hydrate is accompanied by clearly evident acid formation, but that (as others have pointed out) certainty as to utilization of carbohydrate can not be attained by methods relying alone upon such formation. This work is being continued.

6822

Skin Tests for Sensitivity to Virus of Poliomyelitis.

PAUL H. HARMON, JAMES A. HARRISON AND GRAHAM KERNWEIN.
(Introduced by Lester R. Dragstedt.)

From the Department of Surgery, Division of Orthopedics and the Milbank International Fund for the Study of Infantile Paralysis, Department of Hygiene and Bacteriology, University of Chicago.

Early investigators[1,2] denied that the convalescent state in monkeys recovering from poliomyelitis was accompanied by cutaneous hypersensitiveness. Aycock[3] has stated that he has been unable to detect skin hypersensitiveness to monkey passage virus in human convalescents. Both Aycock and Kagan[4] and Stewart and Rhoads[5] while actively immunizing monkeys by the intracutaneous method, failed to note cutaneous allergic reactions. Recently, Jungeblut[6] has found that while cutaneous reactions of hypersensitiveness are lacking in convalescent monkeys, there is a high degree of generalized hypersensitiveness in these animals as judged by the occurrence of an immediate thermic response to the introduction of virus either intracerebrally or subcutaneously. Such a response was lacking in animals that had been uninfected. According to this author[7] the reverse situation obtains in man, as he observed a definite specific cutaneous reaction to an emulsion of virus-bearing monkey spinal cord in each of 27 human individuals with residual paralysis due to poliomyelitis. Sabin[8] has failed to find evidence of an allergic skin reaction in normal adults or in human convalescents either recent or of long stand-

[1] Römer, P. H., "Die Epidemische Kinderlähmung", Berlin, Springer, 1911.
[2] Leiner, C., and von Wiesner, R., *Wien. klin. Wchnschr.*, 1909, **46**, 2331.
[3] Aycock, W. L., personal communication.
[4] Aycock, W. L., and Kagan, J. R., *J. Immunol.*, 1927, **14**, 85.
[5] Stewart, F. W., and Rhoads, C. P., *J. Exp. Med.*, 1929, **49**, 959.
[6] Jungeblut, C. W., *J. Exp. Med.*, 1931, **53**, 159.
[7] Jungeblut, C. W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1072.
[8] Sabin, A. B., quoted by Harrington, H., in "Poliomyelitis", Baltimore, The Williams and Wilkins Company, 1932, 126.

ing, when tested with fresh heat inactivated preparations of monkey virus, human virus and normal monkey cord.

Since one of us (J. A. H.) had prepared a concentrated and purified virus from which a large share of proteins, phospholipins and lecithins had been eliminated, we decided to test the cutaneous reactions of poliomyelitis convalescents with this product. Using a similarly prepared concentrate of normal monkey spinal cord as a control, we have carried out intradermal injections upon 17 children having residual paralysis from poliomyelitis. These children varied from 2 to 19 years of age. The acute attack of poliomyelitis had been passed from 5 months in 2 instances to 14 years in one instance, the majority being fewer years remote from the acute stage of the disease. Tests were also done upon the same group of convalescents with crude 5% monkey virus and with a 5% suspension of normal monkey spinal cord. All preparations were heated upon 2 occasions to 55°C. for 30 minutes, a temperature that had been demonstrated to be well beyond the thermal death time for poliomyelitis virus.[9] Another group of 23 non-poliomyelitic children convalescent from miscellaneous orthopedic complaints received intracutaneous inoculations of all 4 preparations.

That none of these preparations contained viable virus was demonstrated by intracerebral injection of 1.0 cc. of both the concentrated and unconcentrated virus into unused *Macacus rhesus* monkeys by the intracerebral method. No paralysis was observed in either animal during one month subsequent to inoculation. All preparations were shown to be sterile by the usual bacteriological methods.

Very few cutaneous reactions were observed with crude poliomyelitis virus preparations and when present there was always a cutaneous reaction to the corresponding control injection of normal monkey spinal cord emulsion. One-third of both groups of children demonstrated a definite cutaneous erythema reaching its height 24 hours after injection when the concentrated preparations were used. Again there were no differences observed between the virus cord concentrate and the similar preparation of normal monkey spinal cord. No immediate cutaneous reactions were observed.

Eight children convalescent from poliomyelitis were also tested in groups of 2 to each of the 4 preparations given subcutaneously. Following inoculation of 0.5 cc. by this method the rectal temper-

[9] Shaughnessy, H. J., Harmon, P. H., and Gordon, F. B., *J. Prev. Med.*, 1930, **4**, 149.

ature of these 8 children was followed by 2 hourly periods for 24 hours. No deviations from a normal temperature were observed in any instance and no induration developed about the site of inoculation.

6823

On the Ionization of Calcium Citrate.

FRANKLIN C. MCLEAN, A. BAIRD HASTINGS, LILLIAN EICHELBERGER AND JAMES LOWELL HALL.

From the Physiological Laboratory and the Lasker Foundation for Medical Research, the University of Chicago.

The fact that the addition of sodium citrate to calcium containing solutions reduces the concentration of ionic calcium is well known. The nature of the combination between calcium and citrate has not been definitely established. It has been sometimes thought that a neutral compound $Ca_3(citrate)_2$, exists, but this is not consistent with the evidence that the combination between calcium and citrate is negatively charged.[1]

For this reason the frog heart preparation which had been shown to be sensitive to calcium ion changes under controlled conditions was used to assay the calcium ion concentration in solutions of varying total calcium and citrate content.

The results of such experiments are shown in the accompanying figure. The experimental points show the concentrations of calcium and citrate present in solutions which are iso-active with corresponding solutions containing no citrate, but containing a certain concentration of calcium ions. Each series of symbols represents physiologically isoactive points. The relationship for a given concentration of calcium ions appears to be linear for the limits within which this technique is accurate, *i. e.*, calcium ion concentrations from 0.3 to 1.2 millimols per liter. This has been found to be true when the total calcium concentration is as high as 30 millimols per liter.

These results are consistent with the interpretation that (1) calcium combined with citrate does not affect the amplitude of contraction of the frog heart and (2) that one mol of calcium combines with one mol of citrate to form a calcium citrate compound

[1] Greenberg, D. M., and Greenberg, L. D., *J. Biol. Chem.*, 1932, **99**, 1.