Of these only 2 showed diminution of the area of the skin reaction of more than 0.1 square inch during aspirin administration, one of these had marked signs of salicylism and a level of 507 γ/ml. Seven individuals had no difference in area with and without salicylates and 4 had increases of more than 0.1 square inch despite comparable doses of salicylate. With tripelennamine, the C18K reaction was approximately the same as the control reaction in 5 individuals and increased 0.1 square inch or more in 4 individuals. A similar degree of variation is present when the reactions with salicylate are compared with those during tripelennamine administration. Four individuals did not show any change, 2 had lesions which diminished by more than 0.1 square inch and four showed areas which increased by more than 0.1 square inch. With the tuberculin test, 22 subjects were given aspirin; 14 showed no appreciable change from their control skin test, 5 showed a more severe reaction, and three had a less severe reaction.

Discussion. The Arthus reaction is a manifestation of the union of certain antigens with antibodies, and its severity can be related to the amount of antibody uniting with the antigen.[16,22,23,24] Since the severity of the Arthus reaction was not altered appreciably it does not appear that the drugs employed inhibit the union of antigen with antibody *in vivo*. However, the moderately diminished edema at the end of 6 hours in the salicylate treated group may be of interest in view of the demonstrated inhibition by salicylate of spreading due to hyaluronidase.[25]

Tripelennamine has been shown to exhibit a significant antihistamine action.[3,4] Since it did not lessen the severity of either lesion studied, it appears that histamine does not play a significant role in the pathogenesis of necrotizing allergic reactions.

Summary. A study was made of the effect of salicylate and of a synthetic antihistamine compound, tripelennamine hydrochloride, on necrotizing allergic reactions of the Arthus and "bacterial" types. The Arthus reaction was induced passively in rabbits by quantitative methods. "Bacterial" sensitivity to a streptococcus nucleoprotein fraction and to tuberculin was observed in human subjects. Neither type of reaction appeared to be altered by the administration of salicylate or tripelennamine. However, in the early development of the Arthus phenomenon, salicylate treated animals exhibited less edema at the site of the lesion.

[22] Opie, E. L., *J. Immunol.*, 1924, **9**, 231.

[23] Culbertson, J. T., *J. Immunol.*, 1935, **29**, 29.

[24] Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1941, **40**, 127.

[25] Guerra, F., *J. Pharm. and Exp. Therap.*, 1946, **87**, 193.

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Further Observations on the Cultivation of Strains of Poliomyelitis Virus in Developing Eggs.*

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In an earlier paper[1] we reported the successful cultivation of the murine SK strain in developing eggs. In the present paper we wish to summarize observations made since then on this and other strains.

Murine SK Strain. The SK strain has now

* Supported by the Howard Frost Poliomyelitis Research Fund.

[1] Schultz, E. W., and Enright, J. B., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 8.

been carried through 30 passages in fertile eggs. Its ID_{50} for mice in 15th, 21st, 24th and 30th egg passages was 10^{-7}, based on titrations of Chamberland L_3 filtrates of 1% suspensions of whole embryo tissues in half-strength Martin's peptone solution, the filtrates themselves showing a titer of 10^{-5}. This titer was reached by the 4th or 5th day. The average incidence of embryo deaths between the 2nd and 6th days was 79% in inoculated eggs incubated at 35°C, and 46% in eggs incubated at 37.5°C. The virus content of the embryo tissue did not appear to be influenced by the temperature at which the inoculated eggs were incubated.

Up to the 18th passage, the material used to inoculate the next group of eggs consisted of the heads and necks only of the embryos from the preceding passage. After the 18th passage, the entire embryo was used in preparing the passage material. Nine-day eggs were employed in making the passages. All were inoculated by the chorioallantoic route. The inoculum in all passages consisted of 0.1 ml of Chamberland L_3 filtrates of 1% suspensions of the chick embryo tissue in half-strength Martin's peptone solution. Twelve supplementary passages were made by the yolk sac route. In the latter, the passage material consisted of filtrates prepared from suspensions of abdominal viscera only. This series showed about the same virus content at the end of the 12th passage as the series carried with material from the heads and necks only. In the series inoculated by the chorioallantoic route, the allantoic fluid, unfiltered, showed an ID_{50} of 10^{-5} in the 9th passage, the chorioallantois itself an ID_{50} of 10^{-4} in the 5th passage, and the yolk only in the 15th passage titered only 10^{-1}.

Embryos from 5 to 14 days of age seemed to serve equally well for the propagation of the virus. However, in eggs containing 15- and 16-day-old embryos the ID_{50} dropped from the usual level of 10^{-7} to 10^{-4}, and in 17-day-old eggs to zero. With this drop in titer the incidence of embryo deaths also fell off. Attempts to adapt the virus to older embryos failed.

Neutralization tests on material from the 24th egg passage did not show any appreciable change in the antigenic properties of the virus.

Murine MM Strain. This strain, described by Jungeblut and Dalldorf[2] was obtained from Dr. Jungeblut in 1946 in its 107th mouse passage. Jungeblut[3] briefly mentions that this strain had been "carried over five serial passages in fertilized hen's eggs." In a later paper, Brutsaert, Jungeblut and Knox[4] state that the virus could not be recovered after the 5th or 6th serial passage.

We succeeded in carrying the MM strain through 10 egg passages under conditions similar to those employed for the SK strain. The ID_{50} for mice of material from the 10th egg passage was about 10^{-6}, based, as in the preceding series, on serial dilutions of Chamberland L_3 filtrates of 1% suspensions of whole embryo tissue. At 35°C the average incidence of embryo deaths was 75%, based on a total of 52 eggs employed; at 37.5°C this incidence dropped to about 20%.

Murine Lansing or C(M) Strain. Of especial interest have been the results obtained with what appears to be a murine high titer variant of the Lansing strain. This strain prior to its trial in eggs had been passed continuously in mice over a period of more than four years. Just when it first showed itself as a high titer virus we are unable to say since actual titrations were not carried out during most of this period. Up to 1943 it behaved like a typical "low titer" Lansing strain. However, shortly before the present studies were undertaken in 1946, it showed an ID_{50} of 10^{-7} for mice. The possibility that the supposed variant was actually a foreign virus obviously had to be considered. An investigation was therefore undertaken to determine its relationships to Lansing "passage strains" from other laboratories. This study is still in progress, and will be reported later.[5] Its inclusion in this report—as a probable variant of the Lansing strain—seems

[2] Jungeblut, C. W., and Dalldorf, G., *Am. J. Pub. Health*, 1943, **33**, 169.

[3] Jungeblut, C. W., *J. Exp. Med.*, 1945, **81**, 275.

[4] Brutsaert, P., Jungeblut, C. W., and Knox, Alice, *J. Pediat.*, 1946, **29**, 350.

to be justified by the results of serological observations thus far and by the fact that it has produced characteristic flaccid paralysis in individual monkeys when these were inoculated intracranially with mouse brain-cord virus suspensions. In one group of 3 monkeys inoculated with "autolyzed" brain-cord material from recent mouse passage prepared according to Milzer and Byrd,[6] all developed typical paralysis with characteristic cord lesions.

This virus has been carried through 15 egg passages. As in the murine SK series the passages were initiated with a Chamberland L_3 filtrate of a 1% suspension of mouse brain-cord tissue in half-strength Martin's peptone solution. The ID_{50} of this filtrate was 10^{-5}, or not less than 10^{-7} for the unfiltered material. This was seeded to 5 eggs in 0.1 ml amounts, by the chorioallantoic route. All subsequent passages were made as described for the preceding viruses. The virus has been carried through the 15 passages without apparent loss of its ID_{50} for mice. Up to the 5th passage, the material passed consisted of heads and necks only of the preceding embryos. In all subsequent passages filtrates of ground whole embryo tissue were employed. Of a total of 78 eggs inoculated in making these passages, 76% developed dead embryos between the 2nd and 6th days.

While the infectivity for mice of the egg passage material has remained high (10^{-7}), its infectivity for monkeys has proved to be low. An undiluted L_3 filtrate of a 1% suspension of material harvested from the 5th egg passage was inoculated into 4 Rhesus monkeys, each animal by both the intracerebral and intraperitoneal routes. No symptoms were observed until the 3rd week, at which time all 4 of the animals developed a well-defined awkwardness in their movements. None developed a definite paralysis, however. Three of the animals were sacrificed soon after the onset. No well-defined cord lesions were observed in any of them, and only one showed perivascular cuffing in the brain. The 4th animal recovered without any residual manifestations. A similar filtrate from the 10th egg passage was inoculated, undiluted, into 2 monkeys. Both failed to develop symptoms, despite the fact that the ID_{50} of this particular filtrate, for cotton rats, was 10^{-4} and 10^{-5} for mice (or not less than 10^{-6} and 10^{-7} respectively, for the unfiltered material). Employed in a dilution of 10^{-4}, this filtrate was neutralized by an equal volume of anti-C(M) serum produced in a rabbit (final serum dilution 1:5), but not by antisera against the murine SK strain and the GD VII strain of Theiler's virus when these antisera were employed in similar dilutions. Certain tests carried out with mouse brain-cord suspensions have shown, however, that there is some cross reaction with the murine SK strain.[5]

Theiler's Encephalomyelitis Virus. The GD VII strain of Theiler's virus was carried through 10 egg passages. Success with strains of Theiler's virus had been reported, however, by earlier workers.[7,8,9] The ID_{50} of an L_3 filtrate of material from the 10th passage, prepared from a pool of whole embryos, was 10^{-5}. With this particular virus the average incidence of embryo deaths was only 23%, based on a total of 55 eggs employed. The virus was neutralized by an homologous antiserum produced in a rabbit but not by anti-murine SK serum nor by anti C(M) serum.

Strains Which Failed to Grow in Fertile Eggs. The following strains failed to show growth in developing eggs: (1) the cavian SK strain,[10] obtained from Dr. Claus W. Jungeblut in 1946 as his 74th serial passage in guinea pigs, (2) the original monkey passage SK strain,[11] obtained from Dr. John

[5] Schultz, E. W., and White, S. C., unpublished observations.

[6] Milzer, A., and Byrd, C. L., *Science*, 1947, **105**, 70.

[7] Gard, S., *Acta Med. Scand.* (Suppl), 1943, 1943.

[8] Dunham, W. B., and Parker, Sue, *J. Bact.*, 1943, **45**, 80.

[9] Riordan, J. T., and Sá-Fleitas, M. J., *Science*, 1946, **103**, 449.

[10] Jungeblut, C. W., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 177.

[11] Trask, J. D., Vignec, A. J., and Paul, J. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 147; 1939, **41**, 241; *J. A. M. A.*, 1938, **111**, 6.

R. Paul in 1946 in its 22nd passage, (3) a cotton rat passage strain of the Lansing virus, obtained from Dr. Hubert Loring in 1946, (4) the MV strain, and (5) the FW strain isolated by one of us (E.W.S.) from the stools of a poliomyelitis patient in 1941. All 5 of these strains failed to prove infectious for test animals after the 5th or 6th egg passages. Since the L_3 filtrate of the Lansing strain had failed to induce infection in mice, the egg passages in this series were initiated with 0.2 ml of an unfiltered 1% suspension mouse brain-cord tissue in half-strength Martin's peptone solution of the passage material, which did prove infectious for 5 out of 6 cotton rats.

After we had completed this work a paper by Riordan and Sá-Fleitas[12] appeared in which they also report having obtained negative results with certain "monkey pathogenic mouse adapted strains" of poliomyelitis virus.

Summary. Further observations on the cultivation of the murine SK strain of poliomyelitis virus in fertile eggs are reported. This strain has now been carried through 30 passages in eggs. The virus has been found to be widely distributed in the infected embryo. It is easily transmitted from egg to egg by different routes. Embryos from 5 to about 14 days of age seem to serve equally well for the propagation of the virus, but those above 16 days of age fail to support its growth.

The murine MM strain was carried through 10 egg passages; a Stanford mouse passage strain, believed to be a high titer variant of the Lansing strain, tentatively labeled C(M) virus, was carried through 15 egg passages. The GD VII strain of Theiler's mouse encephalomyelitis virus was carried through 10 passages. It caused the lowest incidence of embryo deaths of the three viruses cultivated. Five strains of poliomyelitis virus failed to show growth in eggs.

12 Riordan, J. T., and Sá-Fleitas, M. J., *J. Immunol., Virus Research and Exp. Chemotherapy*, 1947, **56**, 263.

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Ability of Diisopropylfluorophosphonate (D.F.P.) to Produce Antidromic Activity in Motor Nerves.

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Masland and Wigton[1] demonstrated antidromic activity in motor nerves, occurring in association with fascicular twitching of the muscles produced by neostigmin (Prostigmin), or by eserin. They concluded that the cholinesterase inhibiting action of these drugs led to an accumulation of acetylcholine, which stimulated the motor nerve at its ending, and initiated antidromic impulses. This work was further extended by Eccles, Katz, and Kuffler.[2] These investigators demonstrated that the antidromic activity observed in the motor nerve sometimes originated in the muscle fibre, and at other times originated in the nerve ending itself.

In view of the development of new anticholinesterase drugs, it was of interest to determine whether the new drugs produce similar antidromic activity. A study similar to that previously reported with neostigmin has therefore been made using diisopropylfluorophosphonate (D.F.P.). The drug was injected intramuscularly or intraperitoneally in cats, in doses of 5 to 10 mg, as a 1% solution in peanut oil. Active fascicular twitching of the muscles was usually observed in about 40 minutes from the time of injection. When the drug was injected intramuscularly,

1 Masland, R. L., and Wigton, R. S., *J. Neurophysiol.*, 1940, **3**, 269.

2 Eccles, J. C., Katz, B., and Kuffler, S. W., *J. Neurophysiol.*, 1942, **5**, 211.