several days. Type A, B, and AB cells, strongly sensitized in a saline eluate, react in the usual manner with α and β isoagglutinins as specifically and intensely as prior to their being sensitized. Rh+ cells similarly sensitized likewise are not affected in the reactivity with Rh agglutinins. This is interpreted as showing that the A. G. antibody does not block the Rh and major blood-group receptors. This antibody is not reduced in potency at exposure to 70°C. for 10 minutes, but cannot be demonstrated by the indirect developing test after exposure of the serum to 80°C. for 10 minutes. As shown in the above tables, (in distinction to the Rh antibody) this antibody reacts with red cells irrespective of Rh type, is also an autoantibody and a panantibody, but does not react with rhesus cells.

Hematological, histological, and clinical data, as well as further immunological studies concerning these three patients will be submitted in greater detail in a future publication.

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Further Observations on Leptospirosis in Micronesia

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Murine leptospirosis has given rise to a public health problem in many parts of the world (*2*) because the etiologic agent, *Leptospira icterohemorrhagiae*, also causes Weil's disease in man. Since the United States now has control of many islands in the Pacific, it seemed advisable to start work of a survey nature on these islands to find out whether leptospirosis constitutes a menace to the native peoples and our own personnel. Such investigation was started during the summer of 1946, when the University of Hawaii, in cooperation with the Navy, sent a number of biologists into the field.

In a recent report by Alicata (*1*), covering a part of the first summer's work, it was pointed out that 5 out of 40 rats trapped on Moen and Ponape, in the eastern Carolines, were infected with leptospirae. It is the purpose of this communication to note survey findings on the island of Yap, in the western Carolines. Yap, it will be remembered, lies south and west of Guam, about 9 degrees north of the equator. Prior to World War II it was one of the territories mandated to Japan.

A number of traps were put out near the site of the military government unit at Yap Town. The 28 rats which were taken alive were members of 3 species, *Rattus alexandrinus*, *R. norvegicus*, and a third, tentatively identified by Harvey Fisher, of the University of Hawaii, as *R. exulans micronesiensis*. Each animal was killed by drowning the morning after being caught, and a piece of kidney was removed at autopsy immediately after death. The tissue was preserved in 10 per cent formalin for several months before being sectioned and stained by a modification of the Warthin-Starry silver precipitation technic.

Careful microscopic examination of several sections from each rat kidney failed to reveal any spirochetes in the urinary tubules or elsewhere. Although the number of animals is small, it would seem that murine leptospirosis is not present in the area studied. It may be that the geographic isolation of Yap and its lack of shipping facilities have prevented the introduction of *L. icterohemorrhagiae*. Once introduced into this region, which offers much rain and a large rat population, the organism might gain a foothold quickly.

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Triphenyltetrazolium Chloride as a Dye for Vital Tissues

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The use of triphenyltetrazolium chloride as a test reagent for seed germinability was brought to the attention of one of us (R. A. D.) while on a tour of duty in Germany in 1945 as scientific consultant for the Technical Industrial Intelligence Branch of the Joint Intelligence Objectives Agency. The use of this compound for predicting seed germination was based on its ability to stain only those parts of seed embryos which were capable of growth. This fact suggested that the tetrazolium salt might have a wider application as a test reagent for the vitality of tissues other than seeds.

Tetrazolium salts, including 2,3,5-triphenyltetrazolium chloride, were first prepared by Pechman and Runge (*5*) in 1894. In 1941 Kuhn and Jerchel (*2*) synthesized a number of tetrazolium salts by an improved procedure and called attention to the fact that dilute solutions of 5-methyl- and 5-hendecyl-2,3-diphenyl salts stained yeast, garden cress, and bacteria (*3*). These workers believed that the reduction of the colorless salt solutions to a red compound which dyed the plant tissues was not due to the presence of glutathione, ascorbic acid, or cysteine, for the latter substances did not reduce these salts below a pH of 9.0, whereas the characteristic reductions observed on yeast, garden cress, and bacteria took place in neutral solutions.

As a result of these studies Lakon (*4*) substituted triphenyltetrazolium chloride for the toxic sodium selenite in his "topographic method" for testing the germinating ability of seeds. By a comprehensive series of comparative staining and germination tests he was able to show that it is possible to predict the germinability of corn, oats, rye, wheat, and barley by observing the embryo parts which are stained by the red, insoluble formazan deposited in viable tissues. The unstained portions of the embryo were shown to be incapable of growth.

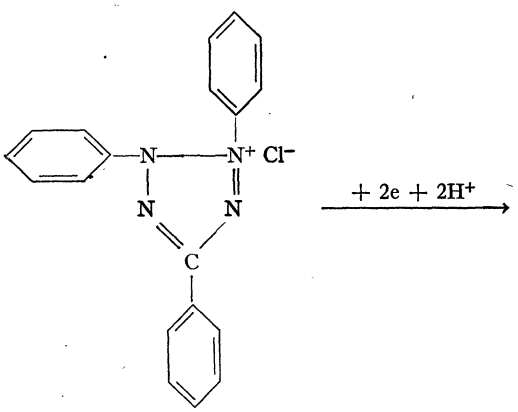
Porter, Durrell, and Romm (*6*) used Lakon's tetrazolium method and found a close agreement between the percentage of stained embryos and the percentage of normal sprouts obtained in standard germination tests with corn, wheat, rice, buckwheat, popcorn, soybeans, and Bahia grass. Less satisfactory agreement was found in a comparison of the two methods when applied to vetch and sorgo and to some lots of oats, peas, and barley.

Since tetrazolium salts were not available in this country, we synthesized the triphenyl compound and the 5-furfuryl-2,3-diphenyl derivative and have used these compounds in preliminary studies on various types of viable and nonviable

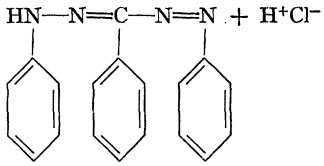
tissues. The furfuryl derivative appeared to give the same tests as the triphenyl compound. In order to prepare tetrazolium salts of suitable purity in satisfactory yields, it was necessary to make certain modifications in the method of Kuhn and Jerchel. This phase of our work will be published later.

Our work also confirms the work of Lakon with seed corn and the observations of Kuhn and Jerchel with yeast. However, we have been interested in the potentialities of the tetrazolium salt as a test reagent for living tissues in general. We have found that many other viable materials, in addition to seeds and yeast, will reduce the triphenyltetrazolium chloride at pH 6.9: the fleshy parts of apples, oranges, and grapes; the gill area of mushrooms; carrot roots, white and sweet potatoes; young leaves; the stigmas and ovaries of certain pollinated flowers; bull sperm and the blastoderm of hen's eggs. Much to our surprise, the serum of bull sperm and the chalazae of egg white give a positive reaction. The reduction of the tetrazolium salt is not due to sugars, for subsequent work has shown that reducing sugars form the red formazan only above pH 11.0, whereas the above-mentioned materials will reduce the triphenyl compound at acidities below pH 7.0.

The use of the tetrazolium reagent should have a distinct advantage over many indicators as a viability test, since it is one of the comparatively few organic compounds which is colored in the reduced state. In the presence of viable tissue the colorless solution of triphenyltetrazolium chloride forms the insoluble red triphenyl formazan by the following reaction:



2,3,5-Triphenyltetrazolium chloride



Triphenyl formazan

It is quite evident that enzyme systems are responsible when this reduction takes place in plant and animal materials, since tissues heated at 82° C. or higher lose their ability to reduce this salt. Furthermore, it is probable that this reduction is caused by dehydrogenase systems requiring coenzymes I or II, for Jerchel and Möhle (*1*) have shown that the apparent redox potential of 2,3,5-triphenyltetrazolium chloride is about −0.08 volt. Thus, it is possible for this compound to act as an electron acceptor for many pyridine nucleotide dehydrogenases. We have found that one of these holoenzymes, glucose dehydrogenase-coenzyme I, in the presence of its substrate, reduces the salt at pH 6.6.[1] Work is being continued to determine if other enzyme systems possess similar properties when treated with this reagent.

Preliminary experiments have indicated that the enzyme systems responsible for the reduction of the tetrazolium salts are present in a wide variety of living tissues. In all probability, the reduction of these compounds by enzymes of living cells cannot be considered a general test for life. Nevertheless, the unusual properties of these reagents suggest that they might be utilized in many types of biological research involving differences in tissue viability.

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Oxygen and Air Pressure Effects Upon the Early Development of the Frog's Egg

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The following constitutes a report on a part of the author's studies of the effects upon early embryological development of (1) different pressures of normal air and (2) various proportions of the constituent substances of normal air under pressure.

Eggs of three species of frogs (*Rana pipiens*, *R. sylvatica*, and *R. palustris*) were used in these observations. Two sets were employed: normal eggs deposited under natural conditions and, in the case of *R. pipiens*, pituitary-stimulated eggs from females brought in shortly before the breeding season and kept in a state of prolonged hibernation in a cold room at approximately 1° C. In the case of eggs deposited under natural conditions, the period of development during which experiment was initiated varied between the 4- and the 16-cell condition. The pituitary-induced eggs were placed in the pressure chambers 15–20 minutes after the sperm suspension was added. Eggs from two separate females and sperm from two males were used in each experiment.

Pressures up to 3 atm. of oxygen were added to the normal air pressure in the pressure chambers. Small egg masses were placed in small glass dishes and covered with water to a depth ranging from $\frac{1}{8}$ to $\frac{3}{8}$ inch above the surface of the mass. The water remained unchanged while the eggs were in the pressure chambers. Control eggs were kept in finger bowls or crystallizing dishes in shallow water which was changed daily. The control embryos were watched until they reached the late yolk plug and early neuralation state, after which the pressure

[1] We are indebted to F. A. Baldauski, who made the glucose dehydrogenase and coenzyme I preparations.