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**Effect of Fluorine on Life Span of Rachitic Rats.\***

SIDNEY B. FINN† AND MORTON KRAMER.‡ (Introduced by H. C. Hodge.)

*From the School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.*

Although considerable study has been made of the toxic effects of fluorine upon the bones and teeth, little attention has been directed to its rôle in modifying phosphorus metabolism in certain of the deficiency diseases. The effect of fluorine upon the calcification of the long bones of rachitic rats has recently been studied roentgenographically by Morgareidge and Finn.<sup>1</sup> It was found that when rats were fed 300 p.p.m. of fluorine incorporated into a rachitogenic diet (Steenbock & Black 2965)<sup>2</sup> the rickets resulting was markedly less severe as evidenced by the narrower zone of hypertrophic metaphyseal cartilage and by the increased overall density of the tibial bone. With this apparent reduction in the severity of rickets it was interesting to speculate whether the increased density of the bone might be due to formation of a more radiopaque compound with fluorine in the bone or whether there was an actual lessening of the severity of the disease.

This experiment was undertaken to show the effect of fluorine upon the life span of rats fed a rachitogenic diet and to offer additional evidence on the specific effects of fluorine when added to such a diet. Twenty-six albino rats from our own inbred stock were divided equally at weaning age (21 days) into 2 groups. Litter mates and sexes were distributed equally. The first group was fed the rachitogenic diet (Steenbock & Black 2965) consisting of yellow corn 76%, wheat gluten 20%, CaCO<sub>3</sub> 3%, and NaCl 1%. The second group of rats was placed upon a similar ration plus 300 p.p.m. of dietary fluorine. This was incorporated by thoroughly mixing a saturated solution of NH<sub>4</sub>F in 95% alcohol with the dry diet, spreading the wet mixture in thin layers on large sheets of wrapping paper and allowing the alcohol to evaporate. The amounts of inorganic phosphorus in each diet were determined by the colori-

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† Now associated with the Maternity, Infancy and Child Hygiene Division, New York State Department of Health.

‡ Assistant Statistician, New York State Department of Health.

<sup>1</sup> Morgareidge, K., and Finn, S. B., *J. Nutrition*, 1940, **20**, 75.

<sup>2</sup> Steenbock, H., and Black, A., *J. Biol. Chem.*, 1925, **64**, 263.

metric method of Berenblum and Chain<sup>3</sup> and found to be equal, thus precluding any possible conversion of the organic phosphorus to the inorganic form by the action of the  $\text{NH}_4\text{F}$  and alcohol. Since the fluorine ration loses its efficacy on standing, this portion of the experimental diet was made up fresh every few weeks. The rats were given the diets and distilled water *ad lib.* until death occurred. Mottled enamel was observed to occur on the incisors of the rats on the fluorine supplemented diet. The age at death of each of the rats was recorded.

*Findings.* Table I gives the age at death (in days) of each of the rats and reveals that 10 of the rats fed the rachitogenic diet died before they reached the 68th day of life, whereas before that age no death occurred in the group of rats fed the rachitogenic diet supplemented with fluorine. By the end of the 75th day of life none of the rats fed the rachitogenic diet survived, while 9 of the rats fed the fluorine supplemented diet were still living.

The average age at death of the rats fed the rachitogenic diet was 65.23 days while for the rats fed the fluorine supplemented diet

TABLE I.  
The Age at Death (in Days) of Each of the 13 Rats Fed the Rachitogenic Diet and of Each of the 13 Rats Fed the Rachitogenic Diet Supplemented with 300 p.p.m. Fluorine.

	Rachitogenic diet Age at death (in days)	Rachitogenic diet supplemented with fluorine Age at death (in days)
	59	68
	60	73
	63	74
	63	75
	64	76
	64	76
	64	76
	64	78
	66	78
	67	80
	68	80
	71	82
	75	82
	—	—
Mean age at death (days)	65.23 ± 0.81	76.77 ± 0.73
Standard deviation (days)*	4.31 ± 0.57	3.90 ± 0.52

$$t = \frac{76.77 - 65.23}{1.61} = 7.16; \text{degrees of freedom} = 24; P < 0.0001.$$

$$* \text{The standard deviation is computed by the formula } s = \sqrt{\frac{\sum(x - \bar{x})^2}{N - 1}}$$

where  $\bar{x}$  is the mean of the set of observations and  $N$  is the number of observations.

<sup>3</sup> Berenblum, I., and Chain, E., *Biochem. J.*, 1938, **32**, 295.

it was 76.77 days. When the difference between the means is tested by the t-Test,<sup>4, 5, 6</sup> § 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.

SAM C. WONG AND GEORGE OUYANG.

*From the Departments of Bacteriology and Immunology and Public Health, Peiping Union Medical College, Peiping, China.*

Recent attempts<sup>1, 2</sup> 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.<sup>3</sup> 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

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

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

<sup>6</sup> 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.

<sup>1</sup> White, W. C., *Am. Rev. Tuberc.*, 1934, **30**, 707.

<sup>2</sup> Wong, S. C., and Weinzirl, J., *Ibid.*, 1936, **33**, 577.

<sup>3</sup> Wong, S. C., *J. Bact.*, 1937, **33**, 451.