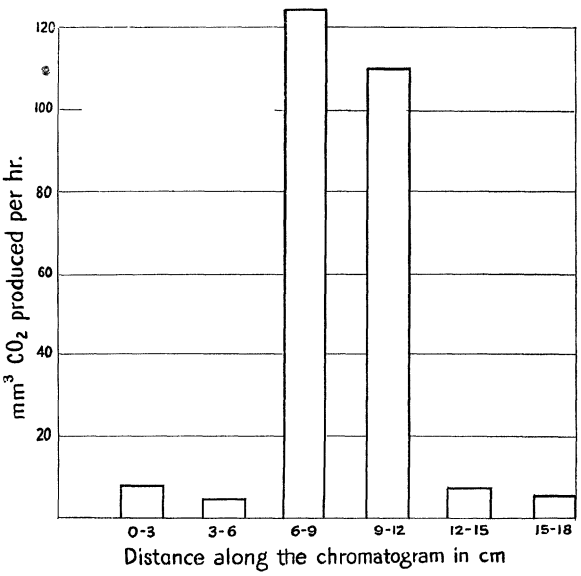

FIG. 15. Urease activity along the chromatogram.

used (diluted with physiological saline) and 0.4 ml of it placed in the Warburg vessel with 2.6 ml buffer and 0.2 ml 10% urea in the side arm. A period of 30 min was allowed for the contents of the vessel to attain thermal equilibrium (37° C). The urea in the side tube was then tipped into the vessels, and carbon dioxide output rate measured manometrically in the usual way for 1 hr.

The results show the existence of a distribution curve of urease on the paper strip. Traces of enzyme are left at the point of origin, but a fair proportion ascends the paper, whose maximum urease activity lies between 6 and 12 cm from the origin. On either side of the maximum there is a drop in enzymic activity. These results are shown in Fig. 15. If a parallel chromatogram is streaked with the freshly prepared benzidine reagent, it is found that the greatest extent of the colorless region on the colored background is 6–12 cm from the origin.

On assessing the activity of the urease over the entire strip, by adding together the activities of the various parts of the strip it is found that the total activity amounted to 85% of that expected from the amount of urease placed on the paper. Another assessment of the total urease activity over the entire strip showed a recovery of 110%, the assessment being made from a calibration curve previously prepared, relating activity (rates of CO_2 production) to the quantity of urease. These results show that urease activity is not diminished within experimental error by our chromatographic technique, and that movement of the urease molecule on filter paper can be followed. It is evident, however, from our preliminary results that metallic constituents of the filter paper may appreciably affect the rate of movement of urease and possibly other proteins. This needs more study.

Chromatography of human serum. A few experiments have been carried out on the chromatography of human serum. The results show the presence, at pH 6, of a complex mixture of hemin-reacting proteins. Three of the fractions appear to give R_f values identical with those found with a preparation of crystalline human serum albumin. Human serum globulins do not seem to move appreciably in the second dimension. We estimate that, with our technique, between 6 and 10 protein fractions appear. This work is now being extended with a view to discovering whether paper chromatography of blood serum may be used for diagnostic purposes.

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Tetrazolium Chloride as a Test for Damage in Artificially Cured Peanuts

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The artificial curing of peanuts is being investigated in several Southeastern states as one phase of a mechanization program designed to reduce production costs and improve nut quality. Drying methods found to be practicable for certain other oil crops and for cereal crops could not be applied empirically to the peanut because of the uncertainty of curing effects upon flavor of nuts for the food trade and because of the heat- and moisture-insulating properties of the peanut shell. Large scale experiments on artificial curing of peanuts, therefore, have had to await results of preliminary trials.

In a series of exploratory curing tests at the Alabama Agricultural Experiment Station, using different drying temperatures and rates of air flow, the desirability of a quick test of curing effects upon peanut quality soon became apparent. It was evident that the standard tests of quality in sound, mature peanut kernels—free fatty acid percentage, iodine value, and germinability—all required more time and analytical apparatus than were practical for rapid estimates of heating effects upon peanuts. Furthermore, germinability could be used at time of harvest only for the Spanish variety of peanut. The runner variety, which constitutes the great bulk of the Alabama crop, germinates only after a period of dormancy of several weeks following harvest.

However, it was assumed that seed viability might be correlated with some of those properties of flavor and oil quality which characterize peanuts of high market value both at time of harvest and after storage. Successful use of tetrazolium salts as viability stains in seed germination experiments (*2, 3, 5, 7*), in determination of vitality in miscellaneous plant and animal cells and tissues (*6*), and in demonstration of injury by heat in stem tissues (*8*) and by freezing in maize (*1, 4*) suggested their possible application as a quick test for heat and drying damage in artificially cured peanuts. Preliminary results indicate that they may be so used.

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TABLE 1

EFFECT OF HEATING UNSHELLED SPANISH PEANUTS FOR 1 HR AT VARIOUS TEMPERATURES UPON VITALITY AS SHOWN BY TETRAZOLIUM CHLORIDE AND GERMINABILITY TESTS

Temperature of treatment °F.	Degree of injury shown by tetrazolium vitality test				Germinability
	Killed	Serious damage	Slight damage	Undamaged	
	%	%	%	%	%
115	0	0	3	97	92
130	0	0	4	96	93
145	0	0	4	96	92
150	0	2	8	90	81
155	4	69	21	6	46
160	100	0	0	0	0

The peanut seed is exceptionally well suited to the vitality stain technique. Porter *et al.* (*7*) pointed out the practical difficulty of interpreting vitality stain tests in some other leguminous seeds because the large, thick cotyledons are stained along with the rest of the embryo, thus requiring more time and care for making a separate analysis of the radicle and plumule. The peanut is unlike other common leguminous seeds in that the torpedo-shaped "germ" (embryonic axis including plumule, hypocotyl, and radicle) is quite readily detached from the cotyledons without overnight soaking or other preliminary treatment. Also, the plumule is well developed in the mature peanut seed, permitting more accurate prediction of normal shoot development than is possible with seeds of such legumes as soybeans (*7*).

Investigators (*2, 3, 5, 7*) have recommended soaking the seeds of other legumes in water for several hours or overnight before testing, and immersing them in the tetrazolium salt solution for several more hours before analyzing them. An accelerated test modified after Cottrell (*2*) was used for the peanut experiments described in this paper, in which the separated embryonic axis was held in warm water (115° F) for an hour, then immersed in a 2% solution of 2,3,5-triphenyl-tetrazolium chloride and incubated in the dark at 115° F for another hour, after which readings were made.

Following such treatment, the germs from green or naturally dried peanuts give a bright red stain to the plumule, as well as to the entire cross section of the interior of the hypocotyl and radicle. The surface cells of the hypocotyl and radicle show only light staining or none, but the heavily stained interior is plainly visible through them. In interpreting heating effects of artificial curing upon the peanut kernels in these studies, four criteria of staining were considered indicative of four degrees of damage. An unstained germ was judged to be evidence of complete killing. An unstained plumule of a germ, which otherwise showed red or pink coloring of the interior of radicle and hypocotyl, was considered to indicate serious heat damage. A pale red or pink staining of both plumule and radicle was interpreted as showing medium to slight curing damage. Peanuts undamaged by the artificial curing were believed to be those whose germs showed a bright red stain throughout, as previously described for the green or field-cured peanuts.

Table 1 presents results of the tetrazolium staining tests on unshelled Spanish peanuts (initial moisture range 22–25%) that were run immediately after each drying experiment was completed, and the corresponding tests of germinability were made on like peanut samples at a later time. The utility of the tetrazolium staining test in determining the temperature range in which serious heating damage in peanuts first occurs is evident from data in the table. Tetrazolium tests and germination counts for untreated peanuts showed essentially the same percentages as those listed for temperatures of 115, 130, and 145° F.

The studies to determine the relationship between vitality as shown by the tetrazolium staining reaction and chemical deterioration of the seeds, as indicated by free fatty acid percentage, were inconclusive for the number of tests made. However, there was strong evidence that the decline in seed vitality accompanying an increase in free fatty acids can be accurately estimated by a further refinement of the tetrazolium technique. It also has been shown by the author in other experiments, results from which are to be published elsewhere, that the first marked increase in the percentage of such undesirable physical properties of peanuts as slipping of skins and breaking of kernels during shelling appears in essentially the same temperature range (150–160° F), which has been shown here to be associated with the first rapid loss of viability. It therefore appears that the tetrazolium test may be used as an indirect measure of heating effects upon biological, physical, and chemical properties of peanut seeds.

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Radioiodine and Paper Chromatography Technique in the Study of Thyroid Metabolism[1]

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Preliminary studies in this laboratory employing the procedure of Fink, Dent, and Fink (*3*) with radioiodine and paper partition chromatography have indicated that several of the radioiodine products of unknown nature

[1] Based in part on work under contract with the AEC, University of Rochester, Atomic Energy Project.