Plasma Albumin Synthesis during Neonatal Development of the Rat

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1. Liver slices were incubated with ^{14}C -labelled amino acids. Albumin was isolated from the slices by precipitation with specific antibody and the incorporated radioactivity measured. 2. The rate of synthesis was seen to be equal in liver slices from adult and late-stage foetal rats. 3. Synthesis was very high in the pregnant rat (three times the normal adult value) and in the 5–15-day post-natal rat (twice the normal adult value). 4. The post-natal increase may be due to the disappearance of haemopoietic tissue and its replacement by active parenchymal cells.

Previous work (Oliver, Ballard, Shield & Bentley, 1962) has shown that, while the rat doubles its body weight in the first 10 days of life, the liver weight increases only slightly. The weight of the liver at 5 days is less than at birth. However, it was also noted that, during this period of overall body growth and consequent increase in plasma volume, the plasma protein concentration remains at approx. 30% of the adult level, until the tenth day, after which it increases rapidly to the adult level. Despite this apparent lag period it has been shown in recent experiments (Wise & Oliver, 1966) that plasma-protein synthesis is initiated early in foetal life, since the foetal rat liver at 16 days was shown to synthesize foetal-plasma proteins *in vitro*. However, quantitative data on the rate of synthesis during development were not obtained.

In the present work, the rate of synthesis of plasma albumin during neonatal development in the rat has been determined with liver slices *in vitro*. This protein was selected for study since it is the major protein component of adult plasma and is synthesized exclusively by the liver.

MATERIALS AND METHODS

Isotope-incorporation experiments. Liver slices were prepared as described previously (Ballard & Oliver, 1964). As a routine, 100 ± 2 mg. of sliced tissue, which had been rinsed in chilled buffer and blotted, was incubated with 2 ml. of buffer containing $0.5 \mu\text{C}$ of ^{14}C -labelled algal-protein hydrolysate ($200 \mu\text{C}/\text{mg}$. from The Radiochemical Laboratory, Amersham, Bucks.). Incubation was carried out for 1 hr. at 37° . The flasks were chilled in ice and the entire contents

transferred to a chilled co-axial homogenizer constructed from Perspex. The slices were homogenized by 10 strokes of the pestle driven at about 2000 rev./min. After homogenization, each sample was transferred to a 5.5 cm. $\times$ 1.5 cm. nitrocellulose centrifuge tube, placed in an ice bath and subjected to ultrasonic treatment with a Dawe Sonifier for 4 min. at maximum energy with a $\frac{1}{2}$ in. probe. Such treatment gave maximum yield of albumin from the liver slices. After centrifugation at 20000g for 3 hr. each supernatant was assayed for ^{14}C -labelled amino acids incorporated into plasma albumin.

Estimation of radioactivity incorporated into albumin. Rat plasma albumin and a rabbit antiserum were prepared as described previously (Wise & Oliver, 1966). The radioactivity incorporated into albumin was determined after precipitation of the albumin as an antibody complex (Campbell & Stone, 1957). To 1 ml. of each extract of liver sample was added 0.5 ml. of antiserum. After 35 min. incubation at 37° the samples were stored at 2° overnight. The precipitate was centrifuged for 15 min. at 3000g. The supernatant was removed by suction and the precipitate washed three times with 1.5 ml. of cold 0.15M-NaCl. The washed precipitate was dispersed in 0.1 ml. of Hyamine hydroxide (1M solution of di-isobutylresorcyethoxyethyl-dimethylbenzylammonium hydroxide in methanol; Nuclear-Chicago Corp., Des Plaines, Ill., U.S.A.) and dissolved by heating for 2 min. at 55° ; 5 ml. of Ditol (Herberg, 1960) was added and the radioactivity determined with an Ekco liquid-scintillation counter at 20° . The addition of internal standards of ^{14}C -labelled amino acids indicated that 10% quenching occurred in all samples, due to Hyamine hydroxide. All results have been corrected for this factor. The efficiency of the counter for ^{14}C was 50%.

The results have been expressed as counts/min. incorporated into the albumin obtained from 100 mg. of tissue. The specific radioactivity was not used as a measure of amino acid incorporation since the value obtained would be dependent on the concentration of endogenous albumin in the slices and this parameter may reasonably be expected to vary with age.

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RESULTS

For the assays to have a quantitative meaning, the following conditions are necessary: (i) the uptake of ^{14}C -labelled amino acids must be linear with time for at least 1 hr.; (ii) the precipitation of albumin must be specific; (iii) the amount of albumin precipitated in the antigen-antibody complex must be a linear function of the albumin concentration.

The time-course of ^{14}C -labelled amino acid uptake into albumin was determined with a litter of animals 10 days of age and the liver slices were extracted as for routine analyses. A sample (0.5 ml.) of each liver extract (plus 0.25 ml. of 0.15 M-sodium chloride) was treated with 0.25 ml. of antiserum as described above. To a second 1 ml. sample of each extract was added 10 μg . of ovalbumin and 0.5 ml. of a rabbit antiserum to ovalbumin. After incubation for 35 min. at 37° and then 2 hr. at 2° the precipitate was removed by centrifugation. To 0.75 ml. of supernatant (equivalent to 0.5 ml. of each liver extract) was added 0.25 ml. of anti-albumin and the incorporated radioactivity in the precipitate determined in the standard manner. Fig. 1 shows that incorporation was linear for at least 90 min. and since the two sets of data agree within 10%, the 'clearing' procedure was omitted from routine analyses.

Fig. 1. Synthesis of rat plasma albumin by liver slices *in vitro*. Incubations were carried out for the times shown. ●, Counts/min. in albumin obtained from 100 mg. of liver; liver-slice extracts were 'cleared' with ovalbumin and anti-ovalbumin before precipitation of albumin with rabbit antiserum. O, Counts/min. in albumin obtained from 100 mg. of liver; liver-slice extracts were treated only with rabbit antiserum to rat albumin.

In other experiments, only one precipitin line has been obtained when liver extracts from animals of various neonatal and adult ages are diffused against antiserum to albumin and hence the precipitation of albumin from the radioactive liver extracts is assumed to be specific.

The amount of albumin precipitated from ultrasonically treated extracts was shown to be a linear function of concentration at all ages tested, by taking 0.5 ml. and 1.0 ml. samples for assay. In all cases the count of ^{14}C obtained was proportional to the volume of extract used. Experiments with ^{131}I -labelled rat albumin also showed precipitation of albumin in the complex to be a linear function of albumin concentration, even though the combining ratio of antibody to antigen decreased from 34:1 to 12:1 as the amount of albumin assayed was increased from 3.7 μg . to 75 μg . (see also Campbell & Stone, 1957).

The results obtained for the synthesis of plasma albumin, by rat-liver slices as a function of age of the donor animals, are summarized in Fig. 2. The rate of synthesis at 5–15 days post-natal is approximately twice the value obtained in foetal slices and there is a subsequent fall to the adult value. The liver slices of pregnant rats have a very high rate of albumin synthesis, about three times the value found in foetal rat liver or adult rat liver.

Fig. 2. Rate of synthesis of rat plasma albumin by liver slices. The incorporation of ^{14}C -labelled amino acids into albumin by rat-liver slices in 1 hr. is plotted against the age of the animals from which slices were obtained. Each point represents the mean of determinations on slices from at least six animals. The vertical bars represent the s.e.m. Δ, Pregnant adult rats; ●, non-pregnant adult rats; ▲, foetal rats (18–21 days gestation); ○, post-natal rats of ages given.

DISCUSSION

The alteration in the observed rate of synthesis of plasma albumin shown in Fig. 2 could have been explained by an age-dependent change in the size of the endogenous amino acid pool, resulting in a change in the specific radioactivity of the ^{14}C -labelled amino acid substrate. This possibility was investigated by measuring the total ninhydrin-positive material (Moore & Stein, 1954) extracted from liver samples in 0.1N-hydrochloric acid in ethanol. Since urea also reacts with ninhydrin, 0.15M-sodium chloride extracts of liver samples were assayed for keto amines (Marsh, Fingerhut & Kirsch, 1957; Skeggs, 1957). At all ages examined (20-day foetal, newborn, 11-day post-natal, pregnant and male adult), the total ninhydrin-positive material was equivalent to 5–6mg. of leucine/g. wet wt. of liver. Keto amines represented less than 0.5mg./g. of liver at the same ages.

The results of Fig. 2, expressed on a wet wt. basis, show that the rate of plasma albumin synthesis by the liver increases about twofold after birth of the rat. However, the changing cytology of the maturing liver makes necessary further comment on the quantitative significance of the results. Oliver, Blumer & Witham (1963) examined the cellular composition of the maturing rat liver and found that, in the 17-day foetal rat, about 60% of the tissue consisted of haemopoietic cells and vascular space. By the sixth post-natal day, the value assigned to non-parenchymal tissue had fallen in an almost linear fashion to the adult level of 10% vascular space, and haemopoietic tissue was absent.

It is apparent that, if the rate of synthesis of plasma albumin was expressed per average parenchymal cell, there would be only a small post-natal increase. That there is an absolute increase in the rate of synthesis per unit weight of liver over the first 10 post-natal days is obvious from the data of Fig. 2. This is consistent with previous data on liver growth and plasma protein concentration, which showed that although the liver weight remains almost constant, the concentration of plasma protein increases slightly during the first 10 post-natal days and the body weight, and consequently the plasma volume,

increase considerably (Oliver *et al.* 1962). It is possible that the observed post-natal increase in plasma albumin synthesis may be explained by the disappearance of haemopoietic tissue and its replacement by active parenchymal cells.

Examination in the electron microscope of liver sections from the foetal, newborn and post-natal rat (W. F. C. Blumer, unpublished work) shows that the endoplasmic reticulum of parenchymal cells is morphologically similar to that of adult liver cells, and on this criterion the liver might be expected to synthesize plasma albumin at these ages. Wise & Oliver (1966) have reported both synthesis *in vivo* and *in vitro* of albumin by foetal rat liver. This aspect of liver cell function appears relatively early in embryological development and appears to show no sharp transitional period as is found with other systems (see Dawkins, 1966).

The rate of synthesis of plasma albumin is lowest in the adult rat liver and highest in the neonatal liver and the liver of animals in an advanced stage of pregnancy. The mechanism that controls the initiation and the rate of plasma albumin synthesis is not obvious from these data, but hormones such as placental oestrogens may control the rate of protein synthesis in foetal and pregnant animals, and be involved in the initiation of the process early in embryogenesis.

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