

enthal concentration values indicated little disturbance in kidney function. However, these tests would not be expected to show much change unless there was a rather extensive impairment in renal function. The phenolsulphonphthalein excretion test was our most sensitive test of tubular function and in several instances showed evidence of dimin-

ished tubular capacity following sodium tetrathionate administration. On the basis of these clinical studies it would seem that sodium tetrathionate probably should not be used if there is any pre-existing renal disease.

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Glycogen in Basophilic Leucocytes in Human Blood Smears. (17381)

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In a recent study of the occurrence of the periodic acid-Schiff reaction in various normal cells of the blood and connective tissue by Wislocki, Rheingold and Dempsey,¹ small clear red dots were observed in the pale pink cytoplasm of the basophilic leucocytes. These investigators were not able to identify the basophiles in control blood smears digested by saliva. They therefore concluded that the red stained material in the undigested smears may have been glycogen but that the "apparent finding needs further verification." Similarly, Gibb, and Stowell² decided that "Because myeloid cells which were free from glycogen were not observed in normal peripheral blood and bone marrow films, basophils, although not specifically identified, may also contain glycogen."

To make certain that basophils could be identified in smears digested by saliva before treatment with periodic acid and leucofuchsin, dried smears of human blood were stained in Wright's blood stain in absolute methyl alcohol for 3 minutes. This step was not followed by the usual one of the diluted stain. For the most part, the smears were very quickly rinsed in 95% ethyl alcohol although at the beginning of the study a few were dipped in running water. Basophils were located and

each cell was ringed by means of an object marker. A map was made giving its position in the circle. The smears were then fixed in absolute ethyl alcohol for 5 minutes, rinsed in 95% and 70% alcohol and stained by the periodic-leucofuchsin technic of McManus³ with $\frac{1}{2}$ % periodic acid, or, as modified by Wislocki, Rheingold and Dempsey,¹ with 1% periodic acid. The smears to be digested were covered with saliva for 45 to 60 minutes after fixation in absolute alcohol and rinsed in running water. After the smears had been examined with no nuclear stain, they were stained in Mayer's hematoxylin for 10 minutes.

In the undigested smears, the glycogen in the neutrophils was stained a deep to pale red-pink, either evenly throughout the cytoplasm, or more deeply either around the periphery of the cell or off at one side. The 29 basophilic leucocytes examined were, in contrast, much paler, a difference seen clearly when the two kinds of cells were found next to each other. There was a marked variation in the amount of glycogen in the basophiles even on the same slide. In some cells rather large clear red granules were seen in groups with finer grains distributed more regularly among the unstained basophilic granules. In others only these dust-like particles of glycogen were visible. A third variety had a hazy pink background which outlined the colorless basophilic granules. A few had no color at all.

¹ Wislocki, G. B., Rheingold, J. J., and Dempsey, E. W., *Blood*, 1949, **4**, 562.

² Gibb, R. P., and Stowell, R. E., *Blood*, 1949, **4**, 569.

³ McManus, J. F. A., *Stain Tech.*, 1948, **23**, 99.

In the digested smears the cytoplasm of the 12 basophiles studied was almost transparent and without color. It is therefore concluded that the material in the basophilic leucocytes which stained red in leucofuchsin after periodic acid is glycogen. Furthermore, since

glycogen can be demonstrated in the human basophilic leucocytes, they do "differ from mast cells which contain periodic acid-Schiff positive material which is insoluble in saliva." (Wislocki, Rheingold and Dempsey¹).

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A Quantitative Method for Measuring Staphylococcal Anticoagulase.* (17382)

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The study of infections produced by *Staphylococcus aureus* in man or in experimental animals has been hampered by the lack of a suitable serological test. Since staphylococcal coagulase will stimulate the production of an inhibitor substance, termed anticoagulase,^{1,2} it has been possible to devise a serological test that could be used in the study of infections caused by this organism.

The mechanism whereby staphylococcal coagulase clots plasma has been partially clarified by the recent work of Smith and Hale,³ Kaplan and Spink,⁴ and Tager.⁵ Most strains of *S. aureus* produce an extracellular substance, coagulase, which, added to plasma, results in the formation of a clot. Coagulase does not clot fibrinogen directly, for it has been found that a second factor, termed acti-

vator³ or reacting factor,⁵ is also required. This substance is found in the blood of man and of certain animal species. Although it is not entirely clear at the moment whether coagulase, activator, or a product of these two substances is responsible for the clotting of fibrinogen, antibodies against coagulase develop following immunization with cell-free coagulase. By employing measured amounts of coagulase, fibrinogen and activator, it has been possible to develop a quantitative method for the measurement of coagulase inhibitor, anticoagulase.

Materials and methods. Buffered Saline. A solution of 0.01 M phosphate in 0.85% sodium chloride at a pH of 7.4 was prepared for dilution of all sera and for preparation of fibrinogen, activator and heparin solutions.

Activator. The source of activator was 300 ml of human serum from one donor. This standard serum was stored in glass containers containing 50-75 ml at 4°C.

Two strengths of activator were employed. For standardization of coagulase, a final dilution of 1:3500 of the serum in a solution of fibrinogen was employed. In the coagulase inhibition test a dilution of standard serum of 1:1000 was used.

Heparin. A solution of heparin containing 100 mg per 10 ml of buffered saline solution was prepared and stored at 4°C until used. A 1:100 dilution of this was added to the fibrinogen-activator mixture.

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¹ Rammelkamp, C. H., *Am. J. Med.*, 1948, **4**, 782.

² Tager, M., and Hales, H. B., *J. Immunol.*, 1948, **60**, 475.

³ Smith, W., and Hale, J. H., *Brit. J. Exp. Path.*, 1944, **25**, 101.

⁴ Kaplan, M. H., and Spink, W. W., *Blood*, 1948, **3**, 573.

⁵ Tager, M., *Yale J. Biol. and Med.*, 1948, **20**, 269.