

Our method consists in repeatedly freezing and thawing a tube of the serum in an ice and salt mixture and recovering the lower portion of the contents of the tube after the last thawing. An indication of what takes place may be seen if, between freezings, the tube is gently tilted back and forth, whereupon a movement of heavy oily-looking streaks occurs between a darker colored lower portion and a lighter colored upper portion. Ultimately the lower portion becomes deeply colored and the upper portion colorless and the lower portion will be found to have a higher agglutinin titer than the whole serum had originally.

By this method we have obtained from a serum which originally did not agglutinate macroscopically in 1-4 dilution a product which agglutinated in 1-16 dilution.

We are informed that this technic was employed in Wassermann's laboratory between 1908 and 1910. It has also been used in chemical research to reduce the water content of certain non-colloid solutions. We owe our acquaintance with the method to a note by Plant² who has shown that it can be used to obtain hemolytic complement of high titer from guinea pig serum. We have confirmed his findings as to complement and also as to rabbit serum anti-sheep hemolysin.

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Takata-Ara Reaction in Obstructive Jaundice.

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The test that Takata¹ proposed was originally used to distinguish between lobar and lobular pneumonia and he reported the reaction with Ara² at this time on the serum in pneumonia and in cerebral spinal fluid as well, where it seemed to be of value in distinguishing between meningitis and luetic involvement of the central nervous system. The reason for the reaction was probably a protein-shift in the blood, according to most investigators and knowing that this occurs in many pathological conditions, Jezler,^{3, 4, 5} Staub⁶ and others

² Plant, Arthur S., *Brit. Med. J.*, 1933, **2**, 414.

¹ Takata, M., Tr. 6th Congress, Tokyo, F.E.A.T.M., 1925, **1**, 693.

² Takata, M., and Ara, K., Tr. 6th Congress, Tokyo, F.E.A.T.M., 1925, **1**, 667.

³ Jezler, A., *Z. f. klin. Med.*, 1929, **111**, 48.

employed the test upon serum or ascitic fluid in liver disease. They found that the test was positive in cases of advanced liver cirrhosis and also in cases of severe toxic parenchymal damage.

Recently, Heath and King⁷ have reported a series of over 400 cases with results which, in general, corroborate these findings. They have obtained positive tests of over 60% of cases of cirrhosis of the liver and also in cases of marked liver damage. They did not, however, get positive reactions in cases of biliary obstruction of the extra-hepatic type.

Having at hand 2 cases where a positive reaction was obtained from serum taken from cases of acute extra-hepatic biliary obstruction due to stones, we made observations on a series of dogs in which the extra-hepatic biliary system had been blocked by ligating the common bile duct. These animals, after operation, were given a balanced diet and blood was drawn weekly for examination. Of a total of 8 animals, 4 were obstructed by common duct ligation and 4 observed as controls. None of the 4 controls exhibited at any time a positive reaction either in serum or plasma. In the 4 animals jaundiced by occlusion of the common bile ducts, jaundice ensued within a few days, gradually deepening during the first 3 weeks, then maintaining a more or less constant level after this time. In 2 of the 4, the Takata-Ara reaction became positive the 45th day after ligating the common duct, in the third animal the reaction was positive on the 24th day, and the fourth showed a positive reaction 65 days after operation. These reactions were read as positive when there was a strong flocculation in 3 tubes, beginning with the second one, any less reaction than this being regarded as negative.

The survival period after development of the positive test was 54 and 148 days, respectively, in the 2 animals with a 45-day interval between operation and positive test, 33 days in the animal with a 24-day development period, and 128 days in the animal in which 65 days had elapsed from operation to positive flocculation.

By these figures it appears that a reaction becomes positive somewhere between one-half and one-third of the total time of survival after ligation of the common duct. It also appears that there is some relation between the rate of development of the test and the survival period.

Pathological changes in the liver at death were the usual and con-

⁴ Jezler, A., *Schweiz. med. Wchnschr.*, 1930, **60**, 52.

⁵ Jezler, A., *Z. f. klin. Med.*, 1930, **114**, 739.

⁶ Staub, J., *Schweiz. med. Wchnschr.*, 1929, **59**, 308.

⁷ Heath, C. W., and King, E. F., *N. E. J. Med.*, 1934, **211**, 1077.

sistent findings with some definite increase in fibrous tissue about the periphery of each liver lobule and especially about the bile duct, a general infiltration of lymphocytes throughout this fibrous tissue and a little cloudy swelling of the cytoplasm of the liver cells.

Other liver function tests were done and many other observations on the blood serum carried out at this time, but the only parallelism existed between the Takata-Ara reaction and the sedimentation time which was materially reduced as the reaction became positive.

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Antigenicity of Streptofibrinolysin.*

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The low antifibrinolytic titer† of most commercial anti-streptococcus serums¹ suggests the possibility that the specific antihuman fibrinolysin formed or secreted by certain strains of *Streptococcus hemolyticus* is not antigenic.

To test this possibility, active antifibrinolytic immunization was attempted with rabbits. The selected vaccines were (a) centrifugates from 24-hour broth cultures of fibrinolytic streptococci, and (b) lytic enzymes isolated from such cultures by the alcohol-precipitation technic.² Control injections were made with (c) heat-killed streptococci centrifuged free from lytic broth. There were also available for comparison (d) a series of rabbit precipitins for the Lancefield streptocarbohydrate A. This specific capsular sugar is apparently genetically linked with the antihuman fibrinolytic function.³

Three methods of immunization were used with these rabbits. Six animals (Group A) received 3 subcutaneous, 3 intraperitoneal and 3 intravenous injections at 3 to 4-day intervals, followed by 6 intra-

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† There is no known parallelism between the antifibrinolytic titer and the therapeutic value of a streptococcus antiserum. (W. H. M.)

¹ Van Deventer, J. K., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1117.

² Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

³ Madison, R. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 49.