creted may, therefore, be simply a result of the decreased urine formation observed in stressed rats. Further studies are being made to determine the disposition of agents in animals under varying physiological conditions.

These results indicate that information derived from certain pharmacological investigations in normal animals may be misleading when applied to agents intended for use in abnormal physiological states.

Summary. Rats subjected to experimental stress (hind leg ligation) excreted significantly smaller amounts of S^{35} in the urine following administration of S^{35}-prochlorperazine than control animals. Evidence is presented that this decreased excretion may be the result of diminished urine formation in stressed rats.

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Measurement of Streptococcal Antigen Synthesis with Fluorescent Antibody.* (27274)

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Previous reports(1,2) have described the various properties of the system in which group A streptococci regenerated the M protein after having been treated with trypsin to destroy that antigen on the surface of the cells. The present communication describes a technic for observing the rate of antigen synthesis photometrically measured by the amount of adsorbed specific fluorescein-conjugated antibody.

Procedure. Type-specific group A streptococcal antisera were prepared according to the method of Swift *et al.*(3); only the highest titer sera, giving strong precipitin reactions with antigen solutions of 1 mg/ml in 10 minutes, were employed. The type 14 streptococcus and the procedures for trypsinization and subsequent M protein synthesis in an amino acid and peptide reaction mixture were as previously described(2).

The gamma-2 fraction of type-specific antisera was isolated by the cold alcohol method of Cohn *et al.* as described by Deutsch(4) and dissolved in a volume of saline equivalent to the original volume of serum. The globulin fraction was reacted with fluorescein isothiocyanate according to the method of Riggs *et al.*, described by Pearse(5). Unreacted fluorescein isothiocyanate was separated from the conjugated globulin by passage through a column of Sephadex. This gel filtration procedure obviated the usual prolonged dialysis; the fluorescent antibody thus obtained contained no dialyzable fluorescent material. The exact procedure was as follows. To a column of Sephadex G-25 (2 × 32 cm) equilibrated with buffered saline, pH 7.0, was added 25 ml of the reacted globulin-fluorescein-isothiocyanate mixture previously centrifuged to remove traces of denatured protein or particles of dye. The solution was applied to the column and eluted with buffered saline at a rate of 2 ml per minute. The first fluorescent band to be eluted

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was the labeled globulin in a volume of about 30 or 35 ml. The effluent protein was concentrated to the desired volume by dialysis against a 20% buffered saline solution of Carbowax 20-M (Union Carbide Chemicals Co.) at 5°C. The latter compound is a nondialyzable ethylene glycol polymer; 5 volumes of Carbowax solution are sufficient to concentrate a dilute protein solution to one-half the original volume in 12 hours. Since the ionic solutes are freely diffusable, the preparation remains isotonic.‡ The original volume of serum was 6 ml and the conjugated globulin was dissolved in a volume of 24 ml of buffered saline, and frozen in 1 ml fractions.

Measurements of fluorescence were performed with the Aminco-Bowman Spectrophotofluorometer. The visible wave length with a maximum excitation of fluorescein at 450 mμ was chosen in preference to the primary ultraviolet excitation maximum wave length to minimize nonspecific fluorescence due to protein and nucleic acid material in the extracts. Fluorescence was measured at the emission wave length of maximum intensity of 525 mμ.

Application of the technic. Cultures of type 14 streptococci grown at 37°C for 16 hours were trypsinized and washed as previously described(2). Control preparations were prepared in the same manner but trypsin was omitted from the solution. The treated cells were suspended in a buffered reaction mixture capable of supporting M protein synthesis in the absence of cellular multiplication(2). The concentration of the suspensions was adjusted with a Klett-Summerson colorimeter to a density value of 260 units, corresponding to approximately 0.9 mg dry weight per ml of reaction mixture. At defined intervals 5 ml and 20 ml aliquots were removed and chilled rapidly to 0°C. The 20 ml samples were centrifuged and the cells were extracted in 1 ml of HCl-saline solution according to standard serological typing procedures(3). The 5 ml aliquots were centrifuged and the cells suspended in 2 ml of buffered saline. Sufficient fluorescent type-specific antibody (or fluorescent normal rabbit gamma globulin for controls) was added to saturate the system when the maximum amount of M antigen was present (0.2 ml of antibody solution sufficed in the experiment presented). The normal gamma globulin solution contained an amount of fluorescein-conjugated protein equal to the conjugated antibody preparation. The cell suspensions were incubated at 37°C for 30 minutes, centrifuged at 8,000 × *g* for 20 minutes at 0°C and washed twice with cold 10 ml aliquots of saline. The cells were resuspended in 2 ml of 0.1 *M* NaOH for 30 minutes at 37°C and centrifuged at 10,000 × *g* for 30 minutes. The supernatant liquid contained all the fluorescein; no additional specific fluorescent material could be obtained by a second alkali treatment. Aliquots of 1.5 ml of the supernates were adjusted to near neutrality with 0.15 ml of 1 *M* HCl, 1 ml of 1.0 *M* sodium borate buffer, pH 8.5,§ was added to each sample and the volumes were adjusted to 3.0 ml with water.

Fig. 1 demonstrates the relationship of the amount of specifically adsorbed and eluted fluorescent antibody as a function of the amount of M protein antigen resynthesized on the surface of trypsinized streptococcal cells over a 180-minute incubation period. The parallel aliquots of cells taken at the given intervals and extracted with acid to solubilize the M protein were used to compare the intensities of the capillary precipitin reactions (shown in parentheses) with the fluorescence values. The base-line control of nonspecific adsorbed fluorescence was obtained with fluorescein-conjugated normal globulin mixed with untreated cells and was of the approximate magnitude of the adsorbed fluorescence of unincubated trypsinized cells plus specific antibody. The maximum value obtained with normal cells and specific antibody demonstrated that the process of resynthesis was nearly complete in trypsinized cells.

Discussion and conclusion. The amount of specific antibody adsorbed by streptococci

‡ During the preparation of this manuscript a note appeared(6) similarly describing this technic of preparing conjugated proteins.

§ The intensity of fluorescence of fluorescein varies with the pH and increases over the alkaline range.

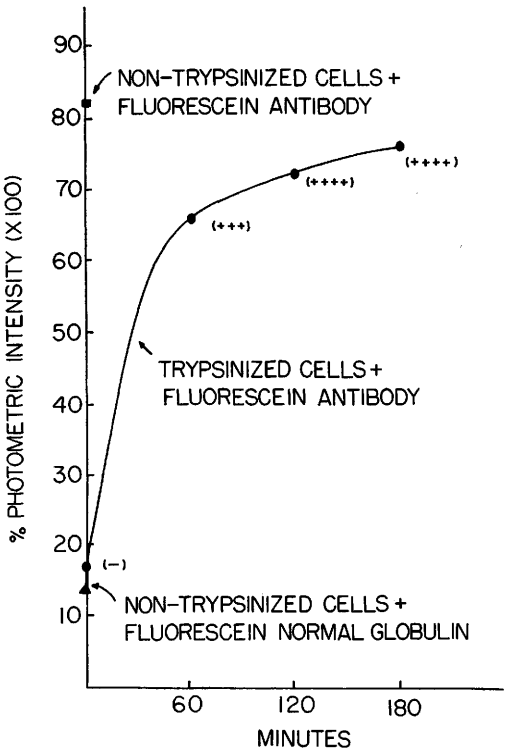

FIG. 1. Intensity of fluorescence of adsorbed and eluted conjugated antibody from streptococci synthesizing M protein for various periods. Figures in parentheses indicate estimated degree of the capillary precipitin reaction from separate aliquots of cells extracted at pH 2.0.

during the process of *de novo* somatic antigen synthesis was a function of the amount of antigen on the cell. The fluorescent technic when compared with acid extraction demonstrated that considerably fewer cells were required to observe the progress of synthesis and, in addition, there was obtained a value more quantitative than the estimation of the degree of precipitation in capillary tubes.

Controls with fluorescein-conjugated normal globulin indicated the degree of nonspecific binding of conjugated protein by the streptococcal cells. The basal limit of nonspecific adsorption was considerably higher when fluorescein-labeled whole sera were employed rather than the globulin fractions.

Standard curves obtained with buffered fluorescein solutions were linear within the total range of the instrument. These values were between 0.02 and 2.0 μg of fluorescein per ml. The fluorescent values of Fig. 1 were within a range of 0.04 to 0.25 μg of fluorescein per ml.

An alternative method proved effective. This was the "sandwich" technic of Weller and Coons(7) in which the cells were first incubated with specific antibody and then reacted with fluorescein-conjugated goat anti-rabbit globulin (Microbiological Associates). However, comparatively large amounts of the latter reagent were required to attain a saturation plateau.

It is conceivable that the aforementioned technics with streptococci may be applicable to other systems where quantitative data are desired with limited amounts of particulate antigenic materials.

Summary. A technic is described for fluorometric estimation of the somatic M antigen of group A streptococci. The amount of fluorescein-conjugated antibody adsorbed and eluted from cells was a function of the amount of antigen present during the process of antigenic protein synthesis.

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