

therefore, between the kinetics of killing and of activation of T4 bacteriophage by urea and the kinetics of protein denaturation by urea, for they exhibit entirely analogous concentration, temperature, and pH dependence.

We conclude that, through a range of values of pH and temperature, urea activates cofactor-requiring phage in a manner similar to its denaturing action—that is, by breaking critical hydrogen bonds in the protein of the phage adsorption organ to produce a new configuration, which is able to form an attachment to the bacterial surface. It seems reasonable to postulate further that tryptophan-like cofactors exert their influence in a similar manner.

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Chenodeoxycholic Acid in Human Blood Serum

For many years chenodeoxycholic acid (3,7-dihydroxycholic acid) was thought to be confined largely to the bile of fowls and to occur only as a minor component of human bile. Recent studies (1) have shown, however, that this bile acid is a prominent constituent of human bile and that it is also one of the principal bile acids in human blood serum (2). After considering the numerous methods for measuring blood cholestes with their highly variable results, which ranged from none at all to values exceeding 100 mg percent, Sobotka (3) concluded that reliable procedures must be preceded by a reasonable amount of evidence that the bile acid being quantitated actually exists in the serum. The present study is designed to furnish some of this necessary evidence and thus to provide a basis for further quantitative studies of the several bile acids in human serum.

An extract of serum was prepared according to methods that have previously been described (4). The procedure employed was as follows: serum was removed from freshly clotted blood, and 25 ml was added dropwise to a mixture of 250 ml of ethanol and 15 ml of a saturated aqueous solution of barium hy-

droxide that contained 1 g per 100 ml of barium acetate. The mixture was heated 10 minutes in a boiling water bath, and the precipitated proteins were removed by filtering through a medium sintered glass funnel. The filtrate was taken to dryness under reduced pressure at 40° to 45°C. The dry residue was stirred with 100 ml of ethyl acetate to which 2.5 ml of a 3.3-percent solution of anhydrous calcium oxide in ethyl acetate had been added. The mixture was heated in a boiling water bath for 2 minutes; it was then centrifuged, and the supernatant was decanted. The ethyl acetate wash was repeated twice more in the same manner, except that the calcium oxide reagent was omitted. The residue was then dissolved in 10 ml of 7N sodium hydroxide and autoclaved for 3 hours at 140°C. After hydrolysis, the alkaline solution was made up to a volume of 50 ml with water, acidified with concentrated hydrochloric acid to a blue reaction with congo red paper (pH less than 3), and extracted four times with 50-ml portions of chloroform. The chloroform extract was concentrated to dryness under reduced pressure, and the residue was taken up in acetone and adjusted to a volume of 1 ml.

Paper chromatography was carried out as described by Sjovall (5) by applying 0.2 ml of the acetone solution to the paper. Solutions of cholic acid (3,7,12-trihydroxycholic acid), deoxycholic acid (3,12-dihydroxycholic acid) and chenodeoxycholic acid in acetone were also applied in amounts ranging from 1 to 40 µg to serve as controls. Antimony trichloride reagent was used for identification as previously described (6). In a system using 20-percent isopropyl ether in heptane, descending for 18 hours, a well-defined spot could be seen that moved at the same rate as the known chenodeoxycholic acid and that also had a purple fluorescence identical with that of the chenodeoxycholic acid control.

By this means chenodeoxycholic acid has been observed repeatedly in both normal and icteric serums. Cholic acid and deoxycholic acid have also been regularly found by employing 60-percent isopropyl ether in heptane. For normal serum it is sometimes necessary to apply larger amounts of the acetone solution in order to detect the minute amounts of bile acid present. If hydrolysis is not done, the bile acids occur as conjugates of glycine and taurine, and a different solvent system is required for chromatography; no free bile acids have been observed in human serum thus far.

In these qualitative studies, no attempt has been made to measure the amounts of bile acids present beyond comparing the size and fluorescent intensity of spots from serum with that of the known amounts of acids that were applied as

controls. By this process, it was observed that cholic acid and chenodeoxycholic acid occur in approximately equal amounts, the former usually predominating in normal serum. Deoxycholic acid occurred in much smaller amounts. Wysocki and associates (7) have noted that an extract of serum dissolved in a mixture of sulfuric and acetic acids had a total absorbancy at 3100 Å that exceeded the values contributed by deoxycholic acid and cholic acid at this wavelength; they concluded that the difference was due to chenodeoxycholic acid. This conclusion is supported by our findings. Their observation that the concentration of chenodeoxycholic acid is approximately 7 times that of cholic acid is probably accounted for by the differences in the methods used (8, 9).

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Lipid Levels in Migrating Birds

Although it is generally known that the body fat content of migratory birds increases greatly at the time of migration, few actual measurements of total lipids have been made. In October 1954, we obtained a sample of birds representing many species that had been killed by flying into obstacles near airport ceilometers. On the nights of 7 and 8 October a large concentration of birds was evidently "riding a cold front" southward when peculiar weather conditions brought about accidents at a number of airports (1). Specimens frozen shortly after death were obtained for us by D. W. Johnston of Mercer University, Macon, Ga., and by Ivan Tomkins at Savannah, Ga. This material has enabled us to compare lipid deposition of birds that were literally "snatched from the air" during migration with previous data that were obtained from premigrant, nonmigrant, and experimental birds. In