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Preparation of Thyroglobulin.

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The method for preparing thyroglobulin has not been modified since Oswald described the procedure in 1899. He made saline extracts of fresh thyroid glands and precipitated the protein with an equal volume of ammonium sulfate. The process was time consuming since the precipitate had to be washed many times. Any other globulins present were also carried down and contaminated the final product. In attempting to prepare hog thyroglobulin, I found that the final preparation was frequently insoluble in saline or even dilute alkali. It was desirable to develop a more rapid method which would give a product suitable for intravenous injection. This has been accomplished by precipitating the thyroglobulin at the isoelectric point.

The fresh glands may be extracted with 0.1 M sodium acetate and the protein precipitated with dilute acetic acid. It has been found that sodium acetate will remove just as much of the iodine from fresh glands as sodium chloride. The optimum mixture for precipitation can be determined by setting up the following series of tubes:

Tube	1	2	3	4	5	6
cc. H_2O	8.75	8.5	8	7	5	1
cc. 0.1 N acetic acid	0.25	0.5	1	2	4	8

To each tube is added 1 cc. of the sodium-acetate extract. In a few minutes the optimum precipitate can be observed. It has been found that the maximum quantity of iodine is precipitated in the tube giving the greatest quantity of precipitate. For hog thyroglobulin, tube No. 3 gives the best results. Using these proportions, a considerable quantity of extract can be precipitated at one time.

If a soluble preparation is desired, it is necessary to remove the precipitate and redissolve it in a short time. If it is allowed to stand for 24 hours, the protein is denatured and cannot be redissolved. However, after one or 2 hours, considerable precipitate has settled out. Most of the supernatant fluid can be withdrawn and the remainder centrifuged. The precipitate is dissolved in 0.1 M sodium acetate by adding a little NaOH to maintain neutrality. The product

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can be further purified by reprecipitation 2 or 3 times. If one is not interested in preparing a soluble preparation, the yield can be greatly increased by allowing a longer time for precipitation with the acetic acid.

This procedure has several advantages over that of Oswald. The time is greatly reduced since no filtering or washing is necessary. The hemoglobin is not precipitated as it is with ammonium sulfate. The dilution 10 times and the slow formation of the precipitate allow many impurities to remain in solution. The cost is negligible even on a large scale. The isoelectric point is being used to purify thyroglobulin.

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Colony Forms of B. Paratyphosus B as Related to Variations in Gas Production.

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It is the purpose of this paper to describe variations in colony form and gas production that occurred in a stock culture of *B. paratyphosus B.* and particularly to emphasize a lack of correlation between these 2 properties.

Variations in properties of organisms of the paratyphoid group have been the subject of numerous studies. Jordan[1] has investigated colony form and its relation to virulence. Variation in the immunological properties of this group has been studied by the English workers.[2] Biochemical variations, particularly the failure of certain strains to form gas from carbohydrates was noted by Oette.[3] Herrmann[4] more recently isolated 16 strains which failed to form gas from dextrose and other fermentable carbohydrates.

During the course of experimental work irregular results in gas production from dextrose by a stock strain of *B. paratyphosus B.* led us to plate out the culture. The colonies were predominantly of the R type with a scattering of the S variety. A preliminary study of cultures of the S and R varieties with respect to their ability to form gas, resulted in subdividing both the R and S forms into

[1] Jordan, *J. Am. Med. Assn.*, 1926, **86**, 177.

[2] White, P. Bruce, A System of Bacteriology, London, 1929, V. 4.

[3] Oette, *Centralbl. f. Bakteriol.*, I. O., 1913, **68**, 1.

[4] Herrmann, *Centralbl. f. Bakteriol.*, I. O., 1929, **113**, 108.