Cantwell even though much weaker solutions were used in our experiments. Their explanation, that the depressant action is probably due to a decrease in pH was checked and found to hold also for the present work. Alanine and phenylalanine exerted only a slight temporary depressing action despite the fact that the molar concentration of the alanine was much higher than that of either aspartic or glutamic acid.

Since the bacterial cultures used in the previous work by Weinstein and Rettger were alkaline in reaction and the amino acids, when neutralized, had no effect on intestinal motility, as judged by experiments of the type here described, it must be concluded that the depressant action of the bacterial cultures was not due to their content of amino acids.

8681 P

Effect of Tannic Acid on Intranasal Infection with Pneumococci.

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It has been shown[1, 2] that certain viruses are infective for mice when instilled into the nares and that this capacity to invade the body of the mouse through the mucosa of the nose can be greatly diminished by preliminary intranasal treatments with tannic acid or alum-solutions. It has also been shown[3] that certain strains of pneumococci are infective for mice by the nasal route and that, under the conditions of the experiment, invasion takes place both through the nasal mucosa and through the pulmonary alveolar walls.[4]

The present experiments were planned to test whether this nasal infectivity of pneumococcus strains is modified by preliminary tannic-acid treatments. For this purpose, 5 experiments were undertaken using groups of young (3 to 6 weeks old) Swiss mice. In each experiment 15 mice were treated with weak solutions of tannic acid for 3 or 4 days before the infecting inoculation was

1 Olitsky, P. K., and Cox, H. R., *Science*, 1934, **80**, 566.

2 Armstrong, C., *Pub. Health Rep.*, 1935, **50**, 43.

3 Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1935, **58**, 465.

4 Rake, G., *J. Exp. Med.*, 1936, **63**, 191.

made. In 2 cases, 0.7 or 1.0% solution of tannic acid in normal saline was used and in the other 3, 0.8% solutions of tannic acid in 1.0% glycerol. In the first test, 7 preliminary treatments, each of 0.03 cc. of the tannic acid, were given intranasally; in the second, 9, in the third, 6, and in the last 2, 8 similar treatments. Fifteen mice remained untreated as controls in each test.

Cultures of strains of pneumococci of both Type III and Type XIX, known to be intranasally virulent, were used. In the past the use of intranasally virulent strains of pneumococci has been attended with the difficulty that this virulence or invasiveness, which is independent of the intraäbdominal virulence is apt to be lost suddenly and without apparent reason during subculture or on animal passage[3] and cannot be regained by any of the means so far adopted. It has now been found, however, (Rake—unpublished) that this intranasal virulence can be maintained over long periods of time in cultures which have been rapidly frozen and dried *in vacuo*. Such cultures have been used in this investigation and in one case the culture was proved to be as virulent intranasally after being frozen and dried for 432 days as it had been prior to freezing and desiccation.

For the investigation 12-hour broth cultures of the heart's blood of a mouse dying from an infection with the strain required were used. Each mouse in both treated and untreated groups of the first 3 tests received 0.03 cc. of undiluted 12-hour culture in pneumococcal broth at pH 7.8. In the other tests the culture was centrifuged rapidly to throw out most of the organisms which were then resuspended in ⅓ the volume of broth. Only 0.01 cc. of this was given intranasally to each mouse to avoid, as far as possible, flow to and absorption through the lungs. The mice were observed, the times of death noted, and the cause of death verified by autopsy and culture.

No significant difference could be shown between treated and untreated groups. Thus, in Experiment 1, using Type XIX, 10 of 15 treated mice and 9 of 16 controls died. In 2 other experiments with a Type III culture, 7 of 15 and 9 of 14 treated mice died, as compared with 8 of 15 and 10 of 15 controls. In the last 2 tests using smaller amounts of Type III culture, 9 of 30 treated mice died, as compared with 13 of 33 controls.

It was also possible to show by use of the technic already described[4] that there was no demonstrable difference in the speed with which invasion of the blood-stream occurred in the treated and untreated groups of mice. In both groups invasion occurred within a very few minutes.

It seems, therefore, that the tannic-acid treatment, which is believed to act principally on the mucosa[1] of the nose, does not influence in any way the course of at least one intranasal bacterial infection. It should, however, be emphasized that pneumococci are believed to invade the body also through the pulmonary alveoli after intranasal instillation.[4]

8682 P

Concentration and Purification of the Gonadotropic Substance in Urine of Ovariectomized and Post-Menopausal Women.*

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While attempting to concentrate the gonadotropic activity of the urine of post-menopausal (or castrate) women, it has been found that the active material may be practically quantitatively concentrated into relatively non-toxic extracts by the use of tannic acid. The method developed for the precipitation and partial purification of the gonadotropic material is as follows.

The chloroform-preserved urine is chilled, siphoned from any sediment and acidified to pH 5 with acetic acid. For each liter of urine, 20 cc. of a freshly prepared aqueous 10% tannic acid solution are added. An immediate precipitate forms. After thorough mixing, the precipitate is allowed to settle in the cold room and is then collected at the centrifuge. The bulky precipitate, which contains considerable water, is extracted once with at least 5 parts of 95% ethyl alcohol followed by 3-4 extractions with 80% alcohol. Alcohol is removed by several washings with acetone and the residue is freed of acetone by reduced pressure. In addition to removing considerable pigment and other inert material, the alcohol-acetone treatment removes any estrogenic substances which may be present.

The resultant powder (100 to 200 mg. per liter of urine) con-

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