the Egyptian material, in spite of its great age, seems to remove both of the kinds of anti-B agglutinin used, and thus displays a specific adsorptive power. It seems quite possible that the Landsteiner blood groups can be determined by such methods; it is unfortunate that M and N do not seem to be demonstrable in muscle.

Work is now in progress to establish more definitely the presence of the agglutinogen B in these specimens, and to determine if an anti-O serum[2] can be prepared which will make possible the decision between a type O and a specimen which has simply lost all of its agglutinogens because of deterioration. Also, it would obviously be desirable to test a great many more samples, and the authors hope to do this if sufficient material can be obtained.

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Further Studies on the Coagulo-Flocculation Test for Malignant Tumors.

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A modified procedure of the coagulo-flocculation test for malignant tumors[1] is described, based on additional studies of protein fractions of normal serums, those in various diseases and malignant tumors. The essential constituents are: (1) Diluted blood serum inactivated at 60°C. for ½ hour, which should be neither contaminated nor hemolized. (2) The antigen is prepared by extracting finely ground beef heart with 95% alcohol in the ratio 1:10 for 3 days at 37°C. and overnight at room temperature and then filtering. (3) A saturated watery solution of sodium chloride.

The serums are diluted with water before their use in the test according to the percentage of hemoglobin. Dare's hemoglobino-

[2] See references in Sasaki, H., *Z. f. Immunitätsf.*, 1932, **77**, 101.

[1] Weiss, E., *Arch. Path.*, 1932, **13**, 106.

meter is used as a standard, and to the percentage of hemoglobin obtained 10 is added and the sum is divided by 20, which gives the dilution for the respective serum. (For instance, the reading was 70% plus 10 = 80 ÷ 20 = 4. The dilution of the serum in this case would be 1:4.) Where the Dare reading is 45% or less, the serums should be diluted only to 1:3. Tallqvist's scales can be used if their average reading is previously compared with Dare and the difference taken into consideration in the calculation of the dilutions of the serums. For instance if the Tallqvist scale shows a 10% higher reading than the Dare, nothing is added to the hemoglobin reading before dividing with 20.

The proper dilution of the serum can be determined also from the red blood count by the following formula: $\frac{\text{Red blood count}}{1{,}000{,}000} - .5$ (*i. e.*, if the red blood count was 4,500,000, then 4,500,000/1,000,000 = 4.5 —.5 = 4.0.) The dilution of the serum in this instance would be 1.4. Dilutions below 1:3 are not made.

Titration of antigens is carried out as follows: Increasing amounts of undiluted antigen (.16 cc., .18 cc., .20 cc., .22 cc., .24 cc., etc.) are placed in the corresponding tubes of 2 rows (each with 8 tubes) of a rack. In each tube of the first row 0.6 cc. of the diluted malignant serum are added and each tube of the back row receives 0.6 cc. of the diluted normal serum. The tubes are thoroughly shaken and then placed in a water bath for 5 minutes at 60°C. After the incubation each tube is diluted with 1.5 cc. of saturated solution of sodium chloride and the results are recorded. The largest amount of antigen which causes only turbidity in the normal tube and a distinct flocculation in the malignant tube is selected as the proper amount for the test (= titre). The titrated amount of antigen should be also tested with syphilitic, jaundice and anemic serums. The titre remains the same for an indefinite period if the antigen is properly preserved.

The routine test: Wassermann tubes are placed in 2 rows in the racks. The tubes of the first row are used for the main test with the unknown serums and also for the malignant, syphilitic, jaundice, and anemic controls. The last tube in the first row contains the antigen control. The tubes in the second row serve as the serum controls for the unknown serums and also for the malignant, syphilitic, icteric and anemic serums. The titrated amount of the diluted antigen is placed in each tube of the first row. The corresponding amount of water is placed in all tubes of the second row. Six-tenths cc. of each diluted serum are added to one tube in the first row and an equal amount of the same serum to the tube behind in the second

row. Six-tenths cc. of water (instead of serum) are added to the antigen control. The ingredients of the serum controls should be the same as those used for the unknown serums. All tubes are then shaken and placed in a water bath at 60°C. for 5 minutes. After the incubation each tube is diluted with 1.5 cc. of saturated solution of sodium chloride and the results are read. If the required amount of the unknown serum is not available, the test may still be performed successfully if the remaining constituents for the reaction are decreased proportionately.

Controls: The following controls are necessary each time the test is carried out: (1) antigen control, (2) serum control (each serum should have a serum control), (3) malignant, syphilitic, icteric and anemic controls.

Interpretation of the results: The controls should be examined before making readings of the unknown serums. The malignant control should show a thick layer of coagulated serum floating on the surface of the salt solution, which contains many large floccula. All other controls should remain uniformly turbid. One tube is read for each unknown serum. Tubes with a distinct flocculation or showing in addition a layer of suspended coagulated serum on the surface of the saline are read as strong positive. Tubes with a fine flocculation are read as weak positive. Tests with doubtful flocculations are repeated. Uniformly turbid tubes are read as negative.

This test applies to all types of malignant tumors. The modification of the original procedure raised the sensitiveness of the test from 79% strong positive reactions to 85% and with the weak positive reactions to 89%.