oxidant since *in vitro* experiments[2] have shown that the unsaturated fatty acid esters cause no direct destruction of carotene with the method of feeding employed. It appears that in the absence of α-tocopherol there is a physiological antagonism between unsaturated fatty acids and carotene which results in the inefficient utilization of carotene.

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Immunological Properties of an Antibody Containing a Fluorescent Group.*

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(Introduced by J. F. Enders.)

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Previous investigations involving chemical derivatives of antibodies usually have been planned either to establish the protein nature of the antibody molecule, or to elucidate the influence of specific polar groups on the mechanism of the antigen-antibody reaction. Reiner,[1] however, prepared serologically active atoxyl-azo conjugates of antipneumococcus I and II antibodies, and suggested that they might be useful in quantitative studies of antigen-antibody reactions. Marrack[2] allowed anti-typhoid and anti-cholera sera to react with diazotized benzidine-azo-R-salt, and demonstrated that homologous organisms were specifically colored pink by the chemically modified antibodies.

The objective of the present investigation is the development of a method by which antigenic substances could be revealed in mammalian tissues.

One of us (A.H.C.)‡ has repeated Marrack's experiment with antipneumococcus II and III rabbit sera,§ and has found that sus-

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1 Reiner, L., *Science*, 1930, **72**, 483.

2 Marrack, J., *Nature*, 1934, **133**, 292.

‡ Unpublished data.

§ Lederle's concentrated therapeutic rabbit sera were used in this work. The pneumococcus-antipneumococcus system was chosen for convenience in developing the method.

pensions of these organisms were agglutinated and colored by the specific azo-serum. The color obtained, however, was insufficiently intense to render the method suitable for the purpose in mind.

Conjugates prepared by the interaction of isocyanates of polynuclear aromatic hydrocarbons with several proteins have been shown to be highly fluorescent.[3] Since such fluorescent labels might be easier to distinguish than colored groups, a β-anthryl-carbamido derivative of antipneumococcus III rabbit serum was prepared. Conjugation was obtained in an aqueous-dioxane medium by the interaction of β-anthryl isocyanate with the serum. Experimental conditions were chosen such that a minimum alteration of the protein molecule would occur. The anthracene content of the conjugate, determined by ultraviolet spectrophotometry, was two groups per molecule of protein (taking 160,000 as the molecular weight of the protein). This conjugate gives an optically clear solution in physiological saline in a concentration corresponding to 1/10th that of the original serum. It has a faint blue fluorescence in daylight, and an intense blue fluorescence in ultraviolet light, even in very dilute solution. Specifically precipitated by pneumococcus III carbohydrate, it agglutinates Type III organisms in the same titer as the original serum (1/800), fixes complement, and passively sensitizes the guinea pig to anaphylactic shock. Parallel opsonocytophagic tests with the anthracene derivative and the original serum showed equal quantitative sensitization of the organisms. These tests, however, do not prove that the antibody molecules themselves are conjugated with the isocyanate, although the quantitative determinations suggest this.

Accordingly the conjugate (diluted 1/50 in terms of the original serum) was mixed in equal proportions with a similar dilution of unaltered Type II antipneumococcus rabbit serum of approximately equal agglutinating titer. Type II pneumococci were added to one aliquot of this mixture, and Type III pneumococci to a second. Agglutination occurred in both tubes. When these two suspensions were illuminated with ultraviolet light in the fluorescence microscope, the clumps of Type III organisms exhibited a bright blue fluorescence. No fluorescence was seen in the Type II clumps. After centrifugation the organisms were washed with 0.9% saline and recentrifuged. Again the Type III organisms showed a bright blue fluorescence macroscopically, whereas the Type II organisms did not. Moreover, when Types II and III organisms were dried on different

[3] Creech, H. J., and Jones, R. N., *J. Am. Chem. Soc.*, 1940, **62**, 1970; *J. Am. Chem. Soc.*, 1941, **63**, 1661, 1670.

parts of the same slide, exposed to the conjugate for 30 minutes, washed in saline and distilled water, and mounted in glycerol, individual Type III organisms could be seen with the fluorescence microscope, whereas the Type II organisms were invisible, although their presence was readily demonstrated at the same focus with visible light. Non-specific adsorption and mechanical occlusion of fluorescent molecules during agglutination would thus seem to be eliminated. Although the isocyanate undoubtedly reacts with other protein molecules in the antibody solution, it seems clear that the antibody molecules also have undergone conjugation without demonstrable impairment of specific function.

Mammalian connective tissue normally exhibits a blue fluorescence which is enhanced by formalin fixation. This particular antibody conjugate, therefore, is inadequate for the demonstration of antigen in tissues, although it might well have other uses. In progress is the preparation of conjugated antibodies in which it is expected fluorescence of a distinctive character will be secured.

Summary. A β-anthryl-carbamido derivative of antipneumococcus III rabbit antibody retains the original immunological properties while rendering Type III pneumococci specifically fluorescent in ultraviolet light.

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Effect of Local Application of Sulfanilamide upon Wound Healing.*

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The efficacy of the sulfonamides in the systemic treatment of certain types of infection has been definitely established. During the past few years several communications[1-6] have described and recom-

* Aided by a grant from the Fluid Research Funds of Yale University School of Medicine.

1 Jaeger, K. H., *Deutsche med. Wchnschr.*, 1936, **62**, 1831.

2 Jensen, N. K., Johnsrud, L. W., and Nelson, M. C., *Surgery*, 1939, **6**, 1.