16. De Venanzi, F., Agüero, O., and Roche, M., unpublished.
17. Drucker, W. R., and Miller, M., Abbot, W. E., Craig, J. W., Jefferies, W. McK., Levey, S., and Woodward, H., *J. Lab. Clin. Med.*, 1952, v40, 794.
18. Levine, R., Loube, S. D., and Weisberg, H. F., *Am. J. Physiol.*, 1949, v159, 107.
19. Daughaday, W. H., and Weichselbaum, T. E., *Metabolism*, 1953, v2, 459.
20. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.
21. Fiske, C. H., and Subbarow, Y., *ibid.*, 1925, v66, 375.
22. Fisher, R. A., Oliver and Boyd, Edinburgh, 1941.
23. Forsham, P. H., and Thorn, G. W., *Proc. Am. Diabetes Assn.*, 1949, v9, 99.
24. Pollack, H., Millet, R. F., Essex, H. E., Mann, F. C., and Bollman, J. L., *Am. J. Physiol.*, 1934, v110, 117.
25. De Venanzi, F., *Rev. Soc. Argent. Biol.*, 1949, v25, 120.
26. De Venanzi, F., and Podrizki, A., *Acta Cient. Venezolana*, 1950, v1, 65.

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Formazan Formation Compared to Phosphatase Activity in Human Serum.* (21271)

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Malignant tissue is stained more intensely with tetrazolium salts than normal tissue according to Straus and his coworkers(1) and Black *et al.*(2). This difference apparently is caused by the higher concentration of the glycolytic enzymes in abnormal tissue(3-7). The question arises whether such enzymatic changes in the tissue are reflected in the blood or serum of the patient. The results of our work indicate no correlation between the reducing power of serum enzymes and malignancy. Instead tetrazolium reduction in serum is apparently correlated with phosphatase activity.

Materials and method. Triphenyltetrazolium reagent: One g of 2,3,5-triphenyl-2-H-tetrazolium chloride (Eastman), 6.7 g potassium dihydrogen phosphate, and 350 ml of 0.1 *N* sodium hydroxide are diluted to one liter with distilled water. The pH of this reagent is 7.2. Two ml of the triphenyltetrazolium reagent, 6.0 ml distilled water, and 0.2 ml of serum are mixed in a test tube, and incubated at 38° C for 24 hours in the dark, because exposure to ultraviolet light itself causes reduction. The tubes are then cooled in an ice-bath and 8.0 ml of acetone is added. This mixture is stoppered and shaken well to dissolve the red formazan. If proteins precipitate and cause cloudiness, the mixture can be centrifuged. A sample of aqueous acetone mixture is pipetted off and placed in a cuvette. The color intensity is measured in a Beckman Model B Spectrophotometer at 485 mμ. Acetone, which has been shaken with an equal volume of water, is used as the reference solution or blank. The alkaline and acid phosphatase analyses were run according to a spectrophotometric modification of the Bodansky(8) and Gutman(9) methods.

Results. Using the method of Straus(1), our results showed that various malignant tumor tissues do not always show a deep red staining, and some normal tissues, such as mucosa of the gastro-intestinal and urinary tracts, hepatic parenchyma, renal cortex, uterine endometrium, and the parenchyma of the testis show a deep staining by formazan.

Sera from patients with cirrhosis, Cushing's syndrome, renal rickets, hypo- and hyperparathyroidism showed as great a reduction in transmission as those patients with neoplasms. However, the lowest per cent transmissions were obtained from patients with cancer of the

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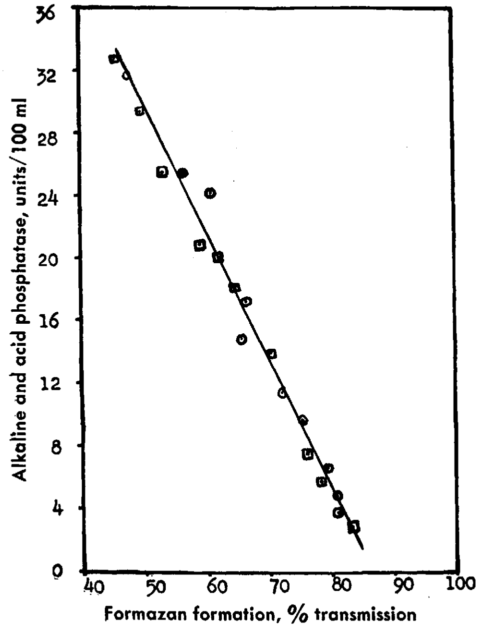

FIG. 1. Phosphatase activity vs. formazan formation. % transmission at 485 mμ. Alkaline phosphatase in Bodansky Units *(dotted square)*. Acid phosphatase in King-Armstrong Units *(dotted circle)*.

prostate and osteogenic sarcoma, neoplasms with high serum phosphatase activity.

This led us to investigate phosphatase activity. Fig. 1 shows the correlation between phosphatase activity of serum and the formation of formazan from triphenyltetrazolium.

Summary. The reduction of tetrazolium by serum is not a specific test for malignancy. It seems however to be correlated with the serum alkaline and acid phosphatase activity.

1. Straus, F. H., Cheronis, N. D., and Straus, E., *Science,* 1948, v108, 113.

2. Black, M. M., Opter, S. R., and Speer, F. D., *Am. J. Path.,* 1949, v26, 1097.

3. Kun, E., and Abood, L. G., *Science,* 1949, v109, 144.

4. Kun, E., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 195.

5. Shelton, E., and Schneider, W. C., *Anat. Rec.,* 1952, v112, 61.

6. Hölscher, H. A., *Naturwissensch,* 1951, v38, 116.

7. Greenstein, J. F., *Biochemistry of Cancer,* Academic Press Inc., New York City, 1947, ed. 1, p. 200.

8. Bodansky, A., *Am. J. Clin. Path., Tech. Suppl.,* 1937, v1, 51.

9. Gutman, A. B., and Gutman, E. B., *J. Clin. Invest.,* 1938, v17, 473.

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Factors Controlling Color Change in the Tree Frog, *Hyla versicolor* Wied. (21272)

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Control of color change in amphibians has been studied by many workers; material published prior to 1943 was summarized by Parker(1). According to Parker's summary, frogs respond to cool dark environments by release of melanophorotropic hormone and darkening of integument, whereas light and warmth result in melanophore contraction and light colors through retention or non-secretion of hormones by the pituitary. Many chemical factors also have melanophorotropic actions and produce darkening of integument, either by stimulating release of melanophorotropic hormone by action on pituitary, or by direct action upon melanophores(2). Recently the study of factors which control color changes in various species has become of considerable practical importance. Johnsson and Högberg(3), Sulman(4) and Thing(5) reported melanophorotropic effects of ACTH

TABLE I. Progressive Color Changes in *Hyla versicolor* and Numerical Assignments.

Color	No.
Green	1
Olive	2
Mottled	3
Beige	4
Brown	5