

13th day and also after it was discontinued. Estrin in oil is poorly absorbed when injected subcutaneously.<sup>11</sup> To avoid this all the injections were made intramuscularly.

The 30 adrenalectomized controls, 10 of which received  $\frac{1}{4}$  cc of sesame oil daily, began to die on the seventh day after the second adrenal had been removed. The numbers alive 5, 10, 15, 20, and 25 days post-adrenalectomy were 30, 24, 11, 8, and 7 respectively. Removal of the adrenals in 2 stages was a factor in prolonging life. Also, different colonies of rats differ in the number of animals dying after adrenalectomy.<sup>10</sup> Our colony seems to be more resistant to the effects of adrenalectomy now than it was a few years ago; and our growth curve for normal rats is now greater (Fig. 1) than it was in 1936.<sup>9</sup>

*Summary.* Our results confirm previous reports that progesterone will keep adrenalectomized rats alive.<sup>1, 2, 12</sup> In our colony, after 2-stage adrenalectomy, doses of one milligram daily or less were usually adequate to keep young, newly-weaned, female rats alive and showing some gain in weight throughout the treatment period.

### 10425

#### Analysis of Present Methods of Collecting Tubercle Bacilli from Urine.\*

HARRY A. FELDMAN AND JOHN H. HANKS. (Introduced by Leland W. Parr.)

*From the Department of Bacteriology, Hygiene and Preventive Medicine, The George Washington University School of Medicine, Washington, D. C.*

In a study on the concentration of tubercle bacilli from urine<sup>1</sup> two standard procedures were used as controls: Petroff's tannic-acid method<sup>†</sup> and direct centrifugation for one hour. Since the detailed

<sup>10</sup> Gaunt, Robert, *Am. J. Physiol.*, 1932, **103**, 494.

<sup>11</sup> Deanesly, R., and Parkes, A. S., *J. Physiol.*, 1933, **78**, 155.

<sup>12</sup> Fischer, A., and Engel, M., *Rev. franc. d'endocrin.*, 1938, **16**, 400.

\* Aided by a grant from the U. S. Public Health Service.

<sup>1</sup> Hanks, J. H., and Feldman, H. A., to be published.

<sup>†</sup> *Petroff's Tannic Acid Method* (Trudeau Routine Laboratory Procedure):

1. Acidify urine with few drops of 30% acetic acid. To each 1000 cc of urine add 2 cc of 5% tannic acid. Store urine in ice-box 24 hours. Decant supernate and centrifuge sediment for 2 minutes at top speed. (In our experience, centrifugation for 5-10 minutes was required to pack the sediment.)

2. Decant supernate and dissolve sediment with N NaOH. Digest and add H<sub>2</sub>O to fill the tube, centrifuge and decant the supernate again. Spread the sediment over clean slides.

3. Add a drop of N HCl and fix the smear with heat.

study of newer methods will not appear for some time, and since the results of one of the present technics are definitely improved by simplification, a brief analysis of this procedure is desirable.

Using Breed's counting method, the 2 procedures were analyzed quantitatively for their ability to collect human tubercle bacilli which had been added from "clump-free" suspensions to clear normal urine in predetermined numbers. The results were expressed as "concentration-factors" which indicated the increase in the average number of bacilli per microscopic field following concentration.

The concentration-factors were determined for direct centrifugation, for each of the steps outlined below for the Petroff procedure, and for a procedure which duplicated Petroff's, except for the omission of the tannic acid. Two methods—milk and HCl—for "fixing" the sediments to the glass slides were compared. The results shown in Fig. 1 are average values from 4 experiments in which different concentrations of bacilli were added to the urines. There were no exceptions to the general ratings as illustrated.

The results in the chart illustrate several useful facts: (a) The Petroff method appears to depend on the spontaneous precipitation of urates from acidified, chilled urine rather than on the use of tannic acid. It could be designated more properly as a "urate" concentration-method. (b) The first "urate" (or tannic acid) sediment produces a higher concentration-factor than is obtained by direct centrifugation. (c) The remainder of the Petroff procedure results

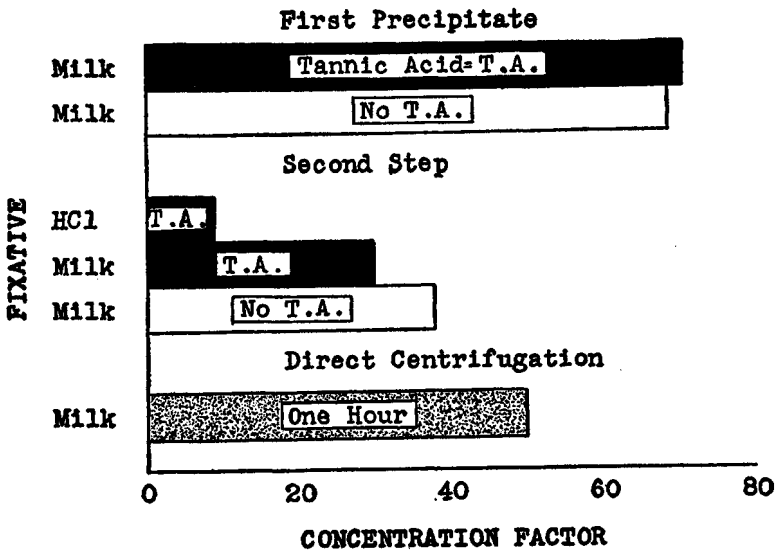


FIG. 1.

in a loss of the majority of the bacilli which were collected in the first step by precipitation. (d) Slide losses are reduced by the use of milk as a fixative.

These findings, in extension of our previous work,<sup>2</sup> demonstrate again that tubercle bacilli cannot be collected efficiently by direct centrifugation. Although the urate sediments in the first stage are extremely bulky, they contain more bacilli per unit volume than the very slight sediments obtained by direct centrifugation. For purposes of guinea pig inoculation or of cultivation, it should be noted that the total bacillary content of the urate sediments exceeds those from centrifugation by approximately one hundred times.

To wash, or to dissolve and recentrifuge a sediment, once collected by any method, results in a marked diminution of the numbers of bacilli collected.

## 10426

### Attempted Transformation of Rabbit-Fibroma Virus into the Virus of Infectious Myxoma.\*

JEAN SINCLAIR MECK AND ELLEN GRAY ACREE. (Introduced by Leland W. Parr.)

*From the Department of Bacteriology, Hygiene and Preventive Medicine, School of Medicine, The George Washington University, Washington, D. C.*

The following experiments were undertaken in an attempt to duplicate the results obtained by Berry and Dedrick,<sup>1</sup> who reported the transformation of fibroma-virus into that of infectious myxoma. The method of procedure followed throughout the experiments was identical with that of the original investigators<sup>2</sup> and consisted in inoculating domestic rabbits with a mixture of active fibroma-virus and heat-inactivated myxoma-virus.

The suspensions of viruses were prepared by grinding 10 to 20 g of virus-bearing tissue with alundum and 100 cc of Locke's solution. Five to 10 minutes before use, the suspension was centrifuged at about 1000rpm. Ten cc ampoules were filled completely with the

<sup>2</sup> Hanks, J. H., Clark, H. F., and Feldman, H., *J. Lab. and Clin. Med.*, 1938, **28**, 736.

\* The work reported in this paper was carried out under the direction of the late Earl B. McKinley.

<sup>1</sup> Berry, G. P., and Dedrick, H. M., *J. Bact.*, 1936, **31**, 50; **32**, 356.

<sup>2</sup> Berry, G. P., personal communication.