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8598 C

Biological Study of "R" and "S" Forms of Chromogenic Acid-Fast Bacillus from Human Leprous Lesion.

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A great deal has been written concerning the various strains of bacilli isolated from leprous patients. Of the many strains, 2 or 3 have most consistently been isolated by investigators. The 3 most commonly cultivated from the leprous lesion are: the organism described by Clegg,[1] which is an acid-fast chromogenic bacillus; Kedrowski's[2] non-acid-fast diphtheroid; and a bacillus isolated by Duval[3] which is permanently acid-fast and maintains *in vitro* the tinctorial and cultural characteristics of the Hansen bacillus of the tissues. At the present time, however, it is almost universally believed that an acid-fast bacillus, which is found in the leprous nodule, is responsible for leprosy.

In spite of the large amount of research on the artificial cultivation of the leprosy bacillus and the variety of forms exhibited by this bacillus comparatively little concerns rough and smooth forms.[4] With this in mind attempts were made to isolate these variants and study them biologically with the purpose of determining cultural and morphological differences, rate of growth and eventually any difference in pathogenicity.

An acid-fast bacillus isolated from a subcutaneous leprous nodule and cultivated according to the method of Duval[5] grew readily upon

1 Clegg, M. T., *Philippine J. Sc.*, 1909, **4**, 77; *ibid.*, 403.

2 Kedrowski, W., *Z. f. Hyg. u. Infektionkr.*, 1901, **37**, 52.

3 Duval, C. W., *J. Exp. Med.*, 1910, **12**, 649.

4 Salle, A. J., *J. Infect. Dis.*, 1934, **54**, 347.

5 Duval, C. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 498.

the ordinary nutrient media, having successfully passed through the transitional period of *in vitro* adaptation. The medium used was glycerol-agar. The Hansen bacillus, after its adaptation to artificial cultivation, is a gram-positive acid-fast rod. The bacilli varied in size and shape, some were short and plump with rounded ends while others were somewhat slender and slightly curved. The distribution of the chromatin varied; some bacilli showed uniform staining, others large bipolar granules, still others small granules or beading, numbering from 2 to 5 and distributed evenly along the entire cell. Growth was visible after 3-4 days at 37.5° and resembled that of the tubercle bacillus in that it was irregular and dull yellow with a wrinkled, dry surface. During its adaptation on special media, however, the growth was moist, pale yellow with a smooth, glistening surface.

A wrinkled culture was streaked several times on glycerol-agar and within 3-4 days many isolated colonies were visible. These were transferred to other plates and subcultured. All colonies were flat with an irregular outline and a dull, wrinkled surface. After 6 subculturings, all colonies had the same characteristics already described. When incubated 6-7 days, there appeared very small, convex, moist colonies somewhat clear and honey-like in color and consistency. These were seen in and about the wrinkled colonies. Several that were separated from the rough colonies were carefully transplanted to glycerol-agar plates and incubated at 37.5° for 6-7 days. Except for a few rough colonies the entire plate showed the same characteristics of the smooth form. Several isolated colonies were again transferred to fresh medium and a pure culture of the "S" form was obtained. An isolated rough colony was carefully replated several times and after incubation for several days no smooth colonies could be seen. These variants remained colonially pure. The "R" form grew more rapidly than the "S."

Tinctorially, the majority of the "S" bacilli were gram-positive slender rods accompanied by a few pale-staining smaller bacilli which appeared gram-negative. Some were slightly curved, others contained small granules varying in number from 2-5 in a cell, still others showed only bipolar staining. The "R" form showed a predominance of gram-positive, short, plump rods. No small granules could be seen. Either the rods were solidly stained or only bipolar staining was present. The bipolar staining was not like that seen in the "S" form. Here the chromatin extended almost to the center of the cell, diplo-bacillary in form, leaving only a faint separation between the 2 large granules. If the light is cut

down, there can be seen about most of the bacilli a definite clear zone not unlike a capsule. While this was present in the "S" form, the halo in the "R" form was definitely much thicker and more conspicuous. With the Ziehl-Neelsen stain the "S" form showed about equal numbers of acid-fast and non-acid-fast rods. The acid-fast rods were slender bacilli, some contained 2-5 small granules others had small bipolar granules while some were uniformly stained. The non-acid-fast rods appeared shorter and stained uniformly. The "R" form showed a predominance of short, plump, acid-fast rods which had large bipolar granules. No small granules were seen in the "R" form. Loeffler's methylene blue rendered the halo about the "R" form more clear and prominent.

Culturally the "R" form is a spreading, flat irregular colony with a dry, wrinkled surface. There is a fairly good growth after 4 days. The "S" form grows more slowly. In about 6-7 days a small circular, convex colony with a smooth, moist, surface becomes visible. If the cultures are allowed to stand at room-temperature for 2 weeks, the growth, especially of the "R" form becomes a deeper yellow. Saline suspensions of the "R" form are granular and flaky, the growth adhering to the sides of the tube; the "S" form yields a uniformly turbid suspension. Both types were subjected to 53°C. for 5 and then for 10 minutes, repeating for every 5 degrees up to 73°. The "R" survived a temperature of 68° after 5 and 10 minutes while the "S" showed presence of growth on glycerol agar plate up to 63°.

Summary. The "R" and "S" forms of the lepra bacillus show definite biological differences. The "S" form is a smooth and moist colony while the "R" form is wrinkled and dry. The "R" form grows the more rapidly, forms a flaky suspension and is more thermostable. After several months of subculturing, both forms remained pure. The "S" form are acid-fast rods the majority of which contain 2-5 small granules. The "R" form are short rods showing a predominance of large bipolar granules and surrounded by a thicker capsular zone. The significance of the small granules in the "S" form and the large polar granules in the "R" form is not fully understood. It is reasonable to believe that the small granules in the "S" form are signs of degeneration while the large granules in the "R" form may represent a stage of quiescence resembling a spore stage. The presence of the much thickened capsular zone and the higher thermal death-point in the "R" form may have some relation to its stability and resistance to disintegration.

Since the "S" form reverts to the "R" during its adaptation on artificial media and the "S" variant can later be isolated from the "R" it must be assumed the "S" and "R" are reversible. The "S" being the typical and the "R" the mutant form.

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Enzymatic Digestion of Desiccated Thyroid.

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In the course of studies on the calorigenic activity of various fractions derived from the enzymatic digestion of desiccated thyroid,[1] we have observed that the organic iodine in the gland is rapidly split into acid soluble and acid insoluble iodine fractions. The 2 fractions are separated by adjusting the pH to 5.0.

A single large lot of desiccated thyroid was used for these experiments (Lot No. 2*). The desiccated thyroid was insoluble at pH 5 and contained 2% of the total iodine in the form of "preformed inorganic" iodine as determined by the method of Lawson.[2] When like volumes of enzyme digest were precipitated in dilutions of one, 2 and 4 volumes, the ratio of acid soluble iodine to total iodine was not increased, indicating that the acid insoluble iodine fraction has a negligible solubility at pH 5. The iodine soluble at pH 5 consisted of organic iodine digested off in the acid soluble form and a small amount of "preformed inorganic" iodine. It was thus possible to measure the rate of hydrolysis of desiccated thyroid into acid soluble and acid insoluble iodine fractions, by measuring the ratio of acid soluble to total iodine in the digest.

Incubation of the desiccated thyroid in one and 2% pepsin at pH 2 resulted in a liberation of 55% of the total iodine in an acid soluble form in 4 hours. (The acid soluble fraction is not increased after several months incubation in pepsin solutions.) In

[1] Thompson, W. O., Thompson, P. K., Taylor, S. G., Nadler, S. B., and Dickie, L. F., *J. A. M. A.*, 1935, **104**, 972.

* We are indebted to Dr. D. Klein of the Wilson Laboratories, Chicago, for the desiccated thyroid and enzyme preparations used in these experiments.

[2] Lawson, W., *Biochem. J.*, 1933, **27**, 112.