chemical treatment. Three plots were thoroughly sprayed with each of four chemical treatments, three plots were flamed with a flame thrower at intervals of three weeks, and one remained untreated.

The results were recorded at the end of 14 weeks on what seemed to us to be a fair approach to a quantitative basis. In Table 1 effectiveness of treatment is given in percentage of destruction of weeds. The percentage of plants remaining alive or arising from unaffected rootstocks or seeds was taken in relation to the number of living plants in the check plot.

TABLE 1

Weed	Percentage destruction by treatment*				
	A	B	C	D	E
Eleusine indica	100	85	98	100	100
Cynodon Dactylon	95	80	90	80	50
Digitaria sanguinalis	100	80	90	85	50
Eriochloa polystachya	90	25	80	80	0
Trichachne insularis	90	50	90	50	100
Cyperus rotundus	90	25	80	90	0

* A = 150 ml of 40% 2,4-D ester/gal of diesel oil; B = 1 lb of pentachlorophenol/15 gals of diesel oil; C = 1 lb of pentachlorophenol, 1 liter of 40% 2,4-D ester, 15 gals of diesel oil; D = Dow G-502, dinitro-*o*-secondary butyl phenol, 1 gal, 1 liter of 40% 2,4-D ester, 10 gals of diesel oil; E = firing.

Effective control was obtained with 2,4-D (150 ml of 40% ester/gal of diesel oil) at the rate of 40–50 gals/acre. Two other treatments applied at the same rate per acre were very effective: (a) dinitro-*o*-secondary butyl phenol (Commercial Dow Contact) in combination with 2,4-D and diesel oil (1 gal of Dow Contact, 500 ml of 40% 2,4-D ester, and 10 gals of diesel oil) and (b) pentachlorophenol with 2,4-D and diesel oil (1 lb of pentachlorophenol, 1 liter of 40% 2,4-D ester, and 15 gals of oil). Firing to destroy all above-ground parts of a grass weed stand proved to be ineffective. *Cyperus rotundus* was rather stimulated in growth. Likewise, there was stimulation in the germination of seeds of *Amaranthus spinosus* L. and other plants.

All the treatments were highly injurious to cane when the spray was applied to plants younger than 4 months of age. Older plants escaped injury when the spray was applied carefully.

More recent experiments have shown that aromatic oils (Shell) are far more effective than diesel oil, making it possible to reduce the quantity of 2,4-D ester to 75 ml/gal of oil.

It is evident that 2,4-D, which alone has little effect on grasses, becomes a very active grass herbicide when dissolved in diesel or aromatic oil and applied as a fine spray. The practical implications will be clear when one considers that grass weeds have become the most pernicious competitors of sugar cane for soil nutrients and soil moisture.

It is recognized that such an active herbicide has to be applied with extraordinary care to growing cane. If the 2,4-D oil spray is applied on a windy day, much injury may result to leaves of cane. To obviate these shortcomings of the treatment, two alternative methods of application have been developed. The first involves a preplanting application. Fields are prepared in the ordinary way, including furrowing and ditching, and irrigation water is then applied. Grass weeds are allowed to grow, and at the end of 3 weeks the 2,4-D oil spray is applied either with knapsack or power sprayers. Cane is planted the day following the herbicide application. This will considerably reduce the grass growth while the young cane is growing. Hoeing or other forms of mechanical cultivation are not eliminated altogether, but much labor saving is effected. The second method requires that the 2,4-D oil spray be applied to fields of growing cane when this is over 3 months of age. A combination of the two methods has been found to reduce effectively the number of hoeings from 6 or 7 to 3 in new plantings.

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Demonstration of Reducing Enzyme Systems in Neoplasms and Living Mammalian Tissues by Triphenyltetrazolium Chloride

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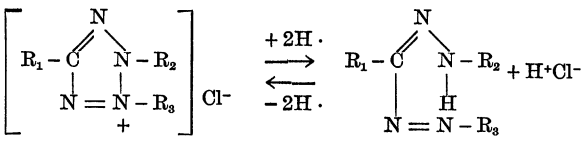
Tetrazolium salts first prepared by Pechman and Runge (*6*) in 1894 and extensively investigated by Kuhn and Jerchel (*3*) were proposed by Lakon (*4*) for testing the viability of seeds. Attention to the usefulness of these reagents was pointed out in this country by the report of Dutcher (*2*), who interrogated Lakon in 1945. Subsequently, Porter, Durrell, and Romm (*7*), Mattson, Jensen, and Dutcher (*5*), Waugh (*9*) and Cottrell (*1*), confirmed the observations of Lakon and indicated that tetrazolium salts may be useful as reagents to detect differences in the viability of seeds and other tissues.

We synthesized a series of tetrazolium salts including the 2,3,5-triphenyl and 2,3-diphenyl-5-methyl compounds. The present paper deals with the demonstration of reducing enzyme systems in neoplasms and living mammalian tissues by means of the triphenyltetrazolium salt.

Warburg and Christian (*8*), in 1943, showed that the plasma of animals bearing large sarcomata contained an increased quantity of zymohexase and postulated that, in contradistinction to normal mammalian cells, the tumor cell obtains energy from glycolysis even in the presence of available oxygen. It appeared to us that, if there were an appreciable difference in the amount of glycolysis in the neoplastic cells as compared to normal cells in contact with available oxygen, such a difference might be demonstrated by reagents capable of reacting with enzymes in the chain of glycolytic fermentation. Tetrazolium salts

are water-soluble, colorless substances; their solutions, in contact with certain reductases in living tissues, are reduced to insoluble, red substances called formazans:



Colorless Tetrazolium Salt Colored Formazan

It is probable that all living cells contain enzymes of the glycolytic system. The concentration of the enzyme varies with the type of cell and the individual characteristics of that cell's demand for energy, and the availability of oxygen for alternative cytochrome oxidation. It is believed that normal mammalian cells will utilize the more efficient cytochrome oxidation preferentially as a source of energy, when the oxygen supply is adequate; further, it is reasonable to assume that a neoplastic cell which obtains energy from glycolysis may reduce tetrazolium salts at a faster rate than normal cells. This possibility may furnish a tool useful in the study of cancer cell metabolism and cellular anoxia in general.

the form of either a 5% or a 10% solution, resulted in immediate collapse. The heart contracted for 5 min after cessation of respiration and then stopped. At this time inspection of the viscera showed no color. Within 10 min the upper small intestine became rose colored. In the next 10 min most of the small bowel, the visible muscles, and heart became pink-red. Later, the skeletal muscles became rose-red, and the upper small bowel became scarlet. At this time the lower small bowel and colon were rose-red, while the liver, spleen, kidneys, and adrenals were not colored. It may be significant that reduction occurs in the vicinity of still living or very recently living cells after cessation of circulation has reduced the oxygen available to the cells.

We have found that there is a differential reduction of the tetrazolium by carcinomatous tissue as compared to the rate exhibited by the surrounding tissues. Segments of freshly removed carcinomatous tissue immersed in 1% tetrazolium chloride at room temperature or at 37° C develop a faint pink color within a few minutes and become ruby-red within 20 min. The uninvolved surrounding tissues remain unstained. Microscopic examination of tumor sections immersed in tetrazolium chloride

TABLE 1

Tissue (human)	Region	Temp. (°C)	Time	Staining
		(Immersion in 1% tetrazolium chloride sol.)		
1. Carcinoma	Breast	Room	20 min	Deep red
2. "	"	37	20 "	Deep red
3. Uninvolved breast	" #2	37	20 "	Light pink
4. " skin	" #2	37	20 "	Basal layer, fine red line (remainder unstained)
5. Carcinoma	"	37	3 hrs	Dark red
6. "	Pancreas	37	15 min	Red-brown
7. Fibroadenoma	Breast	37	3 hrs	No color change
8. Normal jejunum	Gastrojejunostomy	Room	15 min	" " "
9. " "	"	37	3 hrs	Red
10. " skin	Abdomen	37	1 hr	No color change
11. " "	"	37	24 hrs	" " "
12. Duct papilloma (malignant?)	Breast	37	3 "	Ducts faint pink, tumor bright red
13. Uninvolved breast	" #12	37	3 "	No color change
14. Mixed tumor parotid (malignant?)	Neck	37	15 min	" " "
15. Mixed tumor parotid	Case #14	37	2 hrs	" " "

Triphenyltetrazolium chloride, injected intramuscularly in rabbits, causes no apparent injury in doses of 100 mg/kg, using a 2% solution. Injection of 150 mg/kg, using a 10% solution, is lethal within 25–30 min. The serum of a rabbit which received a lethal dose of the salt was colored ruby red by the formazan. Decoloration of the serum and reduction by means of sodium stannite demonstrated the presence of still unreduced tetrazolium salt.

Intravenous injections are more toxic than intramuscular injections. A dose of 25 mg/kg in the form of a 1% solution can be tolerated with no apparent injury. Examination of the anaesthetized animal after 90 min and 24 hrs did not disclose any pigmentation of the tissues. However, intravenous injections of 100 mg/kg, in

shows a diffuse reddish pigment distributed both within the epithelial cells and in the stroma. It has been found that the carcinoma can be frozen upon removal, then sectioned and stained by incubation in tetrazolium chloride solution for 20 min. Mammary carcinoma tissue, allowed to stand for 3 hrs at room temperature (in a moist environment), then immersed in the tetrazolium chloride solution, develops a much fainter color than when utilized immediately after amputation. Freezing immediately after amputation is indicated as a means of preserving the enzyme system which reduces the tetrazolium salt. Heating the carcinoma tissue at 100° C for 5 min entirely inhibits the development of color. Table 1 gives a summary of the staining of human tissues by tetrazolium chloride.

It is of interest to note that tissues from a noncarcinomatous area in a carcinomatous breast were faintly stained, and that uninvolved skin from the same breast showed some reduction, while fibroadenomatous breast and skin from an appendectomy incision showed no color.

The application of the tetrazolium salts as intravital dyes is further suggested by the following observations. A gauze pack saturated with 1% tetrazolium chloride was applied to an ulcer surface which developed after postoperative irradiation of a carcinoma of the anus. It was not possible to determine by inspection whether the ulcer was due to recurrence of the carcinoma or to a late radiation necrosis. After 12 min the ulcer surface was stained bright scarlet-red, as was the gauze surface in contact with it. Removal of the ulcer and surrounding tissue revealed that the staining was limited to the surface of the ulcer to a depth of 2 mm below the surface. Frozen sections showed the presence of squamous cell carcinoma. A similar observation was made on an ulcer of the tongue. A cotton applicator saturated with 1% tetrazolium chloride was held on the ulcer for 10 min. The ulcer surface and the applicator were stained scarlet. Excision of the tongue showed the lesion to be a squamous cell carcinoma. Granulation tissue covering a third-degree burn ulcer was treated similarly for 20 min, but no color developed.

There is a suggestion that the tetrazolium compound is reduced more rapidly than by normal tissues, in regions where a local deficiency of oxygen exists. A leg was amputated for arteriosclerotic ischemia. Skin, deep fascia, and gastrocnemius muscle were taken from the fresh leg at the level of the knee joint and immersed in 1% tetrazolium chloride solution for 20 min at 37° C. No color developed. Similar skin, deep fascia, and muscle were taken from just above the internal malleolus of the tibia, 2″ above a gangrenous ulcer, and also immersed and incubated. The muscle and fascia showed no color although the skin was colored a faint pink, visible through the epidermis. On section it was demonstrated that the superficial layers of the epidermis were not colored. The cutis vera was a moderate pink color, and a fine scarlet line outlined the probable course of the basal layer of the epidermis.

These observations indicate that tetrazolium compounds may be used as a tool in the study of the intracellular metabolism of tissue anoxia. The scarlet-red color which develops on reduction is not ideal, since some mammalian tissues are only a different red. Work is under way not only to synthesize tetrazolium salts which may give formazans of other colors, preferably blue or green, but also to determine the fate of the tetrazolium chloride and to study the enzyme system which affects the rate of reduction in the organism. It is possible that a soluble substance which can be precipitated in differential quantities in some neoplastic tissues may prove a useful agent in the study and treatment of neoplasms.

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Palynological Studies at Sodon Lake: I. Size-Frequency Study of Fossil Spruce Pollen[1]

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In another paper of this series Cain and Slater (*3*) are reporting a pollen analytical study of a 24′ profile of the peat and marl sediments of Sodon Lake; and Cain and Cain (*2*) have made a size-frequency study of the fossil pollen grains at Sodon Lake in relation to the modern species, showing something of the historical successional relationships of *Pinus Banksiana*, *P. resinosa*, and *P. Strobus*. The present paper considers the question of whether it is possible to identify the species of *Picea* which contributed to the fossil pollen of the blue clay layer of the 24′ level at the bottom of the profile.

The vegetational history of Sodon Lake, Oakland County, Michigan, in so far as it is revealed by the stratigraphic column, commences with the pre-Boreal spruce-dominated pollen spectrum referable to Period I in the scheme of Sears (*7*). This is the only level at which spruce is dominant. Of the 322 grains of this level, 85.7% were recognized as spruce, and about 100 of these were in a sufficiently good state of preservation and were oriented properly for measurement of the grain size. Grains seen squarely at right angles to their long axis (in dorsal, ventral, or lateral view) were measured across the maximum dimension of the grain, within the exine. This position is often along a line connecting the points of dorsal insertion of the bladders. The grains were found to range between 61.6 and 97.6 μ. The number of measurable grains was insufficient to produce a smooth size-frequency curve (Fig. 1, heavy-line curve) or to indicate surely whether one or more species of *Picea* were involved in the sedimentation.

[1] In the "Pollen Analysis Circular" Dr. Antevs posed the question: "Is pollen analysis the proper name for the study of pollen and its applications?" In No. 8 of the circular (page 6, October 1944) H. A. Hyde and D. A. Williams, of Wales, suggested the term *palynology* (from Greek *paluno*, to strew or sprinkle; cf. *pale*, fine meal, cognate with Latin *pollen*, flour, dust): the study of pollen and other spores and their dispersal, and applications thereof. Erdtman (1947) has accepted the term. Since it seems appropriate, we are using it formally for a series of papers somewhat broader in scope than those formerly included under "pollen analysis."