

Ltd., South Africa, for generous assistance with hyaluronidase and hyaluronic acid.

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Di- and Mono-Taurobilirubin similar to a Directly Reacting Form of Bilirubin in Serum

IN the course of investigations into the colloidal properties of bilirubin, it was found that inhibition of the diazo-reaction in acid solution is caused by the association of bilirubin with the formation of colloidal solutions of bilirubin. The diazo-reaction at acid pH occurs only if bilirubin remains in the monomolecular state¹.

Bile acids become more soluble after combination with taurin and glycine. The aim of our work was to cause a similar change in the chemical constitution of bilirubin and to combine both free carboxyls of bilirubin with taurin. We used the method described recently for the synthesis of peptides by Boissonnas, Vaughan and Osato². The same method has been used in the synthesis of conjugated bile acids by Bergström, and Norman³. In preparing conjugated bilirubin the experimental conditions were the same as described in that method. We obtained a chemical compound, ditaur bilirubin, which differed from free bilirubin by its solubility in acid solutions. The diazo-reaction at an acid pH was direct. The spectrum of the new compound in dioxan and water was identical with that of free bilirubin. The new pigment gave a positive Gmelin reaction, which is, according to Fischer, typical for bilirubin and other tetra-pyrrolic pigments⁴. The infra-red spectrum of the new pigment confirmed the disappearance of free carboxyl groups as compared with bilirubin. The synthetic pigment changed in water medium, especially when acidified with strong acids, and the yellow colour of the solutions changed into green (possibly taurobiliverdin).

When the experimental conditions for the preparation were slightly changed, two pigments were obtained, one of which, possibly monotaurobilirubin, migrated on paper electrophoresis with half the speed of the other (possibly ditaur bilirubin). The slowly migrating pigment gave a slightly delayed diazo-reaction as compared with the other.

The pigments were separated by paper chromatography with a chloroform/methanol/acetic acid system, and in the same way two pigments in addition to bilirubin were isolated from the serum of a patient with hepato-dystrophy, and also from various specimens of duodenal bile.

In all experiments (using synthetic and natural material) the more polar pigment remained at the starting point and gave a direct diazo-reaction. The

less polar synthetic pigment migrated with a slightly lower R_F than the natural pigment, free bilirubin migrating with the solvent front. As with direct chromatography of serum bilirubin, reversed phase chromatography of synthetic products, according to Cole, Lathe and Billing⁵, yields two pigments besides bilirubin.

The preparation of other similarly substituted bilirubin derivatives has been undertaken. Their chemical properties and elemental analyses will be described elsewhere.

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Production of Opacity on Serum Agar Plates by Some Strains of *Staphylococcus aureus*

IN 1941, one of us (A. J. H. T.) observed that about half the strains of *Staphylococcus aureus* isolated from the skin lesions of impetigo contagiosa had two properties not often seen in strains from other sources, namely, the ability (a) to inhibit the growth of corynebacteria on solid media, and (b) to produce zones of opacity on serum agar plates. In a recent investigation of impetigo in south-east Lancashire, it was found that 80 per cent of strains of *Staph. aureus* belonged to one phage type (Type 71), most members of which inhibited the growth of corynebacteria and were resistant to penicillin¹. More than 95 per cent of the Type 71 strains also produced opacity zones on serum agar plates.

The production of opacity on serum media by *Staph. aureus* has been little investigated, though its occurrence was noted by Woods and Parkin². The zone of opacity extends for 1.0–1.5 mm. beyond the growth and is best seen after 15–18 hr. incubation aerobically at 37° C. The reaction is best demonstrated on infusion broth agar containing 5 per cent fresh horse serum. Substantially the same results are obtained with human serum; but reactions with rabbit serum are rather less frequent and intense. No opacity occurs when the plates are incubated anaerobically or when the infusion agar base is replaced by 'Lemco' broth agar or by 'Oxoid' nutrient agar.

The ability to produce opacity is unevenly distributed among *Staph. aureus* strains. It occurs commonly only among two sorts: Type 71 strains from skin infections and penicillin-resistant Group III strains³ from superficial infections acquired in hospital. Also, it is remarkable that strains producing opacity on serum agar are most uniformly negative to the egg-yolk reaction of Gillespie and