Although this is only a preliminary observation, it is in the direction which would be expected if artificial fluorination ultimately produces a decrease in dental caries.

Klein (*6*, *7*) has recently shown, in higher fluorine concentrations, that a reduction in dental caries from fluorine can be a posteruptive phenomenon. Since all the children examined in Newburgh drank water containing fluorine after many of their teeth had already erupted, the *Lactobacillus* counts bear on the controversial question of the effect of fluorine after eruption of the teeth.

References

1. AST, D. B. *Publ. Hlth Rep.*, 1943, **58**, 857.
2. AST, D. B. *J. dent. Soc., St. N. Y.*, 1944, **10**, 3.
3. BUNTING, R. W., and PALMERLEE, F. *Amer. dent. Ass. J.*, 1925, **12**, 381.
4. DEAN, H. T., JAY, P., ARNOLD, F. A., JR., and ELVOVE, E. *Publ. Hlth Rep.*, 1941, **56**, 761.
5. JAY, P. In *Fluorine and dental health*. F. R. Moulton. (Ed.) (AAAS Publ. No. 19.) Washington, D. C.: American Association for the Advancement of Science, 1942. Pp. 63-67.
6. KLEIN, H. *Publ. Hlth Rep.*, 1945, **60**, 1462.
7. KLEIN, H. *Science*, 1947, **105**, 4-5.

A New Antibody in Serum of Patients With Acquired Hemolytic Anemia

PHILLIP STURGEON

Department of Pathology, Childrens Hospital, Los Angeles

It has been possible to demonstrate an incomplete or "blocking" type of antibody in the sera from three patients (A. G., S. N., and L. H.) with acquired hemolytic anemia. None of these showed autoagglutination; one possibly showed autohemolysis. No significant titers of cold agglutinins were found. Donath-Landsteiner and Ham tests were negative. All had had splenectomy. One (S. N.) died; the other two have made some clinical and hematological improvement. Serum obtained prior to splenectomy was studied in one instance (A. G.).

TABLE 1

IDIOPATHIC ACQUIRED HEMOLYTIC ANEMIAS

Test	Patients		
	A. G.	L. H.	S. N.
Autoagglutination	−	−	−
Panagglutination	−	−	−
Autohemolysis	−	−	+
Direct developing test	++++	+++	++
Pos. indirect developing tests	12	7	3
No. of indirect developing tests performed*	12	7	3
Titer of free antibody	1/4,096	1/256	1/128

*Each test performed with type O cells from different individuals, both Rh− and Rh+.

Serum from rabbits immunized with human serum has been used to demonstrate the presence of the incomplete antibodies, both on the red cells and free in the sera of the patients. It has also been possible to trace the antibodies through several procedures which demonstrate some of their immunological properties. The testing (developing) serum prepared in a manner similar to that described by Coombs, Mourant, and Race, gave a positive interphase precipitin test against human serum diluted 1/20,000 (*2*). A "direct developing test" is performed against thoroughly washed patient's cells. If the cells agglutinate on addition of the developing serum, the test is considered positive. The "indirect" test is performed in the same manner against appropriate normal cells which have first been incubated with the patient's serum. If agglutination occurs, it is presumed to constitute a demonstration of free antibody in the patient's serum (*1*, *3*). A titration of the free antibody in the patient's serum can be made, using the indirect method, against cells sensitized in serial dilutions of that patient's serum. The actual developing test is done by the slide technique. Equal volumes of a 2 per cent sensitized cell suspension and the developing serum are mixed on a slide and allowed to stand for 5 minutes. Table 1 summarizes results common to all three patients. Table 2 lists comparable observations on control cases of hemolytic anemia.

TABLE 2

CONTROL HEMOLYTIC ANEMIAS

	Hereditary forms			Erythroblastosis fetalis	
	Cooley's (2 cases)	Familial jaundice (2 cases)	Sicklemia (1 case)	Patient (3 cases)	Mother (3 cases)
Auto- and panagglutination	−	−	−	+−	−
Auto- and panhemolysis	−	−	−	−	−
Direct developing test	−	−	−	++	−
Titer of free antibody					
Rh+ cells				1/32	1/512
Rh− cells				0	0

Further studies have been carried out with serum from case A. G. The titer of antibody in the presplenectomy serum was found to be 1/512, this serum having been stored at 4°C. for 6 months. The postsplenectomy titer is 1/4,096 in fresh serum. It has been possible to test the thermolability and species specificity and to investigate the relative position of the receptor for this new type of incomplete antibody. A high-titer saline eluate (1/512 at 56°C., 1/32 at 37°C.) of the antibody from A. G. was prepared by incubating patient's cells or sensitized normal cells in equal volumes of saline for ½ hour at 56° or 37°C. Table 3 lists the reactions of this eluate with various receptors as brought out by the developing test.

TABLE 3

REACTION OF THE A. G. ANTIBODY WITH VARIOUS RECEPTORS

Receptor	Developing test
Type O cells	
Rh+	++
Rh−	++
Type B cells	++
" AB cells	++
" A cells	++
Rhesus cells	0*
Sheep cells	0†

* Developing serum first absorbed with "unsensitized" rhesus cells.

† Sheep cells sensitized with human heterophile antibody have been found to give a positive developing test.

Other observations of the A. G. serum have shown that the antibody cannot be demonstrated by the developing test to have reacted with red cells stored in normal saline solution for

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.

References

1. Borman, K. E., Dodd, B. E., and Loutit, J. F. *Lancet*, 1946, I, 812.
2. Coombs, R. R. A., Mourant, A. E., and Race, R. R. *Lancet*, 1945, II, 15.
3. Hill, J. M., and Haberman, S. *J. lab. clin. Med.*, 1946, 31, 1053.

Further Observations on Leptospirosis in Micronesia

F. W. Hartmann

University of Hawaii, Honolulu, T. H.

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.

References

1. Alicata, J. E. *Science*, 1947, 105, 236.
2. Walch-Sorgdrager, G. B. *Bull. Hlth Org., League Nat.*, 1932, 8, 143.

Triphenyltetrazolium Chloride as a Dye for Vital Tissues

A. M. Mattson, C. O. Jensen, and R. A. Dutcher

Department of Agricultural and Biological Chemistry, The Pennsylvania State College

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