acid-water, 4:1:5). The material obtained (ca 400 μg) at the last stage of purification was active *in vivo* to stimulate release of TSH at ≤0.1 μg dry weight. Highly purified TRF has no LH- or ACTH-releasing activity. Amino acids present in this preparation are reported.

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Antigenicity of the Peptide Chains Isolated from Human Hemoglobins A and F.* (30064)

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The conflicting reports concerning the antigenicity of the various human hemoglobins led to a study of the antigenicity of the isolated polypeptide components of hemoglobin. Herein is reported the first phase of this investigation.

Employing the isolated polypeptide subunits of the weakly antigenic human hemoglobins(1,2,3,4,5,6), we have observed the production in rabbits of antibodies specific for the α and β and γ polypeptide chains from hemoglobins A and F. These antibodies form insoluble complexes with their specific antigens and with the hemoglobin containing the peptide antigens.

Materials and methods. Each peptide chain emulsified in Freund's adjuvant, was injected subcutaneously into 2 rabbits at 4 sites. An initial injection of 20 mg (total) was followed for 10 weeks by weekly injections of 10 mg amounts. Two months later, the animals were reinjected at monthly intervals with 50 mg of peptide. The animals were bled biweekly.

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The α and β peptides of hemoglobin A were isolated by countercurrent distribution and the amino acid composition determined (7). Each peptide contained less than 1% of one residue of isoleucine. The β peptide was free of α peptide contaminant. The α peptide was associated with 10 to 15% of β peptide. The γ peptide, isolated from purified hemoglobin F by differential solubility, included approximately 25% α peptide. The amount of contamination of the antigen preparation was estimated using simultaneous equations formulated on the basis of the amino acid residues present in the α, β and γ peptides. The peptide content of each antigen was confirmed, using 7 M urea-starch gel electrophoretic fractionation in 0.1 M veronal buffer, pH 8.0, 350 volts for 24 hours. The antigen-antibody reactions were visualized by the Ouchterlony micro-double-diffusion method(8). The isolated peptide antigens were solubilized in rabbit serum. This serum from control (non-injected) rabbits had been previously tested to show that it did not react with the antiserum. All hemoglobins were purified by starch gel electrophoresis and the hemoglobin (as an antigen)

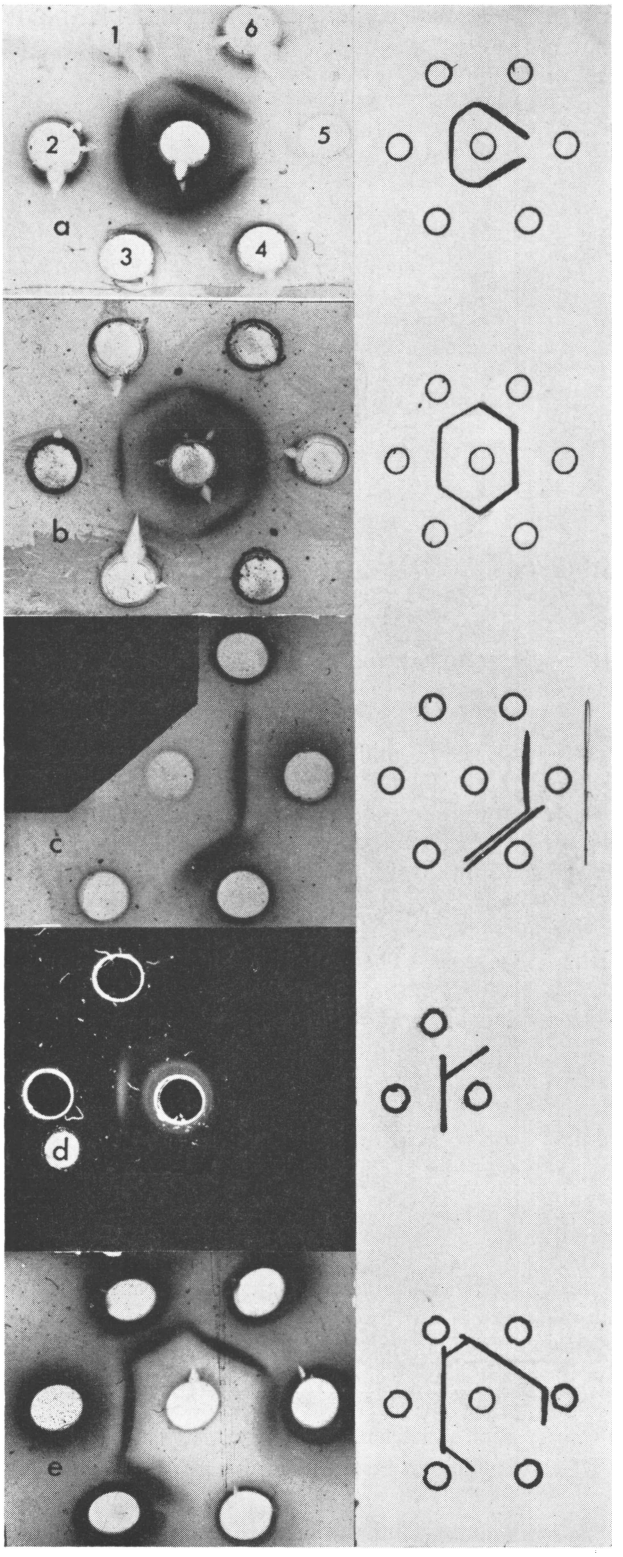

FIG. 1. Precipitin lines formed in the Ouchterlony micro-double-diffusion gel method when the following reactants were tested. The peptide antigen was solubilized in control rabbit serum. Hemoglobin antigen concentration was in the range of 30 to 40 μg per well. All wells numbered as in 1-a.

a. Center well, β antiserum; wells 2, 4, and 6, less than 24 μg of β antigen; well 1, hemoglobin A; well 3, hemoglobin S; well 5, hemoglobin F.

b. Center well, α antiserum; wells 2, 4, and 6, less than 25 μg of α antigen; well 1, hemoglobin A; well 3, hemoglobin S; well 5, hemoglobin F.

c. Center well, hemoglobin F; well 3, control rabbit serum; well 4, γ antiserum; well 5, α antiserum; well 6, β antiserum.

d. Center well, α antiserum; well 1, less than 12 μg β antigen; well 2, less than 25 μg α antigen.

e. Center well, hemoglobin A; wells 1 and 3, β antiserum; wells 2 and 6, α antiserum; well 4, control rabbit serum; well 5, γ antiserum.

within the gel matrix was placed in the Ouchterlony system.

Results. The antibody in the β peptide antiserum (Fig. 1-a) formed a precipitin line of identity with the β antigen, with hemoglobin A, and with hemoglobin S; hemoglobin F (constituted of α and γ peptides only) did not react with the β peptide antiserum, thus demonstrating that β antiserum, unabsorbed, can detect only β peptide antigen either as the isolated peptide or as a part of the hemoglobin molecule.

The α peptide antiserum formed lines of identity (Fig. 1-b) with α antigen and with hemoglobin A, S, and F (all containing α peptides). The same results were obtained using α antiserum from which β antibody had first been removed by absorption with β antigen.

When reacted with hemoglobin F the antiserum to the γ peptide exhibited 2 antibodies (Fig. 1-c), one to the α peptide and one to the γ peptide as demonstrated by the presence of 2 precipitin bands. This is confirmed when the precipitin band resulting from a reaction of α antiserum with hemoglobin F forms a line of identity with the foregoing α antibody. The γ peptide is antigenically distinct and reacts specifically with antibodies directed to this peptide. After the γ antiserum has been absorbed with α antigen, only one precipitin band is seen in the reaction with hemoglobin F and there is no reaction with hemoglobins A and S (containing only α and β antigens).

Since the α antigen contains a small amount of β antigen (a contaminant) the α antiserum contains a small contaminant of β antibody. Two precipitin lines form when α antigen and α antiserum react. One of these, the α antigen-antibody complex forms a heavy line followed by a weak precipitin line, the β complex, which rapidly migrates into the α complex and moves with it thereafter. When this happens the bands which form give the appearance of partial identity (Fig. 1-d) when the isolated antigens or the hemoglobin containing the antigens is reacted with α antiserum. Actually, there is a line of identity of the β reactants which is superimposed upon the line of the α reactants. As the α line continues, it appears to form a spur. This phenomenon then, which simulates a reaction of partial identity, is referable to the contamination of the α component by β peptide. This explanation is confirmed by the fact that a reaction of partial identity did not occur in the reaction of β antiserum with hemoglobin F (Fig. 1-c). The γ peptide, being less soluble, migrates separately from the α peptide and does not exhibit this phenomenon. The simulated partial identity (Fig. 1-d) is not as readily apparent in the reaction with hemoglobin A (Fig. 1-e).

Discussion. Our demonstration of the specific antigenicity of the isolated α and β peptides from hemoglobin A and the γ peptide of hemoglobin F was confirmed by the experiments of Boerma and Huisman(6) in their simultaneous comprehensive study of isolated purified human hemoglobins. By absorption techniques, using a hemoglobin with the appropriate polypeptide chains, antigenic specificity of polypeptide chains was shown. Ovary(9), using α and β peptides supplied by W. Konigsberg, employed guinea pigs to produce antibodies to α and β peptides from human hemoglobin A. Using the passive cutaneous anaphylaxis assay, he determined that these antibodies were specific. In contradistinction, Askonas and Smyth(10) also using peptides supplied by Konigsberg, found only the β peptide and not the α peptide to be antigenic in rabbits.

It is anticipated that the specific antibodies to the α, β, and γ peptides of human hemoglobins A and F will provide an additional tool for the study of antigenic sites and perhaps, in correlation with amino acid sequence studies, another means of studying the evolution of hemoglobin.

Summary. α, β, and γ polypeptide chains isolated from human hemoglobins A and F elicit the production of specific antibodies in rabbits. Antigen-antibody reactions, visualized by the Ouchterlony method, demonstrated that these antibodies form insoluble complexes only with their specific antigens and with the hemoglobins containing the peptide antigens.

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Effect of Triparanol on Synthesis of Squalene and Tetrahymanol by *Tetrahymena pyriformis*.* (30065)

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Triparanol, an inhibitor of cholesterol synthesis in animals, is also an effective inhibitor of growth(1-5) and fatty acid synthesis (4,5) in *Tetrahymena* species. While certain specific sterols and fatty acids reversed the growth-inhibiting effect of triparanol citrate on *T. pyriformis* mating type II, variety 1 (6) a study of the sterols in the inhibited cultures was not made. Pollard *et al*(4) had observed shifts in the concentration of unidentified steroid substances from inhibited *T. pyriformis* W. Shorb(7,8) and Pollard *et al*(4,5) also observed the effect of other growth supplements, acetate, propionate, isobutyrate and α-methyl-*n*-butyrate, on lipid synthesis. The present paper describes studies on the effect of triparanol on some non-saponifiable components of *T. pyriformis*. It also includes a description of some properties of the triterpenoid alcohol, tetrahymanol, the principal component in the non-saponifiable fraction of *Tetrahymena*(9).

Experimental. Stock cultures of *T. pyriformis* were maintained on 1% yeast extract broth containing 10 mg/ml of glucose, sterilized separately and added asceptically. For studies on the isolation and properties of tetrahymanol, cultures of *T. pyriformis* W were grown 5 days in 2 liter batches of proteose peptone broth(10). In one experiment with proteose peptone medium, triparanol citrate was added at 1 μg/ml. For all other triparanol or triparanol citrate inhibition studies, the synthetic medium, growth supplements and the cultivation methods for the W, H and S strains of *T. pyriformis* were the same as those used by Pollard *et al*(5). Other cultural conditions are mentioned in each experiment.

Total lipids were extracted from centrifuged, frozen and thawed cells with chloroform-methanol (2:1, v/v). One aliquot was methylated with BF_3 methylating reagent.‡ This fraction, designated "methylated total lipids," was further partitioned into steroid material and fatty acid methyl esters on a silicic acid column(11), the fatty acid methyl esters and squalene being removed with benzene-hexane solvent (1:1, v/v). The steroid material was eluted with chloroform, followed by methanol. A second aliquot of total lipid was separated on a silicic acid column using chloroform to elute the neutral lipids and methanol to elute the phospholipids(11).

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