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5338

Colloidal Gold Test for Detection and Titration of Immune Bodies in Poliomyelitis.*

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The test depends upon the precipitation of a colloidal gold sol by an electrolyte (sodium chloride) in the presence of serum containing varying amounts of immune bodies specific for poliomyelitis.

Preliminary experiments were made with normal and immune monkey and normal and poliomyelitic human serums in the presence of varying dilutions of Kolmer's cholesterinized antigen and different concentrations of sodium chloride. Other antigens such as plain and cholesterinized extracts of normal rabbit and sheep brain were studied also.

In a manner similar to that described by Mishulow and Krumwiede,[1] Sanderson and Yoe,[2] for the determination of toxicity of diphtheria toxin, and recently by Jungeblut[3] in poliomyelitis, an investigation was made of the possible effect of 1% gold chloride. The results were not specific or consistent and the use of antigens and gold chloride was discarded. A peculiar color change ranging from lilac to deep purple was observed in certain tubes that contained immune serum in the presence of this reagent, however, and the idea of a color reaction suggested itself. Inasmuch as the antigens and gold chloride had seemed to possess no particular merit in such a test, Lange's colloidal gold was used.

The method adopted finally utilized colloidal gold, sodium chlo-

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[1] Mishulow and Krumwiede, *J. Immunol.*, 1927, **14**, 77.

[2] Sanderson and Yoe, *J. Immunol.*, 1929, **16**, 427.

[3] Jungeblut, C. W., Proc. Soc. Exp. Biol. and Med., 1930, **28**, 7.

ride (0.4%) and serum in proportions that were found most effective and clear cut in numerous preliminary determinations. Dilutions of serum were prepared in series in an attempt to detect quantitative differences. Readings were made after 12-16 hours. With cholesterinized sodium chloride, however, results could be read easily after 3 hours at room temperature (22-25°C.).

Composite experiments were repeated with uniform agreement and the results appeared decisive. Poliomyelitic serum from convalescent adult and juvenile patients and immune monkeys consistently produced a rose-colored or violet precipitate with a supernatant fluid graded from clear and colorless to light salmon pink and finally the negative rose color of the control. Most marked precipitation occurred in the tubes containing the lower dilutions of serum. Normal monkey serum and that from normal children in the susceptible age groups (2-7 years) were negative for precipitation effect, as were the serums from convalescent typhoid fever patients and from rabbits immunized with different strains of streptococci and staphylococci. Further studies are in progress with potent typhoid and paratyphoid serums and with antimeningococcus, antipneumococcus, and antitetanus serums.

In a few instances serum from supposedly normal adults showed very slight color changes in the high concentrations of serum and rarely a faint precipitating effect. This observation confirmed the impression that adults may have immune bodies for poliomyelitis as a result of a previous undetected mild attack of the disease or exposure to it.[4]

Positive Wasserman and Kahn reactions in serums did not affect the specificity of the colloidal gold test for poliomyelitis immune bodies.

The pH value of the mixtures used in the test showed that this was not a factor. The controls included a wide range of buffer solutions and the test serums before and after reading the reactions with colloidal gold and electrolyte.

The effect of combining poliomyelitis virus filtrate as a serum diluent before addition of the gold sol and electrolyte was to prevent the precipitation. Such serums behaved like that of the untreated normal monkey and suggested an absorption phenomenon. Other filtrable viruses are being tested in this manner to verify this possibility.

A preliminary application of the colloidal gold test in the study of antibody formation during the course of poliomyelitis was made

[4] Shaughnessy, *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 742.

in experimentally infected *M. rhesus* monkeys. Serum was tested at intervals prior to the appearance of the earliest signs of paralysis or symptoms, and up to the third day of paralysis, with negative results. Further studies are in progress along this line of investigation and in connection with the development of quantitative procedures.

The correlation of immune body content and protective property of serums is also being studied *in vitro* and *in vivo* in monkeys, and serums from all possible sources are in the course of investigation.

5339

A Rapid Method for the Diagnosis of Early Pregnancy from Urine.

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The method is based upon a simple procedure for concentrating the anterior pituitary hormone[1] in the urine and injecting 2 or 3 small doses subcutaneously into immature female rats, 18-21 days old. The animals, including untreated normal controls, are autopsied on the second or third day at the latest and the diagnosis made from the gross changes in the reproductive organs. Serial sections are prepared from the ovaries, tubes, and uterus for confirmation of the macroscopic findings. The procedure shortens the time required for diagnosis to 36-48 hours instead of the usual 4-5 or more days.

Eight ounces of morning urine, preferably not over 8 hours old, are used in the test. Older specimens have been found satisfactory, however, and the results not vitiated by the addition of a preservative or by the reaction of the urine. A preservative such as ether tricresol (4 drops to each 100 cc. of urine) may be necessary in forwarding specimens from a distance.

Two and one-half volumes of 95% alcohol are added to the

[1] Erdheim, J., and Stume, E., *Beitr. z. Path. Anat. u. z. allg. Path.*, 1909, **46**, 1. Evans, H. M., and Long, *Anat. Rec.*, 1921, **21**, 62. Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, **40**, 159; *Am. J. Physiol.*, 1927, **80**, 114. Evans, H. M., and Simpson, M. E., *J. Am. Med. Assn.*, 1928, **91**, 1337. Allen, W. M., *Am. J. Physiol.*, 1930, **92**, 127, 612. Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 806; *Am. J. Physiol.*, 1929, **90**, 329; *J. Biol. Chem.*, 1930, **86**, 499.