the hematocrit, and freshly flowing blood obtained by needle prick is drawn up to the circular mark. Blood adhering to the outside is wiped away, the suction tube is carefully removed, and the pipette encircled from end to end with the rubber band. The hematocrit is now placed in a centrifuge with beveled end down and rotated for 20 minutes at 3000 r.p.m. Centrifugalization at this rate for more than 20 minutes gives constant readings. The red cells are completely sedimented, and clearly and sharply defined from the supernatant plasma. The column of red cells is then measured against a millimeter scale. Since the original length of the column of blood was 100 mm., the length of the packed red cells in millimeters gives directly its percentage. Similarly, the amount of plasma is expressed in per cent. After use, the tube is cleaned in the usual manner with water, alcohol, and ether, and when relined with heparin, is again ready for use.

Rabbit's blood has been employed in the standardization of this hematocrit. A series of consecutive readings on 10 animals, using 10 pipettes for each animal, has given a standard deviation ranging from a minimum of 0.2% to a maximum of 0.8%, with an average for the 100 readings of 0.5%. In terms of coefficient of variation, the range was from a minimum of 0.6% to a maximum of 2.2%, with an average for the 100 readings of 1.2%.

The accuracy of this method, as indicated by the small technical error of 1.2%, together with the simplicity and inexpensiveness of the pipette, and the ease with which readings are made directly in per cent, are features which recommend the hematocrit for use in the clinic and laboratory.

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Absorption and Elution of Antibodies from Various Antisera.

LEO OLITZKI AND MAX FRANKEL. (Introduced by I. J. Kligler.)

From the Departments of Hygiene and Biochemistry, Hebrew University, Jerusalem.

Frankel and Olitzki[1] showed that by specific absorption and elution it is possible to obtain antibodies free from protein by absorbing the antisera with kaoline, centrifugalizing and then eluting the kaoline absorbate with appropriate eluates. The eluates thus ob-

[1] Frankel, M., and Olitzki, L., *Nature*, 1930, **126**, 723.

tained are free from protein, in so far as they can be demonstrated by sensitive chemical tests, but contain active antibodies.

We here report experiments dealing with the elution of antitoxin, agglutinins, complement-fixing and lytic amboceptors and the behavior of these eluted antibodies. Since it is generally accepted that biological sensitization is a more sensitive test for proteins than the most sensitive chemical test, we attempted to ascertain by this method whether the eluates contained demonstrable proteins. The eluting fluid consisted of a 2% solution of glycocol with varying concentrations of sodium chloride. The technique was as follows:

Equal parts of antiserum (diluted 1:10) and kaoline were thoroughly shaken, allowed to stand 24 hours at 37°C. and centrifugalized. The kaolin-protein sediment was then resuspended in the eluting fluid and the suspension kept at 37°C. for 48 hours, then centrifugalized and the supernatant fluid tested for antibodies. The antitoxin content of the protein-free eluates was determined by the intracutaneous neutralization test employed by Roemer[2]; the content of agglutinin, complement-fixing and lytic amboceptors was measured by the methods described by Olitzki.[3]

The results showed that the largest amounts of antitoxin and of the flagellar typhoid agglutinin are obtainable in eluates made with 2% glycocol in 1 to 2% sodium chloride solution, while the largest amounts of the somatic agglutinins as well as the lytic and complement-fixing amboceptors were obtained in eluates made with 0.2 to 0.8% solution of sodium chloride. Attempts to elute by this method the complement-fixing amboceptors from luetic sera giving a strong positive Wasserman and Kahn reaction yielded negative results.

Since antibodies are generally considered to be bound up with globulins, it appeared of some importance to ascertain whether the antibody containing eluates were from the biological viewpoint protein-free as shown by the less sensitive chemical tests. We attempted to test this by sensitization of guinea pigs and subsequent anaphylactic test.

Six guinea pigs received subcutaneous injections of 1 cc. agglutinin-eluate (titre 1000 for *B. typhosus,* H-type) obtained in the manner described above. Sixteen days afterwards 3 of these guinea pigs received an intravenous injection of 0.5 cc. of this eluate, the other 3 an injection of the corresponding amount of the original serum (0.5 cc. serum diluted 1:10). There was a drop in body

[2] Roemer, F., *Z. für Immunitätsforschung und Exp. Therap.*, 1909, **8**, 308.

[3] Olitzki, L., *Centralblatt für Bakteriol.*, 1928, **106**, 247.

temperature in all the animals lasting 15-20 minutes, after which it returned to normal. Three control animals sensitized on the same day with 1.0 cc. of the original serum, diluted 1:10, showed after the intravenous injection of 0.5 cc. of the same serum dilution a heavy anaphylactic shock, associated with a decrease of the body temperature to below 35°C. which returned to normal only after 45 minutes.

In a second set of experiments the sensitizing injections consisted of 3 cc. of the same eluate; a control group received 3 cc. of the serum diluted 1:10. The second intravenous injections were carried out with 1.0 cc. of the eluate and with 0.5 cc. and 1.0 cc. respectively of undiluted serum. The animals treated with eluate showed a small drop in the body temperature lasting 15 minutes, while those treated with the original serum suffered a severe shock accompanied by a decrease of the body temperature to below 35°C. lasting 1 to 1½ hours. An intravenous injection of the eluate, or of a corresponding glycocol-sodium chloride solution, into untreated guinea pigs gave the same drop in body temperature as that noted in treated animals. It is apparent, therefore that the injection of glycocol eluates did not induce sensitization either against the injection of eluate or of original serum.

Repeated injections of eluates into rabbits (*i. v.*, 0.5 cc., 1.0 cc. and 1.5 cc.) did not yield precipitins either against the eluate or against the original serum.

It may be concluded that the eluates of antibodies found by sensitive chemical tests to be free from proteins have neither sensitizing nor precipitinogenic properties.

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Antigenic Power of Ultra-Violet Irradiated Tetanus Toxin.

EMERSON MEGRAIL AND HENRY WELCH.

From the Department of Hygiene and Bacteriology, Medical School, Western Reserve University.

It has been shown by one of us[1] that tetanus toxin is destroyed by a few minutes' exposure to ultra-violet light if toxic broth be diluted to decrease the concentration of protein and reduce the color absorptive factor. Previously, however, it has not been deter-

[1] Welch, H., *J. Prev. Med.*, 1930, **4**, 295.