and in addition, it eliminates approximately 60% of the material which is not significant to diagnosis. In almost every case, all cellular material available from a specimen of sputum could be smeared on one or two slides. This technique has not been in use long enough for accurate evaluation, but there is every reason to hope that a substantial increase in efficiency will be achieved, as well as an increase in accuracy of diagnosis.

Overdigestion phenomenon. A specimen collected from a patient with a known epidermoid carcinoma of the lung and coexisting tuberculosis was overdigested by 1½ hr exposure to trypsin in a running tap-water bath of aproximately 60° C. Stained slides made from the sediment, as was expected, revealed a background of hazy, nondescript cell fragments. Quite unexpectedly, however, numerous malignant cells were seen in an excellent state of preservation and appeared in large clumps. The nuclei of these malignant cells retained their typical dense hyperchromicity and the cytoplasm remained brilliantly orange, as is usual for the highly cornified malignant squamous cells (Figs. 3 and 4).

This phenomenon needs careful evaluation, but it suggests the possibility of a radically new approach to cytology in all its phases. If this observation is confirmed, trypsin could be of great service in the cytologic diagnosis of gastric cancer, where the number of oral squamous and other nonsignificant cells are a great problem. It might be useful as a vaginal douche in cases where it is impossible to obtain satisfactory smears by the usual technique because of postradiation fibrinous and nonviable debris. Trypsin might also be used to lyse clotted pleural and ascitic fluids for cytologic study and, in sufficient concentration, to digest the abundant fecal debris from lower bowel and rectal washings, and thus permit cytologic study of such material.

Summary. By the addition of a solution of trypsin, thick, tenacious sputum can be liquefied and concentrated without loss of significant cellular detail. This compound also digests most of the mature squamous cells which normally make up a large proportion of the cellular elements in sputum, as well as other nonviable materials. As a consequence, it is generally possible to smear all significant cellular material upon only one or two slides. Cell structure is at least as clearly visualized as by the older method, and in the case of pulmonary malignancy the number of malignant cells which can be seen on the slide is enormously increased.

Overdigestion with trypsin was employed on a limited number of specimens. It was found that malignant cells remained intact and easily identifiable after all other cells in the specimen were obliterated by enzymatic digestion.

The utilization of the digestive action of trypsin in the preparation of materials other than sputum for cytologic study has many possibilities, although study of a larger number and variety of cases is necessary before a definite conclusion is warranted as to the range of applicability of this method.

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Manuscript received November 12, 1952.

2,3,5-Triphenyltetrazolium Chloride as a Rapid Indicator of Viability in Cottonseed

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Use of 2,3,5-triphenyltetrazolium chloride for the rapid detection of viability in seeds, first proposed by Lakon (*1*), is based on the capacity of the stain to dye only those parts of the seed embryo that are alive and will grow (*2–5*). The stain, colorless in aqueous solution, is reduced to a red insoluble formazan in the presence of living tissue upon which it is deposited (*4*). A number of investigators have applied this staining technique to cottonseed for the detection of viability, with limited success. Flemion and Poole (*6*) used whole excised embryos and observed a light staining of the entire embryo or most of it. Their results seemed to agree substantially with Flemion's rapid viability test (*7*) in which excised embryos are germinated on moist filter paper in Petri dishes at room temperature. Raggio and de Raggio (*8*) reported results with tetrazolium chloride in good agreement with germination tests. Poe *et al.* (*9*), using whole kernels and an infrared lamp to apply heat and accelerate the reaction, reported that the tetrazolium chloride test agreed with the standard germination tests in only 57% of the individual samples examined. They concluded that the stain could not be relied on to give accurate results for routine laboratory experi-

[1] Appreciation is expressed for assistance in some of the experiments from Miss E. A. Jensen, N.C.P.A. Fellow, and Mrs. J. S. Lambour; and to James B. Dick, Delta Branch Experiment Station, Stoneville, Miss., and Wales Newby, Cotton Oil Products Co., Inc., Opelousas, La., for furnishing the seed. The author acknowledges the helpful suggestions of A. M. Altschul during the preparation of this manuscript.

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ments. Porter *et al.* (*10*) tested longitudinal sections of the whole seed after a presoaking period, but encountered "hard seed" (seed that did not imbibe water) and mechanical difficulties that prevented an accurate evaluation of the color test.

Since the results in the literature were not in agreement, Porter's (*10*) method was selected as most suitable for adaptation since it incorporated one of the more desirable features, that of exposing a longitudinal section of the embryo to the stain. It was modified to obviate the difficulties which he reported and then applied to untreated seed and seed that had been heat-treated, exposed to high energy cathode rays, and stored in a sealed container for several weeks. When available, graded series of samples, consisting of decreasing numbers of viable seeds to increasing numbers of nonviable seeds, were tested. Data are presented which indicate the possibility of using the modified method for rapidly detecting the germinating capacity of cottonseed. Each time the staining technique was applied, the results were compared with those from the standard germination test (*11*) on seed drawn from the same lot.

The following modifications in Porter's (*10*) technique were made. A stronger solution of the stain, 2%, was used. The lint and coat were removed from the kernel since it had been observed that they did not react with the dye. "Hard" seed were excluded from these investigations because Porter found that they did not react. As small a volume of staining solution was used as was required to insure a reaction between it and the cut surface of the section. It was found undesirable to cover the seed section with the stain as there was a tendency for the cottonseed embryos to float and the various parts of the section to separate when an excess of indicator solution was used. Precautionary measures were observed to insure that the cut surface was exposed only momentarily to the atmosphere and that while in contact with the stain both stain and tissue were constantly protected from the light. Differences in the depth of color and the extent to which the cut surface of the tissue became stained were noted.

Small portions of heat-treated samples of cottonseed from other experiments were made available for the tests reported here. They had been heated in a steam-jacketed Baker-Perkins mixer[3] which had been modified to heat rapidly and mix uniformly without crushing approximately 2000-g samples (*12*). Portions of samples from series exposed to increasing dosages of high energy cathode rays (*13*) were also subjected to the tetrazolium chloride staining technique. The cathode rays were produced by a pressure-insulated, electrostatic generator of the Van de Graaff type, operating at 3 million v and capable of emitting a continuous supply of monoenergetic electrons in a magnetically focused beam (*14*).

[3] The use of trade names in this article is for identification and does not imply endorsement by the Department of Agriculture of this product over similar products not mentioned.

All lots of cottonseed were presoaked in distilled water for 16–18 hr. Then 200 seeds from each lot (unless otherwise indicated in the tabulated data below) were sectioned longitudinally approximately through the center of the radicle. Only those embryos that were sound in appearance were exposed to the tetrazolium chloride solution. During the sectioning operation, discolored and soft embryos and empty and shriveled seeds were discarded. The lint and coat were removed from the kernel and half of the kernel was placed, cut-side down, in a Petri dish located in a dark compartment and containing a 2% aqueous solution of the indicator. (The stock dye solution was never more than 4 weeks old and was stored in the dark.) One hundred kernel halves could be accommodated conveniently in one Petri dish which, when full, was placed in a dark cabinet at room temperature during the incubation period of 24 hr.

Complete staining of the sections could be observed in 4 hr. A check count, made after 24 hr, always agreed with the 4-hr count. In some samples, particularly those that had been heat-treated, distinct variations in the depth of color and its location were recognized. Considering all the samples 4 different reactions were observed. They were: (1) an intense carmine red coloring of the entire cut surface; (2) a faint but complete staining of the section; (3) a partial reaction in which cotyledons were stained wholly or in part, but radicals were unaffected by the stain; and (4) no reaction in any portion of the section.

It was first determined that the reaction between the cottonseed kernel and 2,3,5-triphenyltetrazolium chloride was heat labile, as was the case with many other tissues (*15, 16*). Prime fuzzy seed (Delfos 651 variety, 1948 crop), boiled for 15 min, did not reduce the stain, whereas 95% of the unboiled seed from the same lot stained readily. Comparably 95% of the unboiled seed germinated, but none of the boiled ones sprouted during the standard test. When kernel sections from both lots of seed were immersed in tetrazolium chloride solution in the same Petri dish, the boiled seeds were easily distinguishable from the unboiled ones after 4 hr. After 24 hr, counts in duplicate groups of 100 sections each remained unchanged indicating that the formazan once formed and deposited on the tissue did not diffuse into the solution or become deposited on those sections that had not reacted in 4 hr. This exploratory experiment demonstrated that tetrazolium chloride could be used to distinguish between viable and nonviable cottonseed.

Duplicate sets of a lot of naturally moist cottonseed (Delfos variety, 1950 crop, 14% moisture content) were heated at 160° F for 10, 20, and 30 min. The results of the standard germination test and the rapid staining method are shown in Table 1. Observed were seed sections entirely unstained, a few in which only the radicle remained unstained, a substantial percentage that were faintly though completely colored pink, and those intensely stained a carmine red in all parts. When compared with the values obtained from

TABLE 1

CHANGES IN VIABILITY OF HEAT-TREATED COTTONSEED* AS SHOWN BY THE TETRAZOLIUM CHLORIDE AND THE STANDARD GERMINATION TESTS

Duration of heat treatment (min)	Seed discarded (%)	Response to tetrazolium chloride test				Standard germination test (%)
				Completely stained		
		None (%)	Partial (%)	Faint (%)	Intense (%)	
0	30.5	3.5	—	6.0	60.0	52.8
	26.5	11.5	—	—	61.5	56.5
10	31.5	2.0	3.5	19.5	43.5	42.7
	25.0	1.5	0.5	2.5	70.5	61.0
20	22.7	36.7	—	36.7	3.8	2.8
	22.0	21.0	—	46.0	11.0	1.2
30	59.5	14.0	—	26.5	0.0	0.0
	19.0	41.0	—	38.0	2.0	0.5

* Delfos variety, naturally moist, of 14% moisture content, crop year 1950, grown at the Delta Branch Experiment Station, Stoneville, Miss.

TABLE 2

REDUCTION IN VIABILITY OF COTTONSEED* STORED† IN A SEALED CONTAINER AS SHOWN BY THE TETRAZOLIUM CHLORIDE AND STANDARD GERMINATION TESTS

Storage (weeks)	Seed examined (no.)	Seed discarded (%)	Response to tetrazolium chloride test			Standard germination test (%)
				Completely stained		
			None (%)	Faint (%)	Intense (%)	
None	100	24.0	6.0	41.0	29.0	50.3‡
3	408	34.0	36.3	21.8	7.8	7.5

* Mixed variety, 1950 crop, naturally moist, of 20% moisture content.
† Storage in a cabinet maintained at 80° F for 3 weeks.
‡ Average of duplicate tests of 400 seeds each.

TABLE 3

CHANGES IN VIABILITY OF A DELFOS 651 VARIETY OF COTTONSEED IRRADIATED WITH CATHODE RAYS* AS DETECTED BY THE TETRAZOLIUM CHLORIDE AND THE STANDARD GERMINATION TESTS

Dosage (rep†)	Seed examined (no.)	Seed discarded (%)	Response to tetrazolium chloride test		Standard germination test (%)
				Completely stained	
			None (%)	Intense (%)	
None	92	10.8	30.4	58.8	66.7‡
500,000	97	10.3	33.0	56.7	47.2
1,000,000	100	10.0	73.0	17.0	8.5
1,500,000	170	7.0	92.9	0.0	0.0
2,000,000	190	1.0	99.0	0.0	0.0

* High voltage cathode rays produced by a Van de Graaff type generator operating at 3 Mev.
† Rep = roentgen equivalents physical as described by Evans (*21*).
‡ Only 75 seeds available.

standard germination tests, it appeared that those seeds that had reacted completely to give an intense red color could be considered viable.

A sample of naturally moist cottonseed of 20% moisture content was stored in a pint-size sealed glass jar at 80° F for 3 weeks. Germination counts by the standard method showed that during the interval germinability had been reduced from 50.5 to 7.5%. Only 7.6% of the stored seed stained intensely red when exposed to the tetrazolium chloride solution (Table 2). In this sample of seeds staining was complete when it occurred and was either faint pink or intense red. None of the seeds stained heavily in one part of the embryo and lightly in another.

When it was found that increasing dosages of high-voltage cathode rays had adverse effects on the germination and growth of cottonseed (*13*), small samples of the irradiated and unirradiated seeds were examined by the staining technique. The results (Table 3), when compared with those obtained by germinating the seed according to the standard test, indicated again that the seed sections intensely stained could be considered viable. It was interesting to note that there was either an intense response or no reaction. Apparently, loss of reducing capacity took place throughout the entire seed. This might be interpreted as evidence that penetration of the rays was complete when it occurred.

The pH of cottonseed has been found to range between 6.4 and 6.7 (*17, 18*). Hence, complete staining to an intense red cannot be attributed to the presence of reducing sugars which react with the stain only above pH 11 (*19*) nor to that of ascorbic acid, cysteine, and glutathione which react only above pH 9 (*20*).

The fact that the percentage of sectioned embryos which stained completely an intense red showed close agreement with the percentage of normal sprouts obtained by the standard germination test in 16 out of 17 lots of seed indicates that any reaction other than the complete staining to an intense red can be interpreted as a loss of the capacity of the seed to germinate. This close association between the results of the two methods has led to the conclusion that the tetrazolium chloride test when conducted as described, offers promise as a rapid means of detecting changes in viability of cottonseed.

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Manuscript received November 6, 1952.

Effect of Intravenously Injected Bone Marrow Cell Suspensions on Thymic Regeneration in Irradiated C 57 Black Mice[1]

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The development of thymic lymphomas in systemically irradiated C 57 black mice is effectively inhibited by placing a lead shield over one thigh during exposure, despite the fact that the thymus receives the same x-ray dose (*1, 2*). It was recently shown that, although initial radiation injury to the thymus is not prevented, thymic regeneration is significantly accelerated by thigh shielding (*3*). This report is concerned with a series of four experiments indicating that this effect of thigh shielding may be largely reproduced by intravenous injection of homologous bone marrow cell suspensions into systemically irradiated mice. Regeneration of the thymus begins less promptly, however, and requires the injection of relatively large amounts of bone marrow. The intraperitoneal implantation of skeletal muscle and the intravenous injection of exogenous thymic cell suspensions were not effective in promoting thymic recovery.

In each of the four experiments, littermate C 57 black mice of both sexes, aged 33 ± 3 days at the start of treatment, were distributed equally among 3–6 groups. These groups included both untreated and irradiated controls and four types of experimental groups. The irradiated controls received an intravenous placebo injection of Locke's solution, 0.05–0.10 cc, promptly after each of 4 periodic total-body doses of 168 r each at 8-day intervals, on experimental days 1, 9, 17, and 25. Physical factors were: 120 kvp, 9 ma, 0.25 mm Cu + 1.0 mm Al added filter, 30 cm mouse-target distance, 32 r/min. Experimental groups were similarly irradiated and treated in one of the following ways: (*a*) lead shielding of one thigh during each irradiation, as previously described (*2*); (*b*) intravenous injection of bone marrow cells suspended in Locke's solution; (*c*) intravenous injection of thymic cells similarly suspended; and (*d*) intraperitoneal implantation of minced skeletal muscle. The bone marrow suspensions were freshly made each time according to the method of Lorenz *et al.* (*4*). In most instances, the suspension injected into a single recipient contained the femoral marrows of a single young C 57 black donor (1 : 1 concentration), but in one experiment the femoral marrows of each two donor mice were divided among 5 recipients (2 : 5 concentration).

One group of donor animals received 1–4 periodic x-ray exposures (168 r) at 8-day intervals with both thighs shielded by a lead strip 1 cm wide and 3 mm thick. The shielded femoral marrow was removed promptly thereafter, suspended, and injected intravenously (1 : 1) into a correspondingly irradiated recipient. Skeletal muscle from the shielded thighs of the same donors was minced, and small fragments were loaded into trocars for implantation. Thymic cell suspensions were made by mincing thymuses of untreated donors in Locke's solution; they were injected intravenously in 1 : 1 concentration.

At predetermined intervals from 1 to 26 days after the last irradiation (experimental days 26–51), corresponding groups were sacrificed. The thymus, spleen, and pooled superficial lymph nodes (2 axillary, 2 inguinal) were rapidly excised, dissected free of fat and connective tissue, weighed on a torsion balance, and preserved in Bouin's fluid for histologic study.

The mean thymic weights of all groups are summarized in Table 1, and those of the control, marrow-injected, and thigh-shielded groups are compared in Fig. 1. Thymic weight recovered rapidly in the thigh-shielded groups and again overshot normal levels by day 51. The thymuses of animals given marrow cell suspensions began to regenerate appreciably later (day 33), but their rate of recovery thereafter roughly paralleled that of the thigh-shielded groups. The irradiated controls receiving Locke's solution alone showed a slight abortive rise in thymic weight between days 29 and 33, after which there was a secondary fall to initial post-irradiation levels which persisted through day 51. The differences between the mean thymic weights of the marrow and placebo-injected groups are significant at the 0.05 level at day 33 in one experiment, and again at day 40, and become increasingly great thereafter.

Marrow cell suspensions in 2 : 5 concentration did not significantly affect thymic weights at days 33 and 45, nor were muscle implants or thymic cell suspensions effective at day 51. In additional studies, pre-irradiated (450 r) marrow-cell suspensions were ineffective at day 29, but normal marrow cell suspensions were equally so at this time. "Stimulated" marrow from the femurs of thigh-shielded, irradiated

[1] This investigation was supported by a subcontract from the Naval Radiological Defense Laboratory, San Francisco Naval Shipyard, and by grants-in-aid from the National Cancer Institute, National Institutes of Health, USPHS, and more recently from the Jane Coffin Childs Memorial Fund for Medical Research.