develop in size, at the expense of the less dense material (Fig. 8), to emerge as a mature particle. The picture of mature virus forming from larger, less dense bodies imbedded in a matrix material is similar to the situation described by Banfield, Bunting, Strauss, and Melnick (*4*) for molluscum contagiosum. In retrospect, some of their micrographs may be reinterpreted in the light of the present findings. Close re-examination of their original plates (*4*) reveals that in molluscum contagiosum the "holes" in the cytoplasmic matrix contain dense bodies ranging from the barely visible to the size of mature virus particles, with the entire "virus developmental" body seemingly encased in a fine membrane. We have also observed similar developmental forms (precursors of mature virus) in cells infected with ectromelia virus, another member of the pox group.

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Crown Gall Suppression by Anti-Auxin[1]

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The continued depression of auxin level in plants, following exposure to ionizing radiation (*1*), has been suggested (*2*) as a mechanism by which radiation suppresses crown gall caused by *Agrobacterium tumefaciens*. If this suggestion is correct, then other agents that lead to auxin destruction should suppress galls.

This prediction was tested by exposing carrot roots inoculated with *A. tumefaciens* (*3*) to maleic hydrazide (MH), an agent that increases enzymatic destruction of indolacetic acid (IAA) (*4*). A carrot root was surface-sterilized, cut transversely into 12 slices ½ cm thick, and a slice placed apical-end down on 2% water agar in each of 12 Petri plates. Similar slices from 3 other roots were also placed in each plate. The surfaces of all slices were inoculated at the same time with an aqueous suspension of *A. tumefaciens* (Riker's strain A-6). The 4 slices in a plate were transferred at the times shown in Table 1 to another plate, where the agar solution contained a 2.67×10^{-3} *M* concentration of the diethanolamine salt of MH.[2] After incubation at room temperature for 12 days, the galls on the slices from each root were ranked according to size (Table 1).

Evidently crown gall development is suppressed by MH applied as late as 168 hr after inoculation. Similar results were obtained in experiments using plants growing in the greenhouse. When the roots of tomato plants were dipped in 5.24×10^{-3} *M* solution of MH salt 8 days after, or at the time of, inoculation, only small galls developed, whereas large galls developed on untreated plants.

TABLE 1

RANK OF GALL SIZES ON CARROT ROOT SLICES TRANSFERRED TO A 2.67×10^{-3} *M* SOLUTION OF DIETHANOLAMINE SALT OF MH

Transfer, hr before (−) or after (+) inoculation	Root			
	1	2	3	4
−168, −120, −24				
0, +4, +8	0*	0	0	0
+ 24	0	0	4	0
+ 96	1†	2	1	0
+168	2	1	2	0
No. MH	3, 4‡	3, 4, 5	3, 5, 6	1, 2, 3

* No gall.
† Smallest gall on root 1.
‡ Contaminated.

The effect of MH upon multiplication of the pathogen was determined by growing it in a 2% glucose and 0.2% yeast extract medium containing concentrations of the MH salt up to 28.8×10^{-3} *M*. No relation between turbidity and MH concentration was evident after 24 hr at room temperature.

These experiments show that MH, which is known to increase enzymatic destruction of IAA (*4*), suppresses gall formation by affecting tumor enlargement, not by affecting alteration of normal to tumor cells or by affecting pathogen multiplication. In these respects MH suppresses galls in the same way as does ionizing radiation. The hypothesis that radiation suppresses galls by depressing the auxin level has thus led to a prediction borne out by experimentation. Whether the hypothesis accounts for the entire radiation effect remains to be seen.

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[2] The MH was supplied by the Naugatuck Chemical Division, U. S. Rubber Company.

The Effect of Triphenyltetrazolium Chloride on Oat Embryo Respiration[1]

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The reduction of tetrazolium salts has been widely

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used as an indicator of viability and as a measure of metabolic activity in tissues and tissue extracts (*1*). Pyridine nucleotide–linked dehydrogenases (*2, 3*) mediated by diaphorase (*3, 4*), succinic dehydrogenase (*5, 6*), xanthine oxidase (*3*), and cysteine desulfurase (*7*) (by H_2S production) are known to catalyze this reduction *in vitro*. The reaction has been found to be more rapid in the absence of oxygen with liver homogenates (*4, 7*), microorganisms (*8, 9*), and a reduced cozymase-diaphorase system (*2*). This effect has been attributed to competitive action between tetrazolium and the oxidases as hydrogen acceptors (*4, 7*). There is also evidence of toxic effects on several tissues by tetrazolium salts or the process of tetrazolium reduction (*7, 10–14*). In studying the mechanism of the tetrazolium reaction in seeds, changes in respiratory metabolism were observed that may help to explain these toxic effects.

In the present work, embryos of Clinton oats were soaked 3 hr at 30° C, then split longitudinally through the growing axis, and sliced from the seed with as little adhering endosperm tissue as possible. After the embryo slices were washed and blotted, respiratory measurements were made on 40-embryo samples in 0.05 *M* pH 6.0 phosphate buffer in standard Warburg apparatus. The embryos were exposed to tetrazolium (i.e., 2,3,5-triphenyltetrazolium chloride) for about 25 min before measurements began. Oxygen consumption ($Q_{O_2}{}^{air}$), anaerobic carbon dioxide evolution ($Q_{CO_2}{}^{N}$), and aerobic carbon dioxide evolution ($Q_{CO_2}{}^{air}$) were measured for a 1-hr period and calculated on a µl/embryo × hr basis. Under these conditions rates were linear, and standard deviations were about 0.16 µl. The endosperm tissue adhering to the embryo slice was shown to contribute less than 5% to the total respiration, so that the response observed was that of embryo tissue. The effect of tetrazolium on the respiratory capacity of oat embryos is shown in Table 1. At 0.05% tetrazolium there was a slight inhibition of $Q_{O_2}{}^{air}$ and $Q_{CO_2}{}^{N}$ but a definite stimulation of $Q_{CO_2}{}^{air}$. This change resulted in the appearance of aerobic fermentation and in an increase in the RQ ($Q_{CO_2}{}^{air}/Q_{O_2}{}^{air}$) and a decrease in the I/N ratio ($Q_{CO_2}{}^{N}/Q_{CO_2}{}^{air}$). At 1% tetrazolium the increase in $Q_{CO_2}{}^{air}$ remained about the same as at 0.05%, but there was also a significant inhibition of the $Q_{O_2}{}^{air}$ and $Q_{CO_2}{}^{N}$.

TABLE 1

EFFECT OF TETRAZOLIUM ON OAT EMBRYO RESPIRATION

	Control	0.05% tetrazolium	% change	Control	1% tetrazolium	% change
(1) $Q_{O_2}{}^{air}$*	2.30	2.19	− 5	2.27	1.77	−22
(2) $Q_{CO_2}{}^{air}$*	2.20	2.71	+24	2.28	2.94	+29
(3) $Q_{CO_2}{}^{N}$*	2.17	2.02	− 7	2.18	1.55	−29
(4) Aerobic fermentation†	0	0.61	—	0	1.15	—
(5) $RQ = (2)/(1)$	0.96	1.24	—	1.01	1.67	—
(6) $I/N = (3)/(2)$	0.99	0.75	—	0.96	0.53	—

* Av of 4 determinations expressed as µl/embryo × hr.
† Aerobic fermentation = (2) treated − (1) treated × (5) control.

More rapid reduction of tetrazolium under anerobic conditions was also observed with oat embryos. With 1% tetrazolium, the formazan extracted and measured spectrophotometrically after 0.5 and 2 hr in air was only 33% and 55%, respectively, of that in nitrogen. Staining was even more severely inhibited in pure oxygen than in air, especially with oat samples of lowered vitality. With oat embryo slices, this inhibitive effect of oxygen was not due primarily to competitive action, since the formazan produced was equivalent to only about 10% of the decrease in oxygen consumption. It would appear that in the presence of oxygen there is decreased activity of the dehydrogenase systems responsible for tetrazolium reduction.

Similarly, the inhibition of oxygen consumption by 1% tetrazolium (Table 1) would not appear to be due primarily to competitive action. Furthermore, tetrazolium has been found to inhibit the two principal oxidative enzymes of corn embryo homogenates. Cytochrome oxidase, measured colorimetrically (*15*) on the sediment of a high-speed centrifugation, was inhibited 15% and 50% at 0.5% and 1% tetrazolium, respectively; and peroxidase (*16*) in the supernatant was inhibited 35% and 85% at 0.1% and 0.5% tetrazolium.

These data show that under conditions where tetrazolium is used as a hydrogen acceptor it may cause significant alterations in respiratory metabolism. Its effect on oat embryo respiration, in fact, is very similar to that reported on other tissues by agents considered to inhibit the Pasteur effect, such as phenazine, amidine, and guanidine derivatives (*17, 18*) and potassium (*19*). Dickens (*17*) pointed out that several of his most active compounds were quaternary salts with conjugated ring systems, structural features also characteristic of tetrazolium salts. The action of phenosafranine (*20*), as well as of other Pasteur-effect inhibitors, has recently been attributed to interference with transphosphorylation. At present there is insufficient evidence to determine whether tetrazolium acts similarly or whether its influence on the Pasteur mechanism is through its action on oxidative enzymes. The present work, however, indicates that tetrazolium salts may have other effects on respiration than as hydrogen acceptors and that they may be useful in the study of the Pasteur mechanism. Finally, these effects on respiratory metabolism may be the basis for the toxicity of tetrazolium salts observed with some tissues and may affect the usefulness of these compounds as indicators of viability.

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Stabilities of Metal Derivatives of *o*-Substituted Azo Dyes

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The coordinating tendencies with metal ions of azo dyes which contain a hydroxyl, amino, or carboxyl group in one or more of the *ortho* positions have been studied in some detail. Such compounds have found many applications in the dying of fabrics (*1*) and in the preparation of organic pigments (*2*). Although numerous investigations have been carried out as to the light fastness, resistance to laundering, etc., of the metal compounds, almost no measurements have been made on the stability of these compounds toward dissociation into their constituent ions.

Formation constants of the metal derivatives (expressed as the general case)

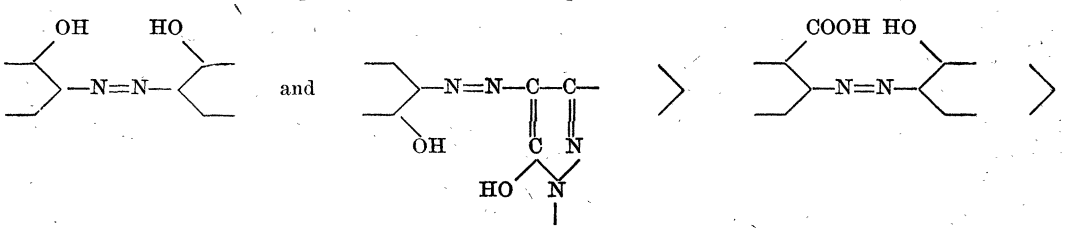
$$M\mathrm{Ch}_{n-1}^{m-(n-1)} + \mathrm{Ch}^- = M\mathrm{Ch}_n^{(m-n)},$$

(where M is the central metal ion, Ch^- is the chelate ion, m is the charge on the metal ion, and n is the number of Ch^- groups) have been measured using the Bjerrum (*3*) technique of potentiometric titration as modified by Calvin (*4*).

The decreasing order of stability of the divalent metal complexes with 4-(2-hydroxybenzeneazo)-3-methyl-1-phenyl-5-pyrazolone, a terdentate group, agrees with those already reported for monodentate and bidentate chelate groups (ammonia [*3, 5*], ethylenediamine [*5, 6*], salicylaldehyde [*5–8*], salicylaldehyde-5-sulfonic acid [*9*], β-diketones [*8, 10, 11*], tropolone [*12*], 8-hydroxyquinoline [*8*], 8-hydroxyquinoline-5-sulfonic acid [*13*], α-amino acids [*13, 14*], and alkyliminodiacetic acids [*15*] and is as follows: Cu > Ni > Co > Zn > Pb > Cd > Mn > Mg > Ca > Sr > Ba. The alkaline earths previously have not been included in such a series, although their noted order is the same as that found by Schwarzenbach (*16*) with iminodiacetic acid and other chelates of the same type.

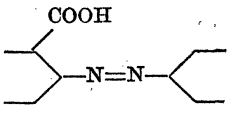
The decreasing order of stability of the metal compounds of the triply charged metal ions with the aforementioned dye is as follows: Fe > Cr > Al, which is the same as that found by Cooperstein (*17*) with the acetylacetonates.

The relative order of coordination of the dyes with a given metal such as copper (II) is



I II III

IV V VI



VII

As the above power of coordination with metal ions falls off, the number of metals which form compounds also decreases, so that the last member of the series

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