Bilirubin and the Polarographic Brdicka Reaction

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Bilirubin added to serum suppresses the protein double wave of the polarographic Brdicka reaction. This effect might be due to the blocking of the -SH groups of albumin by bilirubin.

The polarographic Brdicka reaction detecting protein abnormalities has been used in the study of liver diseases which also often cause an increase of serum bilirubin. The purpose of the present work was to investigate the effect of bilirubin-albumin mixture on the Brdicka reaction.

Materials and Methods

The bilirubin-albumin solution containing 16 mg/100 ml of bilirubin and 2.5 g/100 ml of albumin was made according to Shinowara (1). The blank solution without bilirubin contained the same amount of albumin as the test solution. The Brdicka nonfiltrate reaction was carried out as follows (2): 0.1 ml of test/blank solution was added to 1.4 ml of 0.9% (w/v) NaCl solution, and 1.5 ml of 0.25 N NaOH solution was added. The mixture was placed in a water bath (18–20°C) and a sample of 0.1 ml from the mixture was pipetted to 4.0 ml of Brdicka HCO$_3^-$ solution, and polarographed between −0.8 to −1.7 using silver wire as anode. The samples for polarography were taken immediately, and then 10, 20, 30, and 45 min following the addition of NaOH. The height of the second peak of protein double wave was measured. The results which are means of two series are presented in Fig 1.

Results and Discussion

In serum, most of the bilirubin is known to be bound to albumin. During the denaturation, the height of protein double wave increased.

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in polarograms of both test and blank solutions with increasing denaturation time. Also, in both cases an almost constant level was reached—in test solutions after 30 min, and in blank after 20 min. Thus it might be possible that bilirubin suppresses the protein double wave by blocking the -SH groups of albumin. In this connection, it should be mentioned that the second peak of protein double wave is decreased by oleic acid which is thought (3) to be bound also to albumin. The suppressive effect of bilirubin on the polarographic activity of denatured albumin thus might be only a reflection of the binding of bilirubin to albumin. This effect can occur in cases of erythroblastosis.

References