Albumin and Transferrin Synthesis during Development in the Rat

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In this study, the incorporation of [^{14}C]leucine into albumin and transferrin in early rat foetuses, vitelline plus amniotic membranes, chorioallantoic placenta and perinatal rat liver slices was measured and used to detect and compare the rates of synthesis of the two proteins. Albumin synthesis was detected in the body of foetuses from 13 days gestation onwards. Transferrin synthesis was detected only after day 15. Transferrin synthesis was demonstrable in the membranes but not in the chorioallantoic placenta of all the animals investigated, i.e. from 13 to 19 days gestation. Synthesis of albumin and transferrin by the liver of near-term and postnatal animals was shown to correlate with published data on the parenchymal cell number/unit wet wt. of liver. Near-term foetuses synthesized relatively more transferrin than albumin when compared with 10-day postnatal animals. The serum concentrations of the two plasma proteins were also determined. These increased before term whereas the rate of synthesis of albumin and transferrin declined. Postnatally, plasma albumin concentration increased but transferrin concentration decreased, yet the rates of synthesis of both proteins by the liver increased with age. This lack of correlation between the rates of synthesis of the two proteins and their respective plasma concentrations could be explained in part by their increased stability after birth. There was also evidence that the liver haemopoietic cells took up transferrin although they do not synthesize the protein. Thus the decrease in this population of cells during development could also contribute to the discrepancy between liver synthesis and serum concentrations of transferrin.

Changes in the composition of rat serum proteins during foetal and early postnatal development have been reported by Heim (1961) and Wise *et al.* (1962). Genetic evidence (Rausen *et al.*, 1961; Toivanen *et al.*, 1969), the presence of foetal specific serum proteins (Wise *et al.*, 1962) and isotopic tracer studies (Dancis & Shafran, 1958; Wise & Oliver, 1966; Gitlin & Kitzes, 1967; Gitlin & Perricelli, 1970) confirm that the mammalian foetus is capable of serum-protein synthesis. Kelleher *et al.* (1963) measured incorporation of labelled amino acids into albumin and globulin in the foetal rat *in utero*. Dancis *et al.* (1957) carried out studies *in vitro* with human foetal liver, and Page (1969) investigated albumin synthesis *in vivo* and *in vitro* by the postnatal pig.

There is no report in the literature comparing rates of transferrin synthesis in foetal and neonatal animals. Lane (1968) showed that fluorescent transferrin antibody will bind to the liver of a 2.4 g rat foetus but not to the same tissue from a 1.23 g animal. This evidence suggests that at some stage during its development, the foetal rat liver commences synthesis of transferrin. Gitlin & Boesman (1967) have shown that human and rat foetal membranes are capable of

synthesizing transferrin at an earlier developmental stage. However, the methods used in these experiments did not allow measurement of the rates of protein synthesis by the membranes or other foetal tissues.

The present paper is a study of the synthesis of two specific serum proteins, transferrin and albumin, during foetal and early postnatal life in the rat. There were two major aims. First, to measure the incorporation of [^{14}C]leucine into transferrin and albumin in foetuses and foetal membranes in order to establish the relative importance of these two possible sites of synthesis in early foetal life and the gestational age at which the liver acquires the capacity to synthesize the proteins. Secondly, to determine the changes in liver synthesis of transferrin and albumin which occur during development, and to relate these changes to the serum concentrations of the respective proteins.

Materials and Methods

Materials

Animals. The Wistar albino strain of the species *Rattus norvegicus* was used. These animals have a gestation period of 22 days. Age of foetuses was determined from the mean weight of at least four animals from a litter, by using a previously

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established age-weight relationship for the colony (Yeung *et al.*, 1967). Age of postnatal animals was determined from time of natural birth and measured to an accuracy of ± 0.5 days. Adult rats were male animals weighing 280–350 g; they were not starved before use.

Chemicals. [^{14}C]Leucine, ^{59}Fe and ^{125}I were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. ^{131}I was obtained from the Australian Atomic Energy Commission, Lucas Heights, N.S.W., Australia. Amino acids, sodium deoxycholate and crystalline bovine serum albumin were purchased from the Sigma Chemical Co., St. Louis, Mo., U.S.A. Human transferrin was purchased from Behringwerke A.G., Marburg Lahn, Germany.

Purification of rat proteins and preparation of antisera. Rat transferrin was purified as described by Morgan (1969). Albumin was purified by the method of Michael (1962). Purity of isolated proteins was assessed by cellulose acetate electrophoresis and polyacrylamide-gel electrophoresis. Rabbits were immunized against these proteins and human transferrin (from Behringwerke) by intramuscular injection of three or four doses each of 3 mg of protein in 1 ml of saline and 1 ml of Freund's adjuvant given at 3–4 week intervals. Antisera were obtained by bleeding rabbits from a marginal ear vein. When examined by immunoelectrophoresis against rat serum the antiserum to rat albumin and rat transferrin each showed a single arc.

^{125}I - and ^{131}I -labelling of proteins. Purified transferrin and albumin were labelled with either ^{125}I or ^{131}I by the iodine monochloride method (McFarlane, 1958, 1963). The degree of iodination was 0.5–0.7 iodine atoms/protein molecule. Most of the non-protein-bound iodine was removed by passage through a column of anion-exchange resin IRA 410. Any remaining free iodine was removed by dialysis overnight against 0.15 M-NaCl.

Experimental procedures

[^{14}C]Leucine incorporation into transferrin and albumin in liver slices. Pregnant animals were anaesthetized with ether and foetuses were removed after uterine section. The foetuses were weighed and their livers dissected out immediately and kept chilled in ice-cold 0.9% saline. Livers were sliced with minimum delay by the method of Ballard & Oliver (1963), dried on filter paper and 500 mg of tissue was suspended in 3 ml of Krebs–Henseleit Ringer bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932), which was gassed with $\text{O}_2 + \text{CO}_2$ (95:5). The mM concentration of amino acids present were as follows: alanine, 0.4; arginine, 0.15; aspartic acid, 0.2; asparagine, 0.3; cysteine, 0.1; cystine, 0.05; glutamic acid, 0.2; glutamine, 0.2; glycine, 0.4;

hydroxyproline, 0.2; histidine, 0.1; isoleucine, 0.2; lysine, 0.35; methionine, 0.05; phenylalanine, 0.1; proline, 0.15; serine, 0.2; threonine, 0.2; tryptophan, 0.15; tyrosine, 0.1; valine, 0.15. Incubation was commenced after addition of $1\ \mu\text{Ci}$ of [^{14}C]leucine in a volume of $100\ \mu\text{l}$ (containing $0.6\ \mu\text{mol}$ of leucine giving a final incubation concentration of $0.2\ \text{mM}$ -leucine) and was carried out at 37°C in a shaking (120 oscillations/min) water bath. Incorporation of label into immunoprecipitates and trichloroacetic acid-precipitated protein was shown to be linear over 3 h (see the Results section). An incubation period of 2 h was therefore used as a routine. Reaction was terminated by the addition of $500\ \mu\text{l}$ of unlabelled leucine (0.5 g/l). The slices were then homogenized in a Perspex Potter–Elvehjem homogenizer together with their incubation solution. The suspension was treated by ultrasonic dispersion and centrifuged in an International Centrifuge, model B20 at $18000\ g_{\text{av}}$ for 30 min at 0°C . The supernatant was then prepared for antibody precipitation.

[^{14}C]Leucine incorporation into foetuses and foetal membranes. This was carried out under similar conditions to those used for liver-slice preparations. Depending on their weight, two to four foetuses (about 800 mg total wt.) previously minced with a pair of fine scissors, or three to four membranes (about 150 mg total weight) were used in each flask. Material used was dried on tissue paper before weighing. The membranes were obtained after removal of the placenta and the foetus and therefore included both the yolk sac (vitelline) and amniotic membrane. The chorioallantoic placenta was incubated separately to establish if it synthesized albumin and transferrin. Experiments were also carried out with foetuses from which the liver had been removed, to determine if there were extrahepatic sites of albumin or transferrin synthesis. The homogenized tissue extract was treated with 0.5% sodium deoxycholate (final concentration) and 0.05 M-Tris-HCl, pH 8.0, and stored at 3°C (overnight). The mixture was dialysed against 0.1 M- NaHCO_3 (about 6 h) then against distilled water (overnight) and finally against 0.02 M-sodium phosphate buffer, pH 6.2, (about 6 h) to remove the deoxycholate. The mixture was centrifuged at $18000\ g_{\text{av}}$ for 30 min and the supernatant removed and prepared for antibody precipitation. In some instances, rates of radioactivity incorporation obtained were extremely low, therefore blanks in which $500\ \mu\text{l}$ of unlabelled leucine (0.5 g/l) was added before addition of radioactive leucine, and a time-course (1, 2 and 3 h incubation), were studied in all determinations. Synthesis was only considered to be significant where radioactivity incorporated was above blank values and increased linearly with time.

Immunoprecipitation of transferrin and albumin. The supernatant solutions were 'cleared' (Peters &

Anfinsen, 1950) by the addition of 50 μ l of human serum followed by 0.5 ml of anti-(human transferrin), mixing and incubating at 37°C for 1 h, refrigeration overnight at 3.5°C and centrifugation at 1300 g for 15 min at 0°C. Portions (0.5–1 ml) of the cleared supernatant were then precipitated with 0.5 vol of anti-(rat transferrin) or anti-(rat albumin). It was previously established that this volume of antisera was sufficient to precipitate all the transferrin or albumin present in the portion of cleared supernatant. The solutions were mixed, incubated for 60–90 min at 37°C, then stored overnight at 3.5°C. The next day the precipitates were washed three times with ice-cold 0.15 M-NaCl, dissolved in 0.5 ml of 0.1 M-NaOH, and taken up in 10 ml of diotol (Herberg, 1960) for determination of radioactivity.

The following investigation of the immunoprecipitation procedure was undertaken because of evidence that immunological precipitation of albumin from the liver on to chromatography paper discs does not yield radiochemically pure albumin (Schreiber *et al.*, 1969; Maeno *et al.*, 1970). Albumin immunoprecipitates from livers of foetal (21 day) and adult rats were prepared as described above, air-dried and then dissolved by incubation at 45°C for 1 h in 0.1 ml of a solution of 6 M-urea, 1% (w/v) sodium dodecyl sulphate, 1% 2-mercaptoethanol, 0.1 M-sodium phosphate (pH 7.2). The samples were then subjected to electrophoresis in sodium dodecyl sulphate-polyacrylamide gels by using the same conditions as described by Cashman & Pitot (1971). After electrophoresis the gels were fixed and stained (Vesterberg, 1972) and scanned in a Gifford model 2400 spectrophotometer equipped with a gel-scan adaptor. Subsequently the gels were fractionated with a Savant gel-fractionating apparatus (Savant Instruments Inc., Hicksville, N.Y., U.S.A.). The radioactivity in each fraction was determined by the procedure of Cashman & Pitot (1971) and a portion of the original digest was also counted for radioactivity to determine the percentage recovery.

Incorporation of [14 C]leucine into total soluble protein. Portions (100–200 μ l) of supernatant were treated with 10% (w/v) trichloroacetic acid before clearing. The solution was left to stand overnight and the precipitates were washed with ice-cold 5% (w/v) trichloroacetic acid (three times) and then dissolved in 0.5 ml of 0.1 M-NaOH. A portion (400 μ l) was taken for determination of radioactivity and the rest kept for protein determination. Incorporation of [14 C]leucine is expressed as c.p.m./mg of protein.

Turnover of radioiodine-labelled albumin and transferrin. Two sets of experiments were performed with radioiodine-labelled proteins to study the rates of turnover of albumin and transferrin in prematurely delivered, immediate postnatal, postnatal and adult rats *in vivo*. In one set of experiments 125 I-labelled albumin and 131 I-labelled transferrin were used.

The experiments were then repeated with 131 I-labelled albumin and 125 I-labelled transferrin. For both sets of experiments, adult animals received approx. 1×10^6 c.p.m. of 131 I and 5×10^5 c.p.m. of 125 I.

In the case of adult animals label was injected via the lateral tail vein and blood was sampled from the ventral tail vein directly into micro-haematocrit tubes. Foetal animals delivered by caesarean section received the label intravenously (via the umbilical vein) or intraperitoneally and were returned to foster mothers for the duration of the experiment. Postnatal animals received radioactivity intraperitoneally and were returned to their original mothers. These perinatal animals were bled by severing the jugular vein and collecting blood directly into micro-haematocrit tubes. The tubes were then centrifuged and serum was collected directly into a 40 μ l micropipette. Protein was precipitated from a 100 μ l sample of serum with 10% (w/v) trichloroacetic acid. The pellet was washed the next day with ice-cold 5% trichloroacetic acid (three washes) and 125 I and 131 I radioactivity determined after dissolving the pellets in 1 M-NaOH (2 ml).

Throughout the turnover studies the animals were fed *ad libitum* and their drinking water contained 0.01% KI.

These studies were conducted over a period of 6 days. In the case of postnatal animals one animal from each of four litters was killed each day. The values for plasma protein-bound activity from these animals were corrected for the increase in plasma volume which occurs over this period in neonatal animals [based on the data of Masters *et al.* (1972)]. A line of best-fit through the logarithms of the four results obtained on each day was then calculated by the least-squares method. The half-life of the disappearance of the labelled proteins was determined from this line. Corresponding values were obtained in adult animals by taking daily blood samples from the ventral tail vein of eight rats injected with the labelled proteins and treating the data in a similar manner (expect that no correction was made for change in plasma volume).

Liver intracellular distribution of transferrin and albumin. Fractionation of liver homogenate was carried out as described by Morgan & Peters (1971b). A difficulty encountered when applying the method to foetal liver was the presence of haemopoietic cells. Most of these cell types were not disrupted by homogenization and consequently contaminated the nuclear pellet. This was verified by microscopic examination.

Determination of free leucine pool in adult and foetal rat liver. Livers were removed and immediately weighed and homogenized in an approximately equal volume of 15% trichloroacetic acid. The mixtures were then centrifuged at 1500 g for 30 min

at 0°C, the supernatant was removed with a Pasteur pipette and the volume recorded. The trichloroacetic acid was then extracted with 4 vol. of diethyl ether and the concentration of amino acid in the solution remaining was determined with a Technicon Auto-Analyser.

Incorporation of ^{59}Fe and synthesis of transferrin and albumin in partially purified foetal liver haemopoietic cells. During attempts to fractionate foetal liver homogenate, a 'nuclear' fraction heavily contaminated with whole haemopoietic cells was obtained. The capacity of this fraction to incorporate iron into haem and to synthesize transferrin and albumin was investigated by incubating it with ^{59}Fe -labelled rat serum and with [^{14}C]leucine. The serum was labelled by the addition of ^{59}Fe as its complex with nitrilotriacetic acid. The amount of iron added was less than 50% of the latent iron-binding capacity of the serum. Bates *et al.* (1967) showed that under these conditions iron is very rapidly bound by the specific binding sites of transferrin. The pellet rich in haemopoietic cells was first washed three times with ice-cold Krebs-Henseleit Ringer bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932) containing 10% (w/v) bovine serum albumin (to minimize cell clumping) and was filtered through nylon cloth after each wash. A portion of the washed cells was then incubated in wash medium and an equal volume of ^{59}Fe -labelled serum was introduced. Samples were taken at various times after the addition of ^{59}Fe , washed three times with ice-cold 0.15M-NaCl and the cells haemolysed in water (2ml) and counted for radioactivity to determine ^{59}Fe uptake. Haem was extracted from the remainder of the incubation mixture by the method of Thunell (1965) to confirm that ^{59}Fe was in fact incorporated into haem under the experimental conditions. A second portion of the cell preparation was incubated with [^{14}C]leucine to determine rates of transferrin and albumin synthesis. Precipitation of albumin and transferrin was performed as described above after disruption of cells by sonication.

Analytical methods. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. A range of standards were used in each set of determinations. The concentration of transferrin and albumin in serum and tissue extracts was measured by radial immunodiffusion by the method of Mancini *et al.* (1965) with specific antisera raised in rabbits. Standards were used in all determinations. Serum iron and total iron-binding capacity were measured by the method of Morgan & Carter (1960).

^{59}Fe , ^{125}I and ^{131}I radioactivities were determined in a volume of 2ml of solution (water or 1M-NaOH) in a Packard, model 3002, gamma-scintillation spectrometer. ^{14}C was determined in 10ml of diotol (Herberg, 1960) in a Nuclear-Chicago model 6860 liquid-scintillation spectrometer. Quench corrections were made by the channels-ratio method and count rates were adjusted to a standard 89% efficiency.

Results

Sodium dodecyl sulphate-polyacrylamide-gel electrophoresis of albumin immunoprecipitates

Complete recovery of radioactivity from the gels was achieved when the albumin immunoprecipitates of liver extracts were fractionated by sodium dodecyl sulphate-polyacrylamide-gel electrophoresis. Nearly all of the radioactivity (85–88%, range of four runs, three foetal, one adult) appears as one peak which corresponded to the position of rat serum albumin as demonstrated by running samples of the purified protein. A shoulder was present on the peak in two runs, but it was absent in the other two. The stained gels showed bands corresponding to the heavy chain, light chain and undissociated rabbit immunoglobulin molecules as well as the albumin band.

Protein synthesis by fetuses and foetal membranes

Transferrin and albumin synthesis was measured in whole fetuses at various stages of gestation (Table 1). Data from individual animals indicated that signi-

Table 1. *Transferrin and albumin synthesis in vitro by whole rat fetuses and by foetal membranes in the rat*

Rat fetuses, excluding membranes, were minced and incubated with [^{14}C]leucine as described in the Materials and Methods section. Foetal membranes (vitelline and amniotic membranes, but not the chorioallantoic placenta) were also incubated with [^{14}C]leucine as described in the Materials and Methods section. Transferrin and albumin were then isolated by treatment of extracts with the respective antisera. Results are means $\pm$ S.E.M.

Age (days gestation)	Number of litters	Foetal wt. (mg)	Membrane wt. (mg)	[^{14}C]Leucine incorporated			
				Foetuses (c.p.m./foetus)		Membranes (c.p.m./membrane)	
				Transferrin	Albumin	Transferrin	Albumin
13–14	3	150 $\pm$ 70	27 $\pm$ 10	0 $\pm$ 0	480 $\pm$ 220	170 $\pm$ 10	140 $\pm$ 80
15–16	4	310 $\pm$ 45	45 $\pm$ 7	160 $\pm$ 110	780 $\pm$ 280	520 $\pm$ 120	290 $\pm$ 55
17–19	3	1210 $\pm$ 350	110 $\pm$ 30	10500 $\pm$ 4000	2400 $\pm$ 1700	260 $\pm$ 30	210 $\pm$ 250

ficant transferrin synthesis could be detected in foetuses weighing about 250mg or more, whereas albumin synthesis could be measured in foetuses weighing about 30mg. Incorporation of [^{14}C]leucine into both proteins increased with increase in foetal size.

Table 1 also shows the results obtained when the membranes from foetuses of various ages were incubated with [^{14}C]leucine. Membranes from animals of the age range investigated all synthesized transferrin, but on a per unit weight basis, membranes from earlier embryos were more active. Incorporation of [^{14}C]leucine into albumin was detected in the membranes of all but the more mature foetuses (>1400mg).

Incubation of the chorioallantoic placentas with [^{14}C]leucine revealed that there was no incorporation of the label into either transferrin or albumin. This agrees with data obtained by Wise & Oliver (1966).

Protein synthesis by the liver

Fig. 1 shows the incorporation of [^{14}C]leucine into transferrin in liver slices after various incubation times. Incorporation increased from almost zero at zero time in an essentially linear manner with time over 3h for slices prepared from foetal, postnatal (5 days) or adult rat liver. In view of this, an incubation period of 2h was selected for routine determinations. The free leucine concentration of the liver was measured in two foetal (21 days gestation) and two adult rats. The mean values so obtained were 83.7 and 63.7 $\mu\text{mol/kg}$ of liver respectively. Therefore the leucine that was introduced with the tissue slices represented only a small fraction (<0.1%) of the leucine present in the total system used *in vitro*.

The rate of transferrin and albumin synthesis in liver slices as a function of age is shown in Fig. 2. Incorporation of label into total soluble protein is also shown. Generally, the patterns of transferrin and albumin synthesis followed each other closely. There was a fall just before birth, followed by a postnatal increase which was sustained until 10 days after birth, when there was a gradual decrease in the rate of synthesis of both serum proteins from this age onwards. The correlation between transferrin and albumin synthesis and total soluble-protein synthesis was better for foetal animals than it was for postnatal animals. It should be noted that although there was a similarity in the gross pattern of transferrin and albumin synthesis with respect to age, there was a significant change in the ratio albumin/transferrin synthesis at about 5 days after birth (Table 2).

Experiments with partially purified haemopoietic cells from the liver revealed that these cells incor-

porated ^{59}Fe into haem, indicating that the cells remain viable after being subjected to the purification procedure. However, these cells failed to incorporate any [^{14}C]leucine into albumin or transferrin.

Fig. 3 compares the rate of transferrin synthesis, haemopoietic cell number/ cm^3 of liver and the parenchymal cell number/ cm^3 of liver [from data of Greengard *et al.* (1972)]. There is a good correlation between transferrin synthesis and parenchymal cell number/ cm^3 of liver, particularly at about the time of birth. It therefore follows that a similar correlation exists between albumin synthesis and parenchymal cell numbers, since the pattern of changes of [^{14}C]leucine incorporation into albumin is similar to that of incorporation into transferrin (Fig. 2).

Protein concentration in the liver

Table 3 shows the transferrin and albumin content of foetal, 5-day postnatal and adult rat liver. Data indicate that the transferrin content/unit weight of liver was highest in the foetal liver and declined as the organ matured. In contrast, the albumin content of the liver increased during development. It is noteworthy that the adult values reported in the present study are considerably less than those published by Morgan & Peters (1971a). This is because the tissue used in the present study was in the form of slices which had been washed in ice-cold NaCl

Fig. 1. [^{14}C]Leucine incorporation into albumin and transferrin in foetal, postnatal and adult rat liver slices for various incubation times

Experimental conditions are described in the Materials and Methods section. Albumin and transferrin were isolated by precipitation with the respective specific antiserum. Albumin synthesis in the adult (Δ), postnatal ($\circ$) and foetal ($\square$) rat. Transferrin synthesis in the adult ($\blacktriangle$), postnatal ($\bullet$) and foetal ($\blacksquare$) rat.

Fig. 2. Incorporation of [^{14}C]leucine into albumin, transferrin and total soluble protein in rat liver slices as a function of age of animals

Experimental conditions are described in the Materials and Methods section. Albumin and transferrin were isolated by precipitation with the respective specific antiserum. Soluble protein was precipitated with 10% trichloroacetic acid. T represents term (22 days gestation) and A represents adults. ■, Transferrin synthesis; ●, albumin synthesis; ▲, total soluble-protein synthesis. Vertical bars represent $\pm$ S.E.M. Where no vertical bars are shown the values are the means of two determinations.

Table 2. Relative rates of transferrin and albumin synthesis in liver slices during development

The ratio presented is the [^{14}C]leucine radioactivity incorporated into albumin divided by the [^{14}C]leucine radioactivity incorporated into transferrin for the same tissue-slice preparation. Transferrin and albumin were isolated by immunoprecipitation. The numbers of determinations are indicated in parentheses.

Animals	Albumin/transferrin synthesis
Foetal (19–22 days)	4.8 (8)
Postnatal (5 days)	4.3 (10)
(19 days)	7.1 (3)
(20 days)	7.7 (4)
(40 days)	8.7 (4)
Adult	7.8 (6)

before incubation and subsequent homogenization. Thus most of the protein present in the serum and extracellular fluid was excluded.

Attempts to determine the intracellular distribution of transferrin and albumin of foetal liver was complicated by the presence of haemopoietic cells which contaminated the nuclear pellet. This discrepancy in distribution between adult and foetal liver fractions was not observed with albumin (Table 4).

Serum protein concentrations

The serum albumin concentration of foetal and postnatal animals increased gradually with age until

Fig. 3. Transferrin synthesis, haemopoietic cell numbers and parenchymal cell numbers during development of rat liver

Liver haemopoietic and parenchymal cell numbers are based on the data of Greengard *et al.* (1972). ■, Haemopoietic cell number/ cm^3 of liver; ▲, parenchymal cell number/ cm^3 of liver; ●, transferrin synthesis. T, Term; A, adult.

about 9 days after birth, in much the same way as total protein (Fig. 4). Then there occurred a phase of more rapid increase. The amount of transferrin in the serum appears to follow the same pattern.

However, there was a decrease in serum transferrin concentration postnatally. This was not seen with albumin or total protein.

Transferrin and albumin turnover

The turnover of transferrin and albumin was determined in prematurely delivered, newborn (12h postnatal), 6-day postnatal and adult rats. There was no significant difference between the turnover of proteins administered intraperitoneally and intravenously to prematurely delivered animals, and the rates of turnover of transferrin and albumin in these animals were not significantly different

Table 3. *Transferrin and albumin content of adult, 5-day postnatal and foetal rat liver slices*

Slices were prepared from livers of the various-aged animals, washed in saline, homogenized and then sonicated. The transferrin and albumin content of extracts was determined by radial immunodiffusion. Values are $\pm$ S.E.M. with numbers of determinations indicated in parentheses.

Age of animals (days)		Protein content (μ g/g wet wt. of liver)	
		Transferrin	Albumin
Foetal	(19–22)	(8) 560 $\pm$ 27	1280 $\pm$ 64
Postnatal	(5)	(10) 490 $\pm$ 11	1390 $\pm$ 68
Adult	(6)	300 $\pm$ 5	1770 $\pm$ 103

Table 4. *Intracellular distribution of transferrin and albumin in adult and foetal rat liver*

Liver homogenates were fractionated as described by Morgan & Peters (1971*b*). These results are the means of two sets of determinations. Foetuses used were at 21 days of gestation.

	Transferrin		Albumin	
	(μ g/g wet wt. of liver)	(% of total)	(μ g/g wet wt. of liver)	(% of total)
Adult liver				
Nucleus	110	12	330	9
Mitochondria	60	7	140	4
Microsomal fraction	330	36	560	16
Supernatant	420	45	2580	71
Foetal liver				
Nucleus	370	30	240	11
Mitochondria	130	10	150	7
Microsomal fraction	230	19	360	17
Supernatant	510	41	1380	65

Fig. 4. *Rat plasma albumin, transferrin and protein concentrations as a function of age*

Albumin and transferrin were determined by radial immunodiffusion (Mancini *et al.*, 1965). Protein was determined by the method of Lowry *et al.* (1951); mg of transferrin/ml of plasma ($\blacksquare$); mg of albumin/ml of plasma ($\bullet$); mg of protein/ml of plasma ($\blacktriangle$). Vertical bars represent $\pm$ S.E.M. Where no vertical bars are shown the values are the means of two determinations. T, Term; A, adult.

Table 5. Plasma transferrin and albumin turnover in immediate postnatal, 6-day postnatal and adult rats

Immediate postnatal animals were either naturally born animals less than 12 h old or prematurely delivered animals. The procedure used is detailed in the Materials and Methods section. Numbers in parentheses indicate the numbers of litters (in the case of immediate postnatal and 6-day postnatal) or individual animals (in the case of adults) used. Results are means $\pm$ S.E.

		$t_{\frac{1}{2}}$ (days)	
		Albumin	Transferrin
Immediate postnatal	(4)	1.24 $\pm$ 0.1	0.85 $\pm$ 0.1
Postnatal (6 days)	(4)	2.2 $\pm$ 0.3	1.71 $\pm$ 0.3
Adult	(8)	2.25 $\pm$ 0.1	1.55 $\pm$ 0.1

from the values obtained for 12 h postnatal animals. Table 5 summarizes these results. The values obtained for adult animals agree closely with those obtained by Masters *et al.* (1969). As indicated by the plasma half-life values, the turnover of both serum protein for adults and 6-day postnatal animals is much slower than the turnover in prematurely delivered and newborn animals.

Discussion

Validation of the immunoprecipitation technique

This study was undertaken to obtain information on the synthesis of the serum proteins, transferrin and albumin, in the embryo and during the neonatal period. The method used to isolate the proteins, immunoprecipitation of a mixture of intracellular and extracellular proteins, may be questioned on the basis of the results of Schreiber and his co-workers (Schreiber *et al.*, 1969; Maeno *et al.*, 1970). They found that immunoprecipitation of liver proteins on chromatography paper discs did not yield radiochemically pure albumin, and suggested that this may be due to the co-precipitation of radioactive compounds which were not covalently bound to albumin. However, there are several reasons for believing that the methods used in the present work are reliable and acceptable for their intended purpose, i.e. that of detecting and comparing rates of synthesis of specific proteins. First, it was found that at least 85% of the radioactivity, precipitated from liver extracts with antiserum against rat serum albumin, ran as albumin on sodium dodecyl sulphate-polyacrylamide-gel electrophoresis. This confirms the work of Cashman & Pitot (1971). Secondly co-precipitation of significant amounts of non-covalently bound radioactive compounds along with the specific immunoprecipitates may be ruled out because insignificant radioactivity was found when liver slices were mixed with [14 C]leucine in the cold

and then processed for immunoprecipitation without incubation at 37°C (zero-time values, Fig. 1). Thirdly, it has been shown that the mean rates of albumin synthesis *in vivo* calculated from the incorporation of [14 C]leucine into albumin precipitated immunologically from liver extracts or into albumin extracted chemically from serum differed by only 7% and agreed closely with synthesis rates obtained from albumin-turnover experiments (Morgan & Peters, 1971a). This also applies for transferrin except that in the case of transferrin the protein was extracted from both liver and serum by immunoprecipitation (Morgan & Peters, 1971b).

The results obtained by Schreiber and co-workers (Schreiber *et al.*, 1969; Maeno *et al.*, 1970) may be due to non-specific adsorption of labelled compounds to chromatography paper discs and to the fact that albumin is probably synthesized as a precursor, proalbumin, which differs chemically from albumin but is precipitated by antiserum against albumin, and which is subsequently converted into albumin within the liver cell (Judah *et al.*, 1973; Russell & Geller, 1973). The shoulder in the albumin radioactivity peak which was found in two of the sodium dodecyl sulphate-polyacrylamide-gel-electrophoresis runs performed in the present work may represent this proalbumin. One reason why non-specific adsorption of radioactivity to immunoprecipitates was not a problem in the present investigation may be because the liver extracts were cleared by use of a non-cross-reacting antigen-antibody precipitation before precipitating albumin and transferrin.

Protein synthesis by foetuses and foetal membranes

In the initial studies, embryos between 13 and 19 days of gestation and the membranes of these embryos were used. The results obtained indicated that significant albumin synthesis can be detected in rat foetuses of about 30 mg weight (>13 days gestation) onwards, whereas transferrin synthesis commences at a later stage in development, about 250 mg weight (15 days gestation) (Table 1). Lane (1968), using a fluorescent antibody, could not detect transferrin in the liver of a 1.23 g foetus. The present study which uses a more sensitive technique indicates that transferrin synthesis in the liver begins at an earlier stage. It is noteworthy that embryos weighing 600–1600 mg synthesize more transferrin than albumin. Further, the studies of neonatal liver transferrin and albumin synthesis indicate that during development the albumin/transferrin synthesis ratio in the liver altered from a value of 4.8 (foetus) to 7.1 (10 days postnatal) (Table 2).

Gitlin & Boesman (1967) demonstrated transferrin synthesis in the rat yolk sac but could find no evidence of albumin synthesis, although they found that the chick yolk sac (Gitlin & Kitzes, 1967) and the human

yolk sac (Gitlin & Perricelli, 1970) can synthesize albumin. In the present study, transferrin synthesis was demonstrable in the combined yolk sac and amniotic membranes of foetuses weighing between 30 and 1600 mg. This accounted for most of the transferrin synthesized by the foetus of 400 mg weight or less and a significant proportion (10%) of the total transferrin synthesized by the 600 mg foetus. The data further suggest that transferrin synthesis commences in the foetal membranes before the foetus itself acquires this capacity.

Contrary to the data of Gitlin & Boesman (1967), evidence of albumin synthesis was obtained with foetal rat membranes. Incorporation of [^{14}C]leucine was above blank values and increased linearly with time. In view of the results obtained and those of Gitlin & Boesman (1967), the antiserum to albumin used in the present experiments was rechecked by immunodiffusion and immunoelectrophoresis and was shown to be monospecific.

Protein synthesis by the liver

Morgan (1969) established that the liver is the primary site of transferrin and albumin synthesis in adult rats. It has been reported that in the adult rat, the bone marrow (Phillips & Thorbecke, 1966), spleen (Hochwald *et al.*, 1961; Morgan, 1969), macrophages (Stecher & Thorbecke, 1967) and mammary gland (Jordan & Morgan, 1969) synthesize transferrin. Thorbecke *et al.* (1965) have also shown that the thymus of the newborn rat synthesizes transferrin.

In view of the results obtained by Morgan (1969), further studies were carried out to determine rates of transferrin and albumin synthesis by the rat liver during the neonatal period. In order to make comparisons between the liver of animals of different ages, it was necessary to eliminate the complication of changing free amino acid pools in serum and transplacental loss of radioactive label administered to foetal animals *in utero*. Therefore a liver-slice system *in vitro* was selected. This also avoided the difficulty of administration of radioactive label to foetuses and immediate postnatal animals and the resultant alteration of amino acid pool sizes. As mentioned in the Results section, the amount of leucine introduced with the tissue slices represented less than 0.1% of the amino acid present in the system *in vitro* and should not influence the rates of synthesis obtained.

The changes in transferrin and albumin synthesis in the neonatal rat liver slices (Fig. 2) closely parallel the change in parenchymal cell numbers reported by Greengard *et al.* (1972). The decrease in cell number/cm³ of liver just before birth can account for the decreased synthesis/unit wet wt. of liver of both plasma proteins for the corresponding period.

This may, in part, account for the decrease in plasma transferrin concentrations (Fig. 4) which occurs immediately after birth. These data, and the finding that partially purified haemopoietic cells will incorporate ^{59}Fe into haem but not [^{14}C]leucine into transferrin or albumin, strongly suggest that the liver parenchymal cells are the site of transferrin synthesis in neonatal rat liver.

Liver slices from neonatal rats (5 days postnatal) synthesized more transferrin relative to albumin than animals aged 10 days or more. A good correlation between total soluble-protein synthesis and transferrin and albumin synthesis is obtained in animals from 10 days postnatal onwards, in contrast with the neonatal period. This lack of correlation is possibly due to protein synthesis by liver haemopoietic cells, which is suggested by the rapid postnatal decline in total soluble-protein synthesis which coincides with the decrease in liver haemopoietic cell population which occurs after birth (Lucarelli *et al.*, 1964; Greengard *et al.*, 1972).

Serum protein concentrations

In neonatal animals the serum concentrations of transferrin and albumin do not reflect the rates of synthesis found in liver slices; in fact, after about 10 days of age the serum concentrations rose whereas the synthesis values decreased. This may be due to changes in (i) plasma volume relative to liver weight, (ii) turnover rates of the proteins or (iii) rates of secretion. In the case of transferrin, the additional possibilities of extrahepatic sites of synthesis and of uptake of transferrin by liver haemopoietic cells must also be considered.

On a wet-weight basis foetal liver has nearly twice as much transferrin as adult liver (Table 3). Measurements in partially purified preparations of haemopoietic cells suggest that these cells contain transferrin, although the present study shows that they do not synthesize transferrin. This infers that the haemopoietic cells take up the transferrin that is synthesized by the neighbouring parenchymal cells of the liver. It is therefore proposed that although the 19–21-day foetal liver actively synthesizes transferrin, the animals of this age group have relatively low serum concentrations of the protein because haemopoietic cells present take up transferrin.

The study of turnover rates of serum albumin and transferrin in animals close to term, between 6 and 12 days postnatal and adults reveals that the turnover of the proteins alters after term (Table 4). It is likely that this change occurs gradually and the available evidence suggests that it has taken place by 6–12 days after birth. Thus the increased plasma albumin and transferrin concentrations after about day 7 may in part be due to the increased stability of the circulating proteins. It is unlikely that the short half-lives

of transferrin and albumin found in the immediate postnatal period were due to expansion of the plasma volume as a result of growth, since corrections were made for this change in calculating the half-life (see the Materials and Methods section).

The ratio albumin/transferrin synthesis of 7.8 found with liver slices from adult rats (Table 2) may be compared with the ratio of catabolic rates derived from Table 5:

$$\frac{\text{Albumin catabolism}}{\text{Transferrin catabolism}} = \frac{38}{2.25} \times \frac{1.55}{5} = 5.9$$

where 38 and 5 mg/ml are the mean plasma concentrations of albumin and transferrin respectively in the adult rat (Fig. 4). Presumably the adult animals were in a steady state so that the rates of synthesis of the two proteins were equal to their rates of catabolism. Hence the difference between the two values, 7.8 found with liver slices *in vitro* and 5.9 found *in vivo*, is difficult to explain. Possibly it is a consequence of transferrin synthesis *in vivo* at sites other than the liver (Hochwald *et al.*, 1961; Phillips & Thorbecke, 1966; Stecher & Thorbecke, 1967; Morgan, 1969).

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