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

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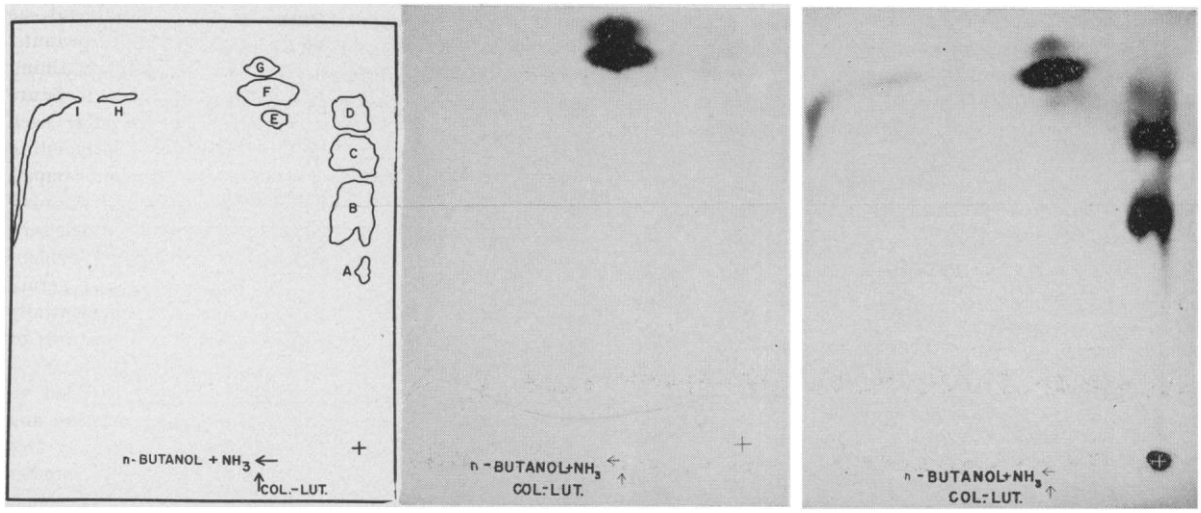

FIG. 1. Distribution on paper chromatogram of iodine-containing constituents obtained from thyroid hydrolyzate: A, unknown; B, diiodotyrosine; C, monoiodotyrosine; D, unknown; E, thyroxine; F and G, iodide; H, diiodothyronine (?); and I, unknown.

FIG. 2. Radioautograph of paper chromatogram run on NaI^{131}.

FIG. 3. Radioautograph of paper chromatogram obtained from alkaline hydrolysis of thyroid gland (48 hr following injection of NaI^{131}).

previously reported in thyroid hydrolyzate as well as the solvent artifact may have arisen from the interaction of radioiodide and the phenol solvent employed. This paper reports the results of a series of experiments aimed at characterizing the iodinated products of thyroid hydrolyzate using a paper partition procedure modified to avoid the interaction of radioiodide and the solvents employed.

The use of two-dimensional paper partition chromatography in conjunction with radioiodine in the study of thyroid metabolism has demonstrated the presence in thyroid hydrolyzates of thyroxine, mono- and diiodotyrosine, and several compounds of undetermined nature (*3*, *4*). Hird and Trikojus (*5*) have demonstrated the presence of thyroxine, diiodothyronine, and possibly triiodothyronine as ninhydrin-reactive products from the hydrolyzate of thyroprotein, using one-dimensional paper partition technique and a solvent consisting of an ammoniacal mixture of *n*-butanol and amyl alcohol.

Rats were given subcutaneously 50 μc of carrier-free I^{131} and sacrificed at 24, 48, and 72 hr. Thyroids were hydrolyzed with 8% $Ba(OH)_2$ for 24 hr in a sealed tube, the excess barium was removed by bubbling CO_2 through the solution, and the supernatant was placed directly on the filter paper. The precipitate was then extracted once with *n*-butanol and the filtrate placed on the paper over the previous spot. Approximately the equivalent of one thyroid gland was used for each paper chromatogram.

In the preparation of the two-dimensional chromatogram, *n*-butanol in an atmosphere of NH_3 was employed as one solvent, and a mixture of collidine and lutidine as the second solvent. Before use, all solvents were treated with a large excess of elementary iodine and then distilled to remove the resulting iodine-reactive products. The filter paper employed was Schleicher and Schuell analytical paper No. 589. The ascending technique of Williams and Kirby (*6*) was employed for the *n*-butanol solvent and the descending technique (*1*) for the collidine solvent. Radioautographs[2] were prepared from the chromatograms in the conventional manner and the paper chromatograms were then developed with ninhydrin to demonstrate the presence of amino acids on the paper.

A diagram showing the radioactive substances in the hydrolyzate is shown in Fig. 1. Spots B and E correspond exactly with ninhydrin spots of 3,5-diiodotyrosine and thyroxine[3] respectively when these substances were added to a control paper. Radioiodide appears on the paper chromatogram as a double spot G and F and these were the sole spots observed with the purified solvents employed (Fig. 2). Spot C was routinely observed as a strong radioactive spot and corresponds in position to monoiodotyrosine as reported by Dent (*2*), the occurrence of which was also shown by Fink and Fink (*4*). Spot H has not been identified but corresponds in position approximately to diiodothyronine, as previously reported (*5*). Spots A and D are unknown iodine-containing substances which appear very faintly, but only A reacted with ninhydrin. Spot I has been consistently observed in all thyroid hydrolyzates and is also of unknown nature.

Fig. 3 shows a radioautograph prepared from a chromatogram of a thyroid hydrolyzate; the thyroid had been dialyzed exhaustively against cold water prior to hydrolysis. The presence of the characteristic iodide double spot indicates that free iodide is formed during alkaline hydrolysis or that the thyroid gland contains undialyzable iodide, possibly in bound form. Mono- and diiodotyrosine appear as strong spots, whereas thyroxine is relatively weak. The radioactive spot remaining at the origin is most likely a small residue of BaI_2, since in other similar

[2] We are indebted to Mr. Robert Hay for the preparation of the radioautographs.

[3] We wish to thank Dr. D. P. Wallach for a generous sample of thyroxine isolated from iodinated casein.

studies careful removal of barium eliminated this spot and did not significantly alter the remainder of the chromatogram. Simple extraction of thyroid glands with collidine-lutidine revealed in the extracts the presence of radioactive spots corresponding to free iodide and mono- and diiodotyrosine, but no thyroxine was detected. This would indicate that mono- and diiodotyrosine exist in free form and are not completely bound to protein.

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The Effects of Antagonists on the Multiplication of Vaccinia Virus *in Vitro*

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A large series of analogues og purine and pyrimidine bases and related substances (*6–9*) have been tested for their ability to interfere with the multiplication of vaccinia virus in tissue culture. The method described by Thompson (*11*) measures the multiplication of the virus in the presence and absence of added substances. The inhibitory effects of substituted amino acids (*11*) and of a phenylalanine analogue (*12*) have been reported.

In the general field of substances which might be expected to interfere with nucleic acid synthesis, many substances diminish the rate of multiplication of the virus virus during the incubation period, thus having an ap- and a few bring about a diminution in the titer of the parent virucidal activity.

In many instances the activities of the analogue on vaccinia virus bear a close resemblance to those on *Lactobacillus casei* (*6, 9*). Thus among the substances structurally related to thymine, 5-bromouracil, 5-nitrouracil, dithiothymine, and isobarbituric acid give small but significant and reproducible inhibitions (Table 1). Certain amides of aminouracil, such as 5-*p*-nitrobenzamidouracil, show similar activity. 2,6-Diaminopurine exhibits strong inhibitory effects, which are reversible by purines and nucleic acid derivatives (*13*).

One outstanding difference between vaccinia virus and the bacterial and other growth systems lies in the failure of folic acid antagonists to show more than minimal inhibitions of the virus. This applies not only to the simpler bases, such as 2,4-diamino-5,6-dimethylpyrimidine, which exhibit antifolic activity in the bacterial systems (*8*), but also to the structural analogues of pteroylglutamic acid (PGA) such as 4-aminofolic acid, as shown in Table 1. The inhibitory effects of the structural analogues of PGA are evidenced in many biological systems which involve the rapid proliferation of cells (*2–4*). In general, diaminopurine has been found to have similar effects (*1, 5, 8*). However, a different locus of action of the two antagonists (*6*) is indicated by reversal studies (*6, 8*), and by the hematological findings (*10*). A possible explanation of the present studies might be that the proliferation of the virus occurs via a pathway which is blocked by diaminopurine but not by the folic acid analogues.

TABLE 1

Influence of Pyrimidine Analogues on Multiplication of Vaccinia Virus

Compound	Conc. mg/ml	Increase in virus titer (logarithm 50% end point)	
		Control	Treated
5-Bromouracil	0.1	2.10	1.70
5-Nitrouracil	0.1	2.20	1.80
5-Hydroxyuracil	0.1	2.20	1.36
2,4-Dithiothymine	0.1	1.96	1.48
5-*p*-Nitrobenzamidouracil	0.1	2.30	1.67
2,6-Diaminopurine	0.05	1.30	−0.69
2,4-Diamino-5,6-dimethylpyrimidine	0.05	1.55	1.59
	0.1	1.55	1.14
4-Aminofolic acid	0.1	2.51	1.92

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