$Hb^{II}$-containing red cells are stained red; $Hb^{III}$-containing cells are eluted and appear as ghosts (Fig. 1). The reaction is not fully understood. Since (*a*) $Hb^{III}$ is not eluted without adding potassium cyanide to the blood; (*b*) both $Hb^{II}$ and $Hb^{III}$ are eluted if hydrogen peroxide is not present in the mixture; (*c*) neither $Hb^{II}$ nor $Hb^{III}$ is eluted without citric acid in the mixture, it seems that peroxidatic or catalatic reaction of hydrogen peroxide with the blood pigment prevents elution by citric acid. The possibility that red cell catalase could play a part was excluded, for acatalatic blood (generously provided by Prof. Takahara (Okayama) and Dr. Lejeune (Lausanne)) behaved like normal blood.

The method is not suitable for the exact estimation of methæmoglobin-levels in blood. However, it gives useful information concerning experimental oxidation and reduction of the blood pigment within the red cells.

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## Bilirubin and Coagulation of Blood

Free bilirubin, dissolved in sodium carbonate, causes, if given intracardially to rabbits, a pronounced lessening of the plasmatic clotting-activity. Among other effects, prolongation of the heparin-tolerance- and recalcification-times (Howell-time) as well as lowering of the factors II and VII occur[1].

We tried to determine if *in vitro* addition of bilirubin to normal human citrated plasma also influences the clotting mechanism[2].

Free bilirubin at physiological *p*H is not very soluble in plasma. Therefore we dissolved bilirubin (50 mg bilirubin 'Homburg') in a sodium carbonate solution (0·185 g in 10 ml. water). This solution was then added to normal plasma (at a ratio of about 1 : 5). Bilirubin- and pure carbonate-plasma (control plasma) were afterwards each dialysed for 24 h, put into barbital buffer-solution *p*H 7·4 and into saline. The *p*H afterwards ranged between 7·1 and 7·5. The bilirubin concentration in the bilirubin plasma figured up to 70 mg per cent; in the control plasma it was less than 1 mg per cent. Excessive, non-albumin-bound, bilirubin was eliminated by centrifugation.

Prior to the test the bilirubin plasma was mixed with normal plasma, resulting in bilirubin concentrations of 10, 20, 30, 40, 50 and 60 mg per cent. The control plasma was added to the same normal plasma in corresponding volume parts. The activity of factors II, V, VII, VIII, IX and X was tested, also the thromboplastin- and recalcification-time as well as antithrombin II and profibrinolysin. The entire number of mixtures tested was 14, that is, bilirubin plasma in normal plasma and control plasma in normal plasma. All reaction-times were measured 10 times, and the results statistically evaluated.

Our results allow comparison between plasmas with and without added bilirubin as regards several qualities of coagulation. The reaction times measured showed no significant differences. Non-conjugated bilirubin remains in our experimental set-up even up to concentrations of 60 mg per cent without provable influence on plasmatic coagulation. Factor V is an exception. This factor displays a slight lessening in activity at concentrations of bilirubin above 20 mg per cent. The prolongation of the reaction-time, however, does not amount to more than 8 per cent.

The differing results of the experiments *in vitro* and those on the living organism demand further clarification. If animal and human plasma do not in principle react differently, one must presume that bilirubin may influence the site of production of certain coagulation factors and thereby cause a weakening of the coagulation potential.

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# IMMUNOLOGY

## Character and Allotypy of an Immune Globulin in Rabbit Colostrum

Rabbit serum $\beta_2$-macroglobulin and its relation to γ-globulin have been previously described[1]. This communication describes the demonstration and characterization of a third immune globulin, found in rabbit colostrum.

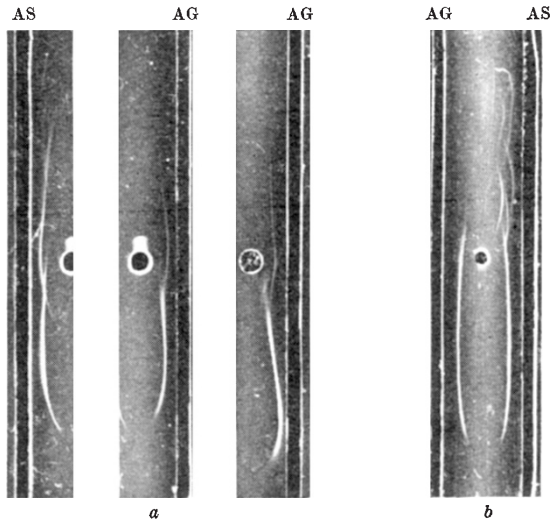


Fig. 1. Immunoelectrophoretic patterns of (*a*) rabbit colostra, (*b*) rabbit serum. Slots were filled with either anti-serum AS (goat anti-rabbit serum globulins) or AG (goat anti-rabbit γ-globulin)

When developed with a highly specific goat anti-serum (AG) to chromatographically purified rabbit γ-globulin, the immunoelectrophoretic pattern of rabbit serum showed only a single γ-globulin arc (Fig. 1*b*). Rabbit colostrum (Fig. 1*a*) gave the γ-globulin arc, but in addition a second arc with the mobility of a fast β-globulin. This immune β-globulin does not, however, precipitate all the anti-γ-globulin, since a forward spur is formed where the two arcs meet. When a less-specific goat anti-serum (AS) to rabbit serum globulins was used to develop the colostral pattern (Fig. 1*a*) the β- and γ-immune globulin arcs crossed; this shows that antibodies specific for the β- and for the γ-immune globulin were present in AS. The β-globulin was evidently present in the rabbit serum globulins used to prepare goat anti-serum AS. When the colostral pattern was developed using anti-serum AG, which had been previously absorbed with either fragment I (ref. 2) of papain hydrolysed γ-globulin or the separated *B* (light) chains[3] of rabbit γ-globulin (Fig. 2), the β-globulin arc did not develop, and only the γ-globulin arc appeared. This shows that the β-immune globulin possesses *B* chains antigenically identical with those of γ-globulin, but lacks other structural components of γ-globulin.

The β-globulin is distinguishable from $\beta_{2M}$ globulin by its faster mobility[1], but might correspond to the third immune globulin, $\beta_{2A}$ globulin, identified in human serum[4], particularly as this serum component has been found to