scribed which, although inactive *in vitro*, are highly effective *in vivo* in certain species of animals.[9] The residual form of streptomycin and crystalline streptomycin also differ quantitatively *in vitro*. The residual form is a more efficient antibacterial agent against the typhoid bacillus *in vitro*, and possesses definite *in vivo* activity as well. Its value as a chemotherapeutic agent, nevertheless, must await further pharmacological and therapeutic tests.

*Summary.* The sensitivity of *E. typhosa* to a residual fraction of streptomycin obtained during the purification process is at least 2 to 5 times greater than to the crystalline $CaCl_2$ double salt of streptomycin. By the Oxford cup plate method, the *E. typhosa-Bc. subtilis* (or *E. coli*) differential ratio of this material is approximately 2.0-3.0. This residual streptomycin is active *in vivo* as well as *in vitro*. Its activity *in vivo* is not as great, however, as might be anticipated from the *in vitro* results.

[9] Meyer, K., Hobby, G. L., and Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 100.

## 15925

## Complement Fixation Studies with Pus Antigen in Granuloma Inguinale.

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Three types of antigens for use in complement fixation tests for granuloma inguinale have been described. Anderson and her associates[1] utilized "capsular substances" obtained by chemical treatment of embryonic yolk cultures of *"Donovania granulomatis."* Dunham and Rake[2] grew a strain obtained from Anderson on yolk-beef heart infusion agar and then on Levinthal's medium, from which they prepared culture antigens. Jennison *et al.*[3] used saline suspensions of Donovan bodies grown in the yolks of fertile eggs.

This report presents data from use of an antigen prepared from pus aspirated from an abscess of Donovan body origin. This abscess was one of 3 which followed an initial lesion of the cervix uteri. The diagnosis of granuloma inguinale of the cervix was established by smear and biopsy.[4]

*Serological Studies.* The pus antigen, used in complement fixation tests for granuloma inguinale, was prepared from pus aspirated from a large fluctuating abscess of the hand. Smears revealed typical Donovan bodies in abundance. No other organism could be demonstrated. The pus was diluted 1:6 with physiological saline solution, shaken thoroughly, and heated at 60°C for 1 hour on 2 successive days. Merthiolate (1:10,000) was added. All tests were negative for organisms which might be expected to grow on the media employed.

The pus antigen was used in complement fixation tests with sera from 25 patients with proven granuloma inguinale, from 14 hospitalized individuals with various infections, other than venereal, from 12 syphilitic patients with no evidence of granuloma inguinale, and from 5 healthy individuals who served as controls.

As shown in Table I, 21 (84%) of the

[1] Anderson, Katherine, Goodpasture, E. W., and De Monbreun, W. A., *J. Exp. Med.*, 1945, **81**, 41.

[2] Dunham, Wolcott, and Rake, Geoffrey, *J. Bact.*, 1946, **51**, 67.

[3] Jennison, David B., Helwig, Elson B., and Milstone, J. H., *Arch. Dermat. and Syph.*, 1947, **55**, 342.

[4] Packer, Henry, Turner, Henry B., and Dulaney, Anna Dean, *J. A. M. A.*, in press.

TABLE I.
Results of Complement Fixation Tests with Antigen Prepared from Pus Containing Donovan Bodies.

| Clinical categories | | No. of patients | C.F. Positive | C.F. Negative |
|---|---|---|---|---|
| Clinical granuloma inguinale, verified by positive smears | Duration of lesions, 6 mo. or longer, with one exception | 25 | 21 | 4* |
| No evidence of clinical granuloma inguinale | Hospital patients, various clinical categories | 14 | 0 | 14 |
| | Syphilitic patients with high Wassermann titers | 12 | 4 | 8 |
| | Healthy individuals, no evidence of disease | 5 | 0 | 5 |

* This group included one patient with a very early discrete lesion, and one whose lesion had been healed for some time.

25 patients with granuloma inguinale gave positive complement fixation tests with the Donovan body antigen. Nineteen of the 21 sera were strongly positive and gave reactions of 3-4+ in dilutions of 1:5 and 1:10. Four sera were positive in a dilution of 1:20 with an antigen dilution of 1:20. One patient with a very early lesion, which was small and discrete, gave a negative reaction in the serum dilution of 1:5. Another patient whose lesions had apparently healed also gave a negative reaction. The highest antibody titers were demonstrated in patients who had been treated but who showed reactivity of lesions after a period of latency. Remissions after treatment and apparent healing occur frequently in this disease.

All of the 14 hospital patients with various types of clinical findings, other than granuloma inguinale, gave negative reactions, as did the 5 healthy individuals who served as controls. Four of the 12 syphilitic patients gave positive reactions with the pus antigen. These patients were under treatment for neurosyphilis, and quantitative Wassermann tests revealed high serum titers. Since one venereal disease (syphilis) was present, the possibility of multiple infection must be recognized even though no history or clinical evidence of granuloma inguinale could be elicited. The paucity of pus antigen prevented more extensive control studies.

Coincident complement fixations for lymphogranuloma venereum and granuloma venereum were carried out with the sera of 13 patients with proven granuloma inguinale and of 6 patients with syphilis. Six of the 13 patients with established granuloma inguinale gave positive reactions for lymphogranuloma venereum in significant serum dilutions. The 7 other sera of this group gave negative reactions, as did the 6 syphilitic sera. These 6 positive reactions for lymphogranuloma venereum were accepted as evidence of multiple infection rather than cross reactivity, on the basis of serum titers. It has been pointed out[5] that a serum titer of 1:40 offers good evidence of infection with the virus of lymphogranuloma venereum. It should be emphasized that the patient from whom the pus for antigen was obtained was serologically negative for this disease and that the Frei test was also negative.

If antigens were available, serological study of granuloma inguinale would undoubtedly afford valuable information. Obviously, cultures of the causative organism would be expected to offer the most dependable source of material for such antigens.

While pus sources of antigens, as used by us, are not practical because of the rare occasions when such material would be available, the sensitivity of this preparation was apparent. Anderson *et al.*[3] found that use of undiluted patient's serum and a 1:10 dilu-

[5] Dulaney, Anna Dean, and Packer, Henry, *J. Immunol.*, 1947, **55**, 53.

tion of the "capsular" antigen gave the best results in complement fixation tests. Direct comparison is impossible because of the differences in antigens and technics, but it would appear that tests carried out in this laboratory using serum dilutions of 1:5 to 1:20 and antigen dilutions of 1:10 (1:60 dilution of pus) and 1:20 (1:120 dilution of pus) gave even more sensitive antigen-antibody reactions.

Additional evidence of the sensitivity of the pus antigen is offered by the following incident. A blood specimen, sent to our laboratory, carried the request for a complement fixation test for "granuloma inguinale." Even though it was believed that a mistake had been made and that the intention was to request an examination for "lymphogranuloma inguinale" (venereum), the serum was tested with the Donovan body antigen. The serum gave a 4+ reaction in a dilution of 1:20. Inquiry revealed that the patient had a perianal ulcerative lesion. A request was made for smears from this lesion, and abundant Donovan bodies were demonstrated. In this instance the patient gave a typical clinical picture of lymphogranuloma venereum with bilateral inguinal buboes and a positive Frei test; the granuloma inguinale had not been recognized.

*Conclusion.* An antigen prepared from pus aspirated from a metastatic abscess containing Donovan bodies yielded highly sensitive and specific serological evidence of granuloma inguinale when used in complement fixation tests. Twenty-one (84%) of 25 patients with established granuloma inguinale (positive smears) gave positive reactions.

## 15926 P

## Enhancement of Diabetes Produced by Adrenocorticotropic Hormone in Rats Maintained on a Carbohydrate Free Diet.*

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*Introduction and Methods.* It has been reported by Ingle, Li and Evans[1] that pure adrenocorticotropic hormone produces glycosuria and increases nitrogen excretion in normal rats forced fed a high carbohydrate diet. Subsequent work from this laboratory has shown that adrenocorticotropic hormone enhances the glycosuria and nitrogen excretion of diabetic rats maintained on a diet containing approximately 52% preformed carbohydrate. It should be noted that the amount of glucose in the urine in the above experiments was less than the ingested preformed carbohydrate, so that gluconeogenesis did not have to be invoked to account for the glycosuria. It therefore seemed that a more severe test of the diabetes-enhancing activity of adrenocorticotropic hormone would be furnished by a study of its metabolic effects in diabetic rats maintained on a carbohydrate-free diet. Under such conditions glycosuria could only be accounted for by gluconeogenesis.

In these experiments rats with alloxan-diabetes were used and the methods and

* Supported by grants from the James Foundation of the Medical School and the Research Board of the University of California.

1 Ingle, Dwight J., Choh Hao Li, and Evans, Herbert M., *Endocrinology,* 1946, **39**, 32.

2 Bennett, Leslie L., and Choh Hao Li, in press.