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The effect of D-penicillamine on the albumin-bilirubin complex

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The effect of D-penicillamine on the albumin-billirubin complex has been investigated. The compound failed in significantly decreasing the free bilirubin level in aqueous albumin or gamma globulin solutions but considerably reduced the dye level in the serum of hyperbilirubinaemic newborns. This finding gives support to the use of D-penicillamine in the treatment of neonatal hyperbilirubinaemia.

When free bilirubin had been shown to diffuse into the brain tissue, hyperbilirubinaemia research has been focussed upon the fate of the albumin-bilirubin complex in serum and it has been suggested that every drug prescribed in the neonatal period and quite especially the compounds applied in the control of hyperbilirubinaemia should be studied for their effect on the said complex [3, 7, 10, 11].

The aim of the present study was to examine the effect of D-penicillamine (DPA), a drug shown to be efficient in lowering the bilirubin level [5], exerted on the albumin-bilirubin complex in hyperbilirubinaemic serum and in solutions of albumin-bilirubin and gammaglobulin-bilirubin.

METHOD

Sephadex G 25 columns measuring 0.6×2.3 cm were prepared. The modified procedure of Chunga and Lardinois [1] was applied using phosphate buffer pH 7.4. A 0.5 ml volume of the substance to be tested was washed with 2.5 ml buffer solution to obtain fraction I containing the large albumin-bilirubin molecule. Subsequent washing with 0.5 ml of 2g/100 ml albumin solution and 1.5 ml of buffer solution yielded fraction II whose middle 1.0 ml volume contained the free bilirubin bound by the albumin of the washing solution. Bilirubin content of the fraction was determined by Mertz's method [9] as modified by Jezerniczky [2] and partly by the procedure of Malloy and Evelyn [8].

Experimental conditions

Model experiments were performed according to Windorfer and Mihailova [11]. The molecular weights of albumin and bilirubin were taken as 1 to 2; the pH was adjusted to 7.4. Identical volumes of 4g/100 ml of human albumin solution or serum and 40 and 80 g/100 ml of bilirubin solutions were mixed and incubated with DPA at concentrations of 0.1 and 1 mg/ml. Incubation was done in the dark at + 4°C for 1, 3, 4, 6 and 8 hr. Free and albumin-bound bilirubin were separated after the 1st or 2nd, 4th and 6th hour of incubation. In some experiment series

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an 8 g/100 ml gammaglobulin solution was applied instead of albumin. In 3 cases the sera of hyperbilirubinaemic newborns were incubated with DPA at 1 mg/ml concentration. The amount of total and free bilirubin was determined after 6 hours.

Results

Preliminary experiments showed that after 6 hour incubation no further changes could be expected from DPA. During that period of time, the free bilirubin level did not rise in the solutions containing DPA in the presence of either albumin or gamma globulin, whilst in experiments with bilirubin dissolved in human adult serum the amount of free bilirubin was found to be lower as in the controls (Fig. 1). The difference was not significant statistically. On increasing the dose of DPA, the free bilirubin level displayed a decreasing tendency. Table I shows the effect of DPA on the total indirect bilirubin level in the presence of serum and of certain serum protein fractions.

Table I

1st solution	2nd solution	DPA D-Penicillamin	Bilirubin mg/100 ml
Serum		0.1 mg/ml	18.5±0.5
		1.0 mg/ml	17.0±0.2
Gammaglobulin 8 g/100 ml	Bilirubin 40 mg/100 ml	1.0 mg/ml	19.5±0.7
Albumin 4 g/100 ml			19.6±0.4

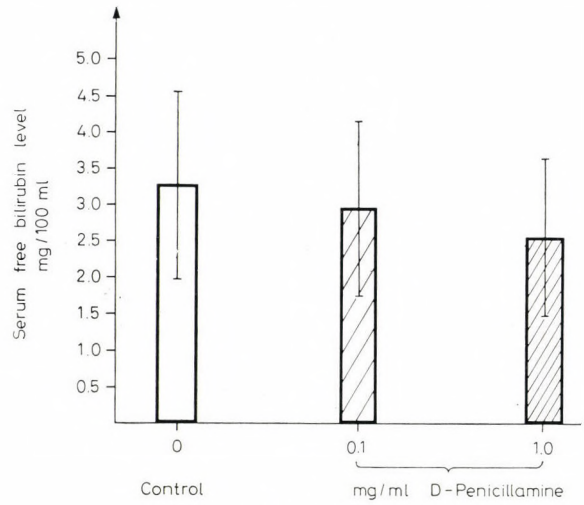

Fig. 1

The data in Table I indicate that DPA is incapable of reducing het total indirect bilirubin level in aqueous solutions of either gamma globulin or albumin. In the presence of serum, a significant fall was, however, noted.

The effect of DPA on the serum of three hyperbilirubinaemic newborns (bilirubin concentration 28 – 30 mg/100 ml) was as follows (Table II).

TABLE II

	Free bilirubin level, mg/100 ml
Without DPA	3.6±0.2
With DPA	2.5±0.6

Incubation with DPA of hyperbilirubinaemic serum for 6 hr thus resulted in a significant lowering of the free bilirubin level ($p < 0.01$).

DISCUSSION

A series of different drugs such as barbiturates, orotic acid, etc. [4, 11], and antibiotics such as ampicillin, carbenicillin, oxacillin, cloxacillin, dicloxacillin, erythromycin, gentamycin, etc., applied in the neonatal period have been examined for their effect on the serum level of free bilirubin. Oxacillin was found to cause a marked dissociation of bilirubin from the albumin–bilirubin complex [11].

In vitro, under the effect of DPA the free bilirubin fraction did not increase; it even showed some decrease. As the drug failed to reduce the total indirect bilirubin level in aqueous solutions of albumin or gamma globulin, for the effect the presence of other serum protein fractions too seems necessary. On the other hand, DPA in vitro caused a significant fall of the free bilirubin level in the serum of hyperbilirubinaemic newborns. This finding supports the value of DPA therapy in neonatal hyperbilirubinaemia.

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