All mouse tumors so far studied, spontaneous or transplanted, primary or metastatic, are deeply stained after injection of T. 1824, and even pin-point metastases in the lung and liver may be readily detected by their blue color. The results are equally definite with the Brown-Pearce epithelioma of the rabbit and a sarcoma of the chicken. Five or 10 cc. of the 1:1,000 solution was injected in these cases.

Examination of the fresh, stained tumor with the naked eye or with a magnifying glass reveals that, although the whole tumor appears blue, the dye is mostly fixed in the stroma of the growth. Microscopic observations have not been extensively carried out as yet. However, the preliminary work so far done seems to indicate that T. 1824 behaves like Trypan blue, according to the careful studies of Ludford.[1] The cancer cell would be surrounded rather than penetrated by the dye.

The dye itself is without effect on the growth rate of the tumor. As it is known to combine readily with protein materials, an attempt was made to combine it with a toxic substance. Both rattlesnake venom and *Bacillus paratyphosus B.* toxin rapidly lose their toxicity in combination with the dye, but in this combination the dye still localizes in the tumors. In spite of the gross loss of toxicity, there appears to be an effect on the growth rate of the tumor following the intravenous injection of the dye in combination with a toxin.

9235 P

Active Immunity to Experimental Poliomyelitis by Intranasal Route in *Macacus rhesus.**

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We wish to report protection against intranasal infection with subsequent development of immunity in monkeys that had received instillations of pituitrin S and adrephine,† followed by intranasal instillations of potent virus suspensions.

[1] Ludford, R. J., *Proc. Royal Society London*, B, 1929, **104**, 493.

* This work was supported by a grant from the Friedsam Foundation, and the President's Birthday Ball Commission for Infantile Paralysis Research.

† The pituitrin S and adrephine was in part supplied to us through the kindness of Parke, Davis & Company, Detroit, Michigan.

The treatment consisted of instilling ½ cc. of surgical pituitrin into each nostril, followed in 5 minutes by instillation of 1 cc. of adrephine (a preparation consisting of a mixture of adrenalin and ephedrine, with a small amount of chloretone).

One group of 16 animals received preliminary treatments with pituitrin S and adrephine twice a day for 5 and 7 days, followed by 3 daily instillations of 1 cc. of a 5% suspension of virus into each nostril (intranasal test). Of the 16 animals, 6 received the first of the instillations of virus 12 hours after the last treatment; 4 received virus 24 hours, 3 received virus 48 hours, and three, 96 hours after the last treatment. The animals were bled from 18 to 57 days after the intranasal test, and neutralization tests were done on their serums. A second intranasal test was performed on the surviving animals from 3 to 14 weeks later, and the animals surviving this test were inoculated intracerebrally with an infective dose of virus.

A second group of 10 animals received the instillations of pituitrin S and adrephine daily, followed in 4 or 6 hours by instillations of virus. Eight of the 10 animals received a second treatment with pituitrin S and adrephine, 4 hours after the virus. These daily treatments and instillations of virus were continued for 20 to 28 days. The surviving animals were bled and neutralization tests done on their serums. Then each animal received an instillation of one cc. of virus into each nostril on 3 successive days without pituitrin S and adrephine. The survivors of this intranasal test were then inoculated intracerebrally with a paralyzing dose of virus.

Eight of 10 of the 16 animals in the first group that received virus 12 and 24 hours after treatment survived the intranasal test and 2 succumbed to the experimental disease. Of the 6 animals that had received the virus 48 and 96 hours after treatment, one monkey, that had received virus 48 hours after treatment, survived, and 5 succumbed. Eight animals survived a second intranasal test. One animal died 25 days after the second intranasal test. Six of the 8 surviving animals neutralized the virus and 2 failed to neutralize. The 8 survivors were given the intracerebral test (.01 cc. of a 5% suspension of potent virus in 1 cc. of saline). Of the 8, two survived and 6 succumbed. One of those that succumbed came down after a prolonged incubation period (27 days), and a second animal developed a mild form of the disease and recovered.

Of the 10 animals that had received daily instillations of both pituitrin S, adrephine, and virus (second group), 6 survived daily treatments and exposure to virus and 4 succumbed to the disease within the incubation period of the virus. Neutralization tests were performed on the serums of these 6 animals. The serums of 3

neutralized the virus; one partially neutralized and 2 failed to neutralize. Five of these 6 animals survived the intracerebral test.

Of the 26 normal controls employed in the intranasal tests, 25 succumbed. Of the 11 normal controls employed in the neutralization tests, all succumbed, as did the 13 normal controls used in the intracerebral tests.

Histologic study of the mucous membranes of the animals treated with pituitrin S and adrephine shows an extensive infiltration of the superficial and deep layers of the mucosa with eosinophiles. Some scattered eosinophiles are present in the submucosa, otherwise the mucous membranes appear normal. Mucous membranes obtained from normal untreated animals do not show eosinophilic infiltrations.

It appears from the results of these experiments, that not only do the majority of the animals develop appreciable protection against intranasal instillations of potent virus, but an appreciable number of these animals, particularly those included in the second group that had received prolonged daily treatments with pituitrin S and adrephine, as well as virus, developed an active immunity as indicated by the neutralization and intracerebral tests. These results help to bring out even a greater similarity between the experimental and the human disease, and perhaps serve to support the epidemiologic concept of the mechanism involved in the production of a widespread immunity to the disease in the normal population.[1] We do not attempt to explain the significance of the eosinophilic infiltration (or "barrier") in the mucous membranes of the treated animals.

9236 P

Effect of Pancreatic Tissue Extract on Cholesterol of Blood in Cardiovascular Arteriosclerosis.* †

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Pancreatic tissue extracts have been studied since 1908 by various investigators. Since 1929, insulin-free extracts have been investigated and independently prepared by Frey, Gley, Kraut, Kisthinios, Vaquez, and Wolffe. Wolffe and his associates reported that their

[1] Kramer, S. D., *J. A. M. A.*, 1932, **99**, 1048.

* Aided by a grant from the Lucius N. Littauer Foundation.

† I wish to thank Dr. Edward P. Flood, Director of Medicine, and Dr. William Aronson, Director of Laboratories, for their assistance and cooperation.