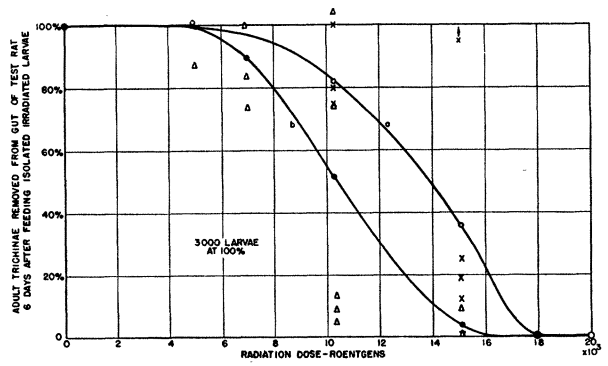

FIG. 2. Inhibition of maturation by Co^{60} gamma irradiation of isolated trichina larvae and of trichina larvae in rat carcass. △ = isolated trichina larvae. × = larvae irradiated in rat carcass.

is indicated in Fig. 1. With a dose of 12,800 r over 99% of adult females were rendered sterile. Complete sterilization was reached by a radiation dose of 15,000 r. To achieve complete sterilization of isolated larvae irradiated *in vitro,* about 12,800 r were required (Fig. 1). These curves are based on the degree of infection found in muscle 30 days after feeding rats 5000 irradiated larvae.

By irradiation, it was possible to prevent larvae from maturing, even though the larvae showed normal motility in the warm-stage test. The amount of irradiation required to do this was somewhat higher than that for sterilization. When trichinous muscle was irradiated, 18,000 r reduced maturation to less than 1%. The variation in inhibition with dosage is shown in Fig. 2. The complete maturation-inhibiting dose for trichina larvae *in vitro* was 18,000 r (Fig. 2).

Radiation dosages required to kill were measured only on isolated larvae. Complete kill, as determined by the motility test, required 750,000 r. When the larvae were examined 2 hr after completion of irradiation, the killing dose had dropped to 700,000 r, and when examined 20 hr after irradiation the killing dose was 400,000 r. From previous results, there is good reason to believe that the killing dose for irradiation of trichinous muscle would be substantially higher than the 700,000 r observed for irradiation *in vitro.*

Work is continuing on the irradiation of pork as a possible method of controlling trichinosis. Undesirable flavor changes occur in many foods when preserving doses of radiation (about 2,000,000 r) (*5*) are applied. However, one-hundredth of that dose is more than adequate to prevent maturation of encysted trichinae. Preliminary tests of pork irradiated with doses up to 38,400 r have shown negligible flavor change (*7*). A detailed report on this work will be published soon.

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Some Applications and Limitations of Tetrazolium Chloride

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The use of 2,3,5-triphenyltetrazolium chloride (TTC) and its various derivatives in seed testing, in viability determinations, and in various other branches of research has become widely known. The reaction involving reduction of TTC seems to be essentially the same as that with methylene blue. However, TTC becomes colored when reduced, instead of colorless, and the oxidation reaction which reverses the color change reaction in methylene blue does not seem to occur or only occurs slowly under certain circumstances in TTC.

Characteristics of the stain. Young, rapidly growing tissues of such plants as corn and onion will show the red color in about 20 min when soaked in a 1% TTC solution. The carmine red color, which may alter to a cherry color in 24 hr, will also appear in solutions without the presence of living cells, e.g., when TTC is heated together with a reducing sugar to 50° C or subjected to strong light. With higher pH the light reaction will occur at lower light intensities for a given length of time. A higher pH also results in a more rapid reduction of TTC in living cells of a uniform kind (Table 1).

TTC-stained cells which appear red macroscopically may not show any red color under the microscope with an incandescent or daylight light source. It may, however, bring out the contour of mitochondria more clearly in such cells as those of corn coleoptiles. Other derivatives of TTC sometimes produce more striking effects.

The change in appearance of mitochondria was first demonstrated by H. Ziegler when the writer worked with him in B. Huber's laboratories in Munich. We have tried to reproduce his results in this country and also have observed the smaller mitochondria in corn coleoptile cells to be red after soaking for 2 hr in a 1% TTC solution. The color is variable depending on the depth of focus so that there may be some doubt as to whether the bodies are refracting light from in back of them or are giving off a color peculiar to the bodies themselves.

Chloroplasts may also become red in such cells as

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TABLE 1

TIME OF APPEARANCE OF COLOR IN CORN ROOT TIPS AFTER IMMERSION IN 1% 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE (TTC) SOLUTIONS OF VARIOUS pH (Temperature about 80° F)

Min	pH			
	3.0	5.4	7.8	8.4
5	0	0	Pink	Red
15	0	Pink	Red	Red
25	Pink	Pink	Red	Red

those of *Chlorella* and in most higher plant cells crystals of formazan later appear. As pointed out by Ziegler, the larger mitochondria of corn root tips, sometimes called statoliths, may also become reddish. If they do reduce TTC then they may serve as more than just starch synthesizing particles. It is not impossible to suppose that the electrical orientation of the tissue is dependent on their position and that this might affect the migration of auxin in roots or coleoptyles or in other tissues adjacent to statenchyma tissue and thus the bending of that tissue. It is known that cytoplasmic grouping to one end or another of a cell does effect the direction of the electric current (*1*) from certain kinds of tissue.

Effect on Growth. TTC may be applied in weak enough solutions and in short enough periods of time so that a stain will appear but the cells continue in a living state. Corn seed were allowed to germinate until the roots were about 5 cm long and then immersed in an 0.05% TTC solution for 5 min, removed, and washed in distilled water. The intact root together with the seed was then placed in a Petri dish on agar and observed for growth. In about 1 hr the color appeared and persisted for several days. It then slowly disappeared. Growth was somewhat reduced by this treatment in comparison to untreated controls. The stained roots responded to gravity by bending downward when the dishes were set on their sides.

TABLE 2

EFFECT ON 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE (TTC) TEST AND ON NORMAL APPEARANCE OF *Pinus strobus* NEEDLES AFTER COOLING TO VARIOUS TEMPERATURES (°C)

In TTC	Treatment	−7°	−10°	−13°
5 min after thawing	TTC test positive	100%	40%	2%
	Untreated leaves	All green	50% light green	All light green
2 hr after thawing	TTC test positive	100%	5%	2%
	Untreated leaves	All green	70% light green	All light green

* Percentage of needles showing positive test 24 hr after placing in TTC. Determinations made in July.

The same general procedure may be used with embryos of conifer seeds, such as those of *Pinus palustris* which are large enough to be easily handled. Excised embryos of this species were washed for 3 min in a 0.5% Chlorox solution, then washed in distilled water, and placed with a sterile wire on sterile glucose-agar in Petri dishes. In cases where fungi and bacteria were kept out, embryos continued growth when soaked in TTC solution for 1 min and yet later turned pink in color. In about 3 days the color faded, the cotyledons turned green, and the entire embryo increased considerably in size.

Viability determinations. One of the best methods of determining the viability of conifer leaves after various drying and freezing treatments is with TTC. In needles that have been dried down over a 20-day period to about 40% of their moisture content based on dry weight, the TTC test showed a slow decline in intensity, also a decline in the total number of leaves showing a positive test. The rate of CO_2 evolution determined with the University of Munich infrared gas analyzer showed a decline at a slower rate than the TTC test, and with more rapid drying showed a slight rise before dropping to zero (*2*).

TABLE 3

TIME OF APPEARANCE OF COLOR IN EXCISED EMBRYOS OF *Pinus strobus* IN 1% 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE (TTC) SOLUTIONS OF VARIOUS pH (Temperature 80° N)

Seed treatment	Time since immersion in TTC	pH			
		3.3	4.2	7.3	8.3
Dry stored seed	½ hr	0	0	0	0
	1 "	0	0	0	0
	20 "	Pink	Pink-red	Pink-red	Pink-red
	TTC test*	40%	50%	50%	50%
Seed soaked in H_2O for 11 days	½ hr	0	Pink	Pink	Pink
	1 "	Pink	Pink	Pink	Pink
	20 "	Red	Red	Red	Red
	TTC test*	100%	100%	80%	75%

* Percentage of 10 excised embryos showing a complete pink or red color in 20 hr. Variations of TTC % tests with pH are not statistically significant.

In determining the viability of frozen needles, the TTC test is less accurate. It is best to allow the material to stand for about 2 hr at room temperature before testing. The rate of CO_2 evolution may, like the TTC test, be about normal just after thawing of frozen damaged tissue, but it then declines to about one-half the normal rate in a half hour and in an hour nearly to zero. These rates were determined with the Duke University infrared gas analyzer. The TTC test showed the same general pattern of decline after thawing, but the TTC determinations took place over a 24-hr period so that results are more difficult to

assess. However, differences did appear depending on the length of time before immersing in the TTC solution after thawing (Table 2). The more badly damaged tissue (−13° C) can be seen to show a nearly negative test sooner than the less damaged tissue (−10°).

In seed testing TTC has been widely and successfully used. Evaluating the intensity and extent of staining so as to predict the germinability of a sampled lot of seed is the main difficulty. Sometimes the test seems to overrate the germinability percentage in certain conifer seed which have a period of dormancy that must be overcome for relatively rapid germination. This discrepancy may be partly the fault of the germination tests which are carried out with samples from the same lot of seed (*3*). We obtained the best predictions by making the TTC test on a sample of seed as they arrived from the seed house before stratification, and comparing these with percentages of germinating seed after stratification in moist cloth at 40° F for 2 months. Evidently there is not only a decrease in time of reacitvity of the seed to TTC during stratification, but there is an increase in the total percentage of seed showing a positive TTC test (Table 3).

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The Influence of a Plasma Factor on *in vitro* Leucocyte Migration[1]

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While the intrinsic mobility of leucocytes is a well-known phenomenon, the influence of the plasma on this mobility has not yet been made clear. Using an *in vitro* method we have been able to show that the rate of migration of leucocytes is strongly influenced by a factor in the plasma.

In this technique, heparinized venous blood is first divided into cells and plasma. The cells are then washed three times by a sequence of suspension and centrifugation in Hanks' solution (*1*), and finally resuspended in autologous or homologous plasma. This suspension is then drawn into thin-walled capillary tubes of the type commonly used for melting point determinations. Each tube is sealed and then centrifuged to subdivide the system into three components; namely, a basal layer of packed red cells, an intermediate buffy coat of leucocytes, and an uppermost layer of plasma (Fig. 1). The initial concentration of heparin is adjusted so that the plasma clots after centrifugation, thus providing a matrix into which the leucocytes can migrate. Tubes are incubated in an upright position for 16 hr at 37° C, and the distance which the leucocytes have migrated into the plasma is then measured with an ocular micrometer. This measurement is therefore an indication of the distance which the fastest-moving leucocytes have traveled from the buffy coat. For each combination of cells and plasma, ten tubes were measured, and an average

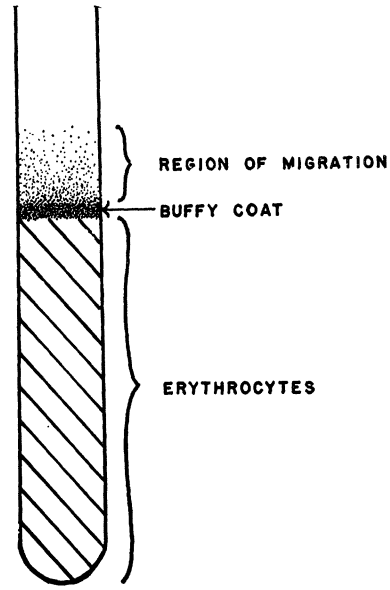

FIG. 1. Diagrammatic representation of the capillary tube method of measuring leucocyte migration.

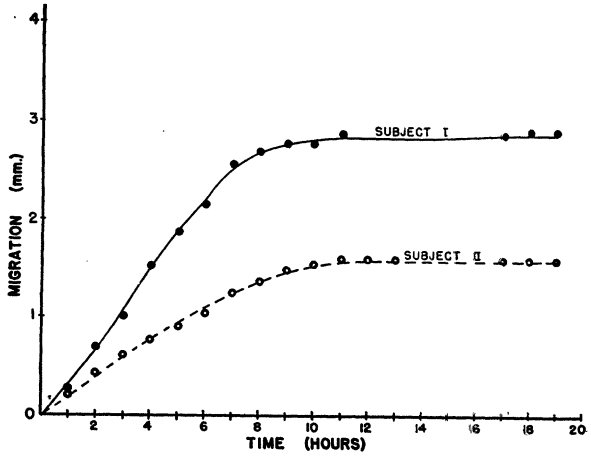

FIG. 2. Rate of leucocyte migration in two subjects with different migration distances. The difference in migration distance is the result of a difference in the rate of migration during the early hours of the experiment.

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