it was 76.77 days. When the difference between the means is tested by the t-Test,[4, 5, 6]§ this difference of 11.5 days is highly significant, the probability of such a difference occurring by chance alone being less than 0.0001. The effect of fluorine on the life span of the rats thus appears to be real.

Summary. Fluorine, when added to a rachitogenic diet, appears to increase the life span of rats.

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Studies on Purified Tuberculin Prepared from Bacterial Bodies.

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Recent attempts[1, 2] to increase the yield of synthetic-medium tuberculin have been based primarily on increasing the growth-rate of the bacilli and on increasing the number of bacilli per unit-volume of medium. While admitting the importance of these factors it is generally agreed that further improvement along these lines is unlikely. A new approach made in this laboratory consists of increasing the production of tuberculin by the creation of an optimal condition in the medium favoring rapid cell-autolysis after maximal growth of organisms has been attained. Some of these findings have been reported.[3] Since the tuberculin thus obtained consists principally of bacillary proteins it seems of importance to compare the potency of purified tuberculin prepared from relatively pure bacillary proteins with that prepared from the soluble parts of the whole culture which is generally assumed to consist of metabolic products in addition to the bacillary proteins. On the other hand it

4 Fischer, R. A., *Statistical Methods for Research Workers*, 5th Edition, pp. 120-125, 1934, Oliver & Boyd, London.

5 Tippett, L. H. C., *The Methods of Statistics*, 2nd Edition, pp. 112-116, 1937, Williams & Norgate, Ltd., London.

6 Yule, G. Y., and Kendall, M. G., *Introduction to the Theory of Statistics*, 11th Edition, pp. 438-443, 1937, Charles Griffin & Company, Ltd., London.

§ A complete discussion of this test for determining the significance of the difference between two means is given in these references.

1 White, W. C., *Am. Rev. Tuberc.*, 1934, **30**, 707.

2 Wong, S. C., and Weinzirl, J., *Ibid.*, 1936, **33**, 577.

3 Wong, S. C., *J. Bact.*, 1937, **33**, 451.

appears also of interest to determine the time required for complete extraction of the active cellular protein.

Methods. Preliminary trials have shown that autolysis of organisms was more rapid in dilute alkali than in the culture medium. In the present investigation studies were made exclusively on bacterial cells. Virulent *Mycobacterium tuberculosis,* H37, was grown for 4 weeks in liter Erlenmeyer flasks containing 300 ml of a malic-acid synthetic medium.[2] At the end of this period the fluid was decanted from each flask after which it was replenished with half of the original volume of sterile 0.05 N NaOH. For controls sterile distilled water was added to some of the flasks. All the flasks were reincubated at 37°C and the contents thoroughly shaken each day. The state of cellular disintegration was followed by examination of smears stained by the Ziehl-Neelsen technic every other day. When a majority of the bacilli had been found to have autolyzed each flask was tested for presence of contamination by culturing the fluid on blood-agar, pH 7.6, for 48 hours. The pH of 10 flasks was adjusted to 6.0 to 6.5 with 1-N HCl before heating at 100°C for 3 hours. The preparation will be designated as tuberculin A. Ten other flasks were heated without adjustment of pH and will be known as tuberculin B. The details of the preparation of purified tuberculin will be described elsewhere. Essentially the method is similar to that of Seibert's for PPD[4] with the exception that glycerol and dialysis in collodion sacs were omitted. Total nitrogen was determined by the micro-Kjeldahl method. The biological activities of both preparations were compared on human subjects with a PPD preparation kindly supplied to us by Dr. Florence Seibert. Only the first strength of the Mantoux test consisting of 0.00002 mg in 0.1 ml was compared. To rule out personal factors dilutions of all the tuberculins were done by one of us and issued to the other with different labels. The key was later supplied. Readings were made 48 hours after the injection by one of us but sometimes by a nurse. Injections were made on the dorsal surface of each forearm in approximately the same position. New tuberculin-syringes were used throughout this study.

Properties. Both preparations are light powders and very soluble in concentrations of 0.5% with the aid of few drops of dilute NaOH. The resulting solutions were pale yellow in color. The total nitrogen of tuberculin A was 13.27% and of tuberculin B, 12.96%. Incidentally it might be mentioned that the nitrogen-content of the former is the same as purified tuberculin prepared from the soluble

[4] Seibert, F. B., *Am. Rev. Tuberc.,* 1934, **30**, 713.

parts of whole cultures. Intravenous injections of 15 mg contained in 1.5 ml into guinea pigs weighing 300-350 g produced no harmful effects. Prolonged immunization of rabbits, 150 mg per animal, showed that tuberculin B was non-antigenic while tuberculin A was weakly antigenic. In the latter case only complement-fixing bodies detectable in 1:5000 dilution of the antigen with serum diluted 1:5 was obtained.

Result. The yield of purified tuberculin in both cases was about 0.5 g. In contrast, the yield from the pooled supernate of 20 flasks was only 0.15 g. Autolysis began to appear on the 3d day of incubation and grossly this was manifested by the disappearance of granular forms. On the 14th day few intact cells could be found. On the other hand, stained specimens from control flasks in which distilled water alone was added showed comparatively little cellular disintegration. The heated tuberculin precipitated by trichloracetic acid in both preparations was practically salt-free, 3 to 4 washings being sufficient to render the precipitate free from chloride and sulfate ions. It was found that tuberculin B which had been heated at an alkaline pH was devoid of activity. This is to be expected since it is the classical procedure for hydrolyzing proteins. The result of the comparative biological activities of PPD and of tuberculin A which is prepared with pH adjusted to about 6 before heating is presented in Table I. From the table it is clear that tuberculin A is identical in activity to Seibert's PPD.

Comment. The above findings show 3 points of interest. First, evidence is at hand which seems to identify tuberculin with tuberculoprotein, since purified tuberculin prepared either from the bacillary protein or from the soluble parts of the whole culture presents similar biological, physical, chemical, and antigenic properties. Second, the method is only applicable if the pH of the crude tuberculin is adjusted

TABLE I.
Table Showing Biological Activity of Tuberculin Prepared from Acidified Lysate.

Subject	Readings in mm	
	Seibert's PPD	Tuberculin A
H.T.C.	12 × 8	12 × 12
W.L.M.	13 × 10	14 × 11
F.H.	13 × 10	12 × 10
C.J.L.	15 × 15	15 × 12
C.T.Y.	0	0
L.C.L.	0	0
T.F.C.	17 × 15	13 × 12
L.L.L.	12 × 10	17 × 13
L.C.L.	17 × 15	15 × 12
F.Y.C.	12 × 11	13 × 12

to below 7 previous to heating. Conversely an alkaline pH at high temperature hydrolyses tuberculin and thereby destroys its biological activity. Third, the complete disintegration of the bacilli is a logical method in further increasing the yield of tuberculin, for the number of organisms present in most cultures grown in synthetic media is large.

Conclusion. From the results presented above it is justifiable to conclude that tuberculoprotein is suitable for the production of purified tuberculin and that a greater yield of tuberculin over a short period of time may be obtained if the organisms are treated with dilute alkali.

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Enhancing Action of Egg-Yolk on Virulence of Meningococcus for Mice.

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Nungester and his coworkers[1] found that gastric mucin of the hog exerts a great enhancing action on the virulence of meningococcus in experimental infection of mice. This observation has been widely confirmed and extended to a number of other microörganisms of low virulence. However, as Miller[2] and Nungester, *et al.*,[3] have pointed out that different lots of mucin varied considerably in their activities and that sterilization by excessive heat might decrease their action, difficulties have been met with in our hands to secure a potent product for experimental work. Recently, in our studies on rickettsiæ cultivated in the yolk-sac of the developing chick,[4] it was accidentally noticed that rickettsia in yolk was more virulent than the same organisms contained in other tissues. It was thought that possibly yolk has an enhancing action on virulence similar to that of mucin. In order to test this supposition, meningococcus has been chosen for study and the results are herewith communicated.

1 Nungester, W. J., Wolf, A. A., and Jourdonais, L. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 120.

2 Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1136.

3 Nungester, W. J., Jourdonais, L. F., and Wolf, A. A., *J. Inf. Dis.*, 1936, **59**, 11.

4 Pang, K. H., and Zia, S. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 76.