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New Colorimetric Method for Determination of Bile Acids in Blood.

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An exhaustive comparative study of the hitherto published methods for the determination of bile acids and salts in the blood demonstrated that they entail a very large loss of these substances by adsorption in the precipitation of the proteins, and in the removal of the bile pigments by charcoal. This fact could have been anticipated, if it is recalled that the bile acids are the most surface-active substances that are present in the blood. We found that by precipitating the proteins with an alcoholic solution of the alkaline $ZnSO_4$-NaOH reagent used in the Hagedorn-Jensen blood sugar method, and by avoiding the use of charcoal, a quantitative recovery (90 to 95 per cent) of bile salts added to blood plasma could be effected. The new color reaction is a modification of the well known Liebermann-Burchard reaction for cholesterol.

The method is as follows: To a mixture consisting of 75 cc. of a freshly prepared 0.45 percent $ZnSO_4$ solution, 15 cc. of 0.1 *N* NaOH solution and 50 cc. of redistilled alcohol, 5 cc. of plasma are added, drop by drop with shaking. The mixture is allowed to stand over night and filtered. The filtrate is evaporated to dryness on the water bath. The protein precipitate is washed on the filter with 50 cc. of boiling water. These hot water washings are used to dissolve out the bile salts from the residue of the alcoholic filtrate, leaving undissolved the bile pigments and some of the lipoids. The filtered aqueous solution is evaporated to dryness, and the residue is washed several times with ether to remove the remaining traces of bile pigments and cholesterol. The remaining dry residue is dissolved in 5 cc. of acetic anhydride, transferred with filtration to a narrow test tube graduated at 5 cc., and 10 drops of the purest concentrated sulfuric acid are added, and the tube shaken. Standards are prepared simultaneously and similarly from known solutions of purified bile salts in acetic anhydride. The clear brown color of the solutions is compared in the usual way in a microcolorimeter after 15 to 20 minutes of standing. By this method from 1 to 10 mg. of added bile salts could be quantitatively recovered from blood,

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with an error of 5 per cent for the larger quantities, the error increasing to 10 per cent for the smallest quantity of 1 mg. The presence of bile salts could not be demonstrated by this method in normal blood, nor in blood drawn from patients without jaundice or liver disease.

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The Behavior of Electrolytes in Valonia.

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The accumulation[1] of strong electrolytes in living cells presents a problem of peculiar interest. An example of this is afforded by *Valonia macrophysa*. The large multinuclear cell of this marine alga consists of a very thin protoplasmic membrane, inside of which is a large central vacuole filled with sap, and outside of which lies a cell wall imbibed with sea water. It is a very striking fact that the sap inside of this very thin film of protoplasm has a concentration of K about forty times as great as that in the sea water which bathes the outside of the film.[2]

This can be explained if we assume that the protoplasm is impermeable[3] (or nearly so) to K^+ and OH^- but that KOH enters in the form of undissociated molecules. This does not require the assumption that molecules of KOH exist as such in the sea water. We assume rather that K^+ and OH^-, striking the surface of the protoplasm, which is presumably non-aqueous, associate to form KOH which then passes through the non-aqueous layer (for the sake of simplicity we will assume for the moment that the entire protoplasm consists of a single non-aqueous layer[4]) and dissociates in the sap inside. Equilibrium will be reached when as many molecules of KOH enter the external surface in unit time as enter the internal surface.

Since the pH value of the sap is about 5.8, while that of the sea water is about 8.2, it is evident that the concentration of OH^- is about 250 times as great in the sea water as in the sap, and that, in consequence, there must be at equilibrium a much higher concentration of K^+ in the sap than in the sea water, to ensure that as many molecules of KOH enter the internal protoplasmic surface, as enter