plasma activity in normal rats, according with FLEISHER and WAKIM[5]. Both the isoenzymes were remarkably increased in plasma, after poisoning with the 2 doses of CCl_4. Increases were directly related to the administered dose of CCl_4, according to the results given by ZIMMERMANN[22].

As far as AAT I is concerned, our data are in agreement with those of the other authors. An increase of plasma levels of AAT II has been also reported by FLEISHER and WAKIM[5], and by GABRIELI and ORFANOS[4], but not by HIRAYAMA et al.[6]. It is possible that negative findings reported by HIRAYAMA et al.[6] depend on a loss of AAT II activity, which is known to be more labile than AAT I[23]. This is supported also by the very low recovery (6–20%) of AAT activity that the authors obtained after the elution. The maximal activity peak of both the isoenzymes appeared in plasma at 24 h after poisoning with 0.1 ml of CCl_4, while with 0.02 ml the highest increase was observed 36 h (AAT II) and 48 h (AAT I), respectively, after the administration of the drug. This suggests that the administration of a high dose of CCl_4 causes a more severe and earlier injury than the administration of a lower dose.

On the other hand, we were able to find a relationship between isoenzyme behaviour and histological changes. These data agree with those of CORNISH and BLOCK[24], but are in contrast with those of DINMAN et al.[25] and ZIMMERMANN et al.[22], who found, respectively in rabbits and in rats, that the dose of CCl_4 has no influence on the time elapsing before the appearance of the peak serum levels of AAT, and of the maximal histological alteration in the liver. These different results may depend on the different animals employed by these authors, and, when rats were used, on the different mode of drug administration[26].

Riassunto. Mediante frazionamento cromatografico su colonna di resina a scambio anionico, abbiamo potuto documentare nel siero di ratti intossicati con dosi diverse di CCl_4, incrementi assai significativi sia della componente mitocondriale che citoplasmatica della aspartato-aminotransferasi.

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An Inheritable Abnormal β-Chain in Rabbit Haemoglobin

By routine use of carboxymethylcellulose column for the separation of α- and β-chain of rabbit haemoglobin[1], we have found an abnormal globin composition in the haemoglobin of one rabbit. The additional globin peak was eluted after the normal β-chain. The abnormal globin is inheritable: the same pattern has been observed in one animal out of a kindred of 5 of the 'mutant' rabbit. Data are presented which indicate that our findings can be accounted for by a mutation affecting the β-chain.

Materials and methods. Rabbit erythrocytes were collected and haemolyzed as previously reported for human red blood cells[2]. The concentration of the haemoglobin cyano derivative (CN Met Hb), used[3] for all the chromatographic and electrophoretic separations, was determined at 541 nm according to SCHNEK and SCHROEDER[4].

Horizontal starch gel electrophoresis of the various haemoglobins was carried out at 4 °C in Na borate-Na bicarbonate buffer pH 9.3 in *Tris*-EDTA-borate buffer pH 8.6, pH 8.3[5] and pH 7.3 and in *Tris*-succinate buffer pH 5.4. Starch gels were stained using benzidine or Amino Black. Electrophoresis of haemoglobin was also performed on cellulose acetate at 18 °C using the same buffers. The strips were stained with Ponceau Red. Chromatography of cyanomethaemoglobin on Amberlite IRC-50 was accomplished according to ALLEN et al.[3].

Free globin chains were prepared by acid-acetone precipitation[6] and α- and β-chains were separated according to DINTZIS[1]. Electrophoretic separation of Hb subunits was carried out on starch gel in 6 *M* urea and 0.05 *M* mercaptoethanol in *Tris*-EDTA-borate buffer pH 7.3[7] and *Tris*-K phosphate buffer pH 6.1.

Results. A typical chromatographic separation on CM cellulose column of the α- and β-globins, prepared from 'normal' rabbit haemoglobin, is reported in Figure 1a. Only 2 peaks can be detected.

The chromatographic pattern of globin from the 'mutant' rabbit haemoglobin reveals on the contrary the presence of 3 peaks well separated one from another (Figure 1b). The first peak has a migration corresponding to that of the α-chain, and the second peak to that of the normal β-chain. In fact, if purified β-chain uniformly labelled with L-Valine C^{14} (U) (New England Nuclear Corp., 0.05 mc/0.0293 mg) is added to the 'mutant' globin before chromatography, the peak of radioactivity is coincident with the second peak (Figure 2). The sum of the β- and the additional chain, as mg of protein (determined by the Folin technique), is equal to the α-chain.

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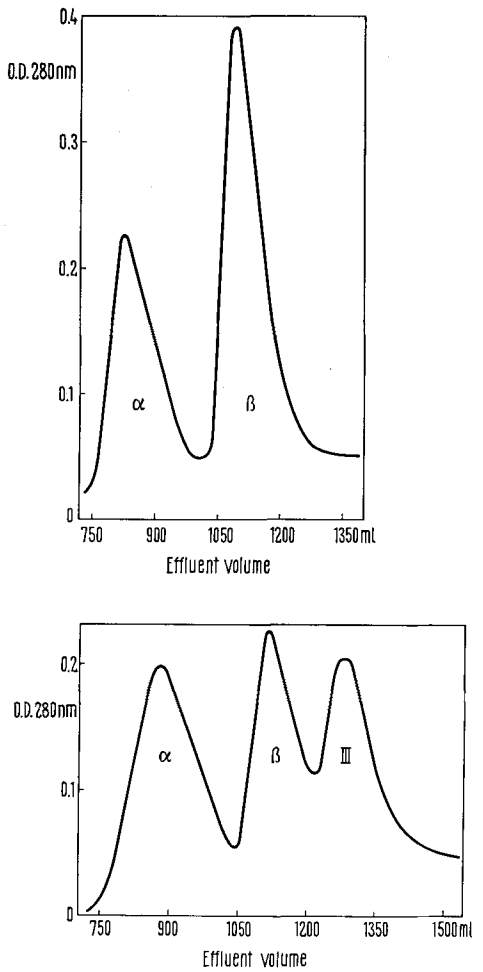

Fig. 1. (a) Separation of α- and β-chain of 'normal' rabbit haemoglobin on carboxymethylcellulose column. (b) Separation of 'mutant' rabbit globins on carboxymethylcellulose column.

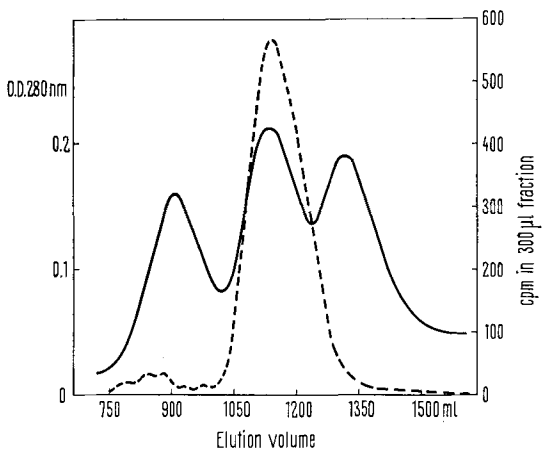

Fig. 2. Carboxymethylcellulose column chromatography of 'mutant' rabbit globin (100 mg) plus β-chain purified from 'normal' rabbit and uniformely labelled with L-Valine C^{14} (G) (6.1 mg; 260,000 cpm). – – – –, O.D. 280 nm; ———, cpm in 0.3 ml fraction.

When free globin chains were subjected to starch gel electrophoresis at pH 6.1 and pH 7.3 in 6*M* urea and mercaptoethanol, the 3 peaks were also separated. The additional peak, which is absent in control samples, has a minor cathodic mobility. Electrophoresis at pH 8.6, 5.4, 4.7, and 1.8 did not separate the 3 protein components.

Starch gel electrophoresis and migration on Amberlite IRC-50 of haemoglobin from 'mutant' rabbit showed only one component with the same characteristics of the 'normal' rabbit haemoglobin.

Discussion. The amino acid sequence of rabbit haemoglobin is not unique and evidence has been reported in favour both of a translational origin of the α-chain multiplicity[8] and of an α-chain heterogeneity due to 2 allelomorphic haemoglobins[9,10]. In our case the abnormal chromatographic pattern of the free globin chain suggests the appearance of a new β-globin. In fact, the additional peak is likely to be due to an amino acid substitution in the β-chain and not in the α-chain, as suggested by the fact that the sum of the β and of the additional peak is equal to the α-chain. The electrophoretic behaviour of the abnormal globin suggests the addition of a negative charge or a substitution of a positively charged amino acid with a neutral one.

The possibility that the pattern might be due to an artefact is ruled out by the fact that out of about 30 outbreed rabbits examined, from various origins, only one male was found to have the described pattern.

The fact that the intact haemoglobins both from the 'mutant' and 'normal' rabbits have the same behaviour when analyzed by electrophoretic and chromatographic techniques may be due to the tridimensional shape of the protein, and to interactions between α- and β-chain so that the substituted amino acid may not be exposed, the difference in charge becoming evident only when the molecular structure is disrupted by urea or acid pH.

Through breeding experiments it has been possible to show that the abnormal pattern is inheritable: the 'mutant' male was mated with a 'normal' female and the same 'mutant' chain was found in 1 animal out of a kindred of 5. This finding makes the heterozygosity for the β-chain mutation the most likely possibility.

Further work is in progress in order to characterize the mutation genetically and biochemically[11].

Riassunto. Mediante tecniche cromatografiche ed elettroforetiche, è state trovata una globina anormale nella emoglobina di un coniglio. La mutazione globinica riguarda verosimilmente la β catena. Questa emoglobina anormale è trasmessa ereditariamente.

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