The Metabolism of Serum Proteins in Neonatal Rabbits

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ABSTRACT 1. Incorporation of S^{35}-labeled amino acids into serum proteins has been studied in neonatal and developing rabbits. It was found that, per unit weight, neonatal rabbits synthesized only about $\frac{1}{36}$ of the gamma globulin, $\frac{1}{7}$ of the beta globulin, $\frac{1}{2}$ of the alpha globulin, and $\frac{1}{8}$ of the albumin that an adult synthesized. The growing rabbit developed the ability to synthesize various serum proteins at different times.

2. Plasma volumes and serum protein concentrations were determined at different times during the growth period of the rabbit. Plasma volumes were found to be 1 and $\frac{1}{2}$ times larger in newborn animals than in adults, with a gradual decline to the adult level. The total serum protein concentration at birth was about 60 to 65 per cent of the adult value and gradually increased with growth as the plasma volume decreased.

3. Half-lives of homologous albumin and gamma globulin were studied. The half-life of albumin in neonates was nearly twice as long as the half-life in adults, the latter value being reached at 1 month of age. The half-life of gamma globulin in neonates was more than twice as long as the half-life in adults and reached adult values at 2 to 3 months.

4. Attempts were made to alter serum protein metabolism. Gamma globulin synthesis early in life was augmented with antigen injections.

INTRODUCTION

Relatively few observations have been made on the metabolism of all serum proteins during the neonatal period. Most of the information available on this subject deals specifically with gamma globulins or antibodies. It has been demonstrated in both mammals and birds that the neonate is capable of very little gamma globulin synthesis and that by one of a variety of means gamma

This research was supported by United States Atomic Energy Commission Contract No. AT (30-1)-1205 and United States Public Health Service Grant E-2052 (C1). Publication No. 234 of the Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh.

Miss Deichmiller has submitted this manuscript as partial fulfillment of requirements for Master's Degree.

Received for publication, November 16, 1959.

globulin or antibody is transferred to the neonate from the mother. Only after weeks or months does the ability to form antibody gradually develop in the offspring. The synthesis of other serum proteins at birth and during the developmental period and the transfer of these proteins from mother to offspring have attracted little attention. It is the purpose of this paper to present observations on the metabolism of all serum proteins in the neonatal and developing rabbit.

Materials and Methods

The non-inbred, neonatal albino rabbits with their respective does used in this study were brought to the laboratory at the age of 1 to 3 days. The lactating does were allowed Wayne rabbit diet and water containing 2 mg. potassium iodide per 100 ml. *ad libitum*. The young rabbits began to supplement their milk diet with laboratory pellets at 3 to 4 weeks of life. Nursing rabbits were separated from the doe 12 to 16 hours prior to bleeding to reduce the level of lipid in the circulation. All bleedings were made by cardiac puncture (IC).

1. MEASUREMENT OF INCORPORATION OF S^{35}-LABELED AMINO ACIDS INTO PROTEINS

The uptake of injected S^{35}-labeled amino acids into serum proteins during the first 26 hours was used as a relative measure of the synthesis of these proteins. It has been shown in adult rabbits that virtually all the initial incorporation of the S^{35}-labeled amino acids into serum proteins occurs within the first 12 hours after injection (1), and similar observations have been made with C^{14}-labeled amino acids (2). Once proteins are so labeled, they retain their labels until degraded (3). Following protein degradation, the S^{35} amino acids are either catabolized or made available for reincorporation into other proteins.

In preliminary studies it was found that IC injections of 0.2 mc. of S^{35} amino acids in the form of a yeast cell hydrolysate[1] per kilo of body weight would give easily measured labeling of serum proteins, and this dose was used throughout these experiments. The animals were exsanguinated 26 hours after injection when serum protein specific activities were still near maximum values. At this point virtually all the S^{35} in the serum was incorporated into protein and the binding of S^{35} amino acids to proteins by dithio bonds was negligible (4). Some of the more rapidly metabolizing proteins could have slightly less than maximum specific activity after 26 hours, but this was considered a small and constant experimental artifact.

The serum was separated into albumin and gamma, beta, and alpha globulins by electrophoresis on a starch-supporting medium using the technique of Kunkel and Slater (5) as modified by Kuhns (6). Two ml. of serum to which was added 0.5 ml.

[1] The S^{35}-labeled amino acids were contained in a yeast cell hydrolysate supplied by Abbott Laboratories. The principal S^{35}-containing constituents of the hydrochloric–formic acid hydrolysate were methionine, cystine, and glutathione. Approximate proportions of these were 55, 15, and 30 per cent respectively.

of the barbital buffer at pH 8.6 and ionic strength 0.1 was applied to a block measuring 46 × 9 × 1 cm. and was allowed to migrate 15 cm. from the site of application. After separation had taken place, the block was cut into 1.0 cm. segments. Each segment was suspended in 5.0 ml. of physiological saline and eluates filtered off by means of a vacuum pump. After dialysis against running tap water for 24 hours followed by distilled water for another 24 hours, volumes of the eluates were measured. Each dialysate was made 0.01 N with NaOH and analyzed for nitrogen by the Markham modification of the micro-Kjeldahl technique (7) and for S^{35} content (8).

Samples ranging up to 1250 μg. of protein per 0.3 ml. were counted on 1.0 inch steel planchets which were preheated in order to lower the surface tension between the metal and the solution, thereby giving a sample of uniform thickness (8). The planchets were dried and counted for a total of 4000 counts. All counts were corrected for self-absorption, background, and S^{35} decay. Specific S^{35} activity of the protein fractions was then determined by dividing the corrected S^{35} counts by the amount of nitrogen in the 0.3 ml. sample.

2. MEASUREMENT OF THE SIZE OF THE PLASMA VOLUME AND RATES OF CATABOLISM OF HOMOLOGOUS ALBUMIN AND GAMMA GLOBULIN

The dilution and disappearance of the I^{131}-labeled homologous proteins from the blood stream of the neonatal and developing rabbits were determined in order to calculate the size of the plasma volume and the rate of catabolism of the proteins. Two mg. of the homologous albumin (RSA) or gamma globulin (RGG) prepared by ethanol fractionation (9), trace-labeled with I^{131} (I*RSA and I*RGG), and containing an average of one or less iodine atoms per molecule of protein (10), were injected by the IC route in doses of 0.2 to 0.3 ml. containing 5 to 10 μc. per 100 gm. of body weight. Five minutes later 0.7 to 1.0 ml. of blood was taken from the heart with a clean syringe and needle for the determination of the circulating plasma volume. For the disappearance studies, similar bleedings were made at approximately 3 day intervals. As the rabbits grew and the I^{131}-labeled proteins decreased, another injection of labeled protein was given to permit continuation of the degradation studies to maturity. This second injection was made when there was 2.5 per cent or less of the original I^{131}-labeled protein remaining in the blood stream.

Two different experimental approaches were used to determine the rate of catabolism of the labeled homologous proteins. First, the rate of loss of I^{131}-labeled protein from the plasma was determined. In calculating the protein half-lives by this method, allowances were made for the decay of the I^{131}, gain in weight of the rabbits, and the removal of labeled protein at each bleeding. Urine samples did not contain labeled protein. Second, the total radioactivity of the entire animal and the amount of the protein bound and non-protein bound I^{131}-labeled protein in the animal were measured. Animals were sacrificed and autopsied at various times after injection. Skin, viscera, stomach, and carcass were counted for total radioactivity, after which each fraction was homogenized and centrifuged and the protein of the supernate was precipitated with an equal volume of 20 per cent TCA for the determination of the protein bound and non-protein bound radioactivity. The decline in protein bound

radioactivity and the accumulation of non-protein bound radioactivity indicated catabolism of labeled protein.[2]

3. PROCEDURES EMPLOYED TO ALTER THE PATTERN OF SERUM PROTEIN METABOLISM IN NEONATAL ANIMALS

Two procedures were employed in attempts to alter the pattern of serum protein metabolism in neonatal rabbits: (*a*) during the first 2 weeks of life, two injections totaling 3.2×10^9 of *Shigella flexneri* type 3 organisms were administered into the footpads and two injections totaling 2×10^9 sheep or human erythrocytes were given intramuscularly; (*b*) sodium ribonucleate (RNA) was injected subcutaneously six times per week from the 1st or 2nd day of life until 60 days of age at a dose of 5.0 ml. of a 5 per cent solution per kilo of rabbit. This schedule of RNA was similar to the one employed by Richter to produce hypergammaglobulinemia and amyloidosis in adult rabbits (11).

Paper electrophoretic analyses were made on the sera of animals treated in each of the above ways at 1 week of age and weekly thereafter. Serum protein nitrogens were determined by the Markham modification of the micro-Kjeldahl technique. In addition, in those neonates injected with bacteria and foreign red blood cells the levels of agglutinins to each of the antigens were determined at weekly intervals. The determination of agglutinin titres for *Shigella* was made according to the method of Harris *et al.* (12). The end-point of the agglutinin titres to the red blood cells was read in the same way but 2 ml. of 5 per cent cell suspension were used as antigen (7). In all these experiments, part of each litter was untreated and served as a control, being bled simultaneously with the treated animals of the same litter. Serum of the does, on arrival at the laboratory, was tested for agglutinins against human or sheep erythrocytes and the *Shigella* bacilli. Paper electrophoretic patterns and protein nitrogen concentrations were also run on this serum.

RESULTS

1. *Incorporation of S^{35}-Labeled Amino Acids into Serum Proteins*

The results of starch electrophoretic analyses of the serum proteins of groups of rabbits from 2 to 100 days of age are shown in Table I. During the 1st weeks of life, the total protein concentrations, beta globulin concentrations, and albumin concentrations were lower than the adult concentrations and gradually increased to mature levels within 3 to 4 months. The alpha globulin concentrations remained about the same throughout life. The gamma globulin concentrations were within the adult range at birth, dropping to less than $\frac{1}{3}$ of their initial value by the 3rd week of life and then gradually increasing to the adult level by 70 to 100 days of age.

[2] This latter procedure was employed at the suggestion of Dr. John Humphrey, Mill Hill, London.

Table II shows the S^{35} specific activities of the various serum proteins. These specific activities are dependent upon the amount of synthesis of the protein at the time the labeled amino acids were injected, the number of sulfur-containing amino acids in the protein, and the concentration of protein in the

TABLE I

SERUM PROTEIN CONCENTRATIONS

Age	Protein/ml. serum				
	Total protein	Gamma globulin	Beta globulin	Alpha globulin	Albumin
days	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
2	39.4	6.7	4.5	6.8	21.4
5	38.0	4.0	9.1	5.5	19.4
6	38.3	2.8	6.3	8.1	21.1
13	40.8	2.2	6.6	7.3	24.7
19	55.0	1.8	8.9	10.3	34.0
50	48.5	3.5	8.3	5.3	31.4
70	59.2	6.6	13.1	5.0	34.5
100	61.8	4.8	11.0	7.6	38.4

TABLE II

S^{35} SPECIFIC ACTIVITIES OF SERUM PROTEINS

S^{35}* counts after injection of 0.2 mc. S^{35} amino acids/kilo of body weight

Age	S^{35} counts/mg. protein			
	Gamma globulin	Beta globulin	Alpha globulin	Albumin
days				
2	0.6	9.4	15.5	3.6
5	1.0	4.3	17.4	5.5
6	5.0	11.4	17.0	5.3
13	9.1	14.4	16.6	9.0
19	14.2	17.2	19.9	10.5
50	34.9	27.9	32.6	12.4
70	20.2	29.2	35.8	18.5
100	39.0	41.8	52.2	26.1

* All counts must be multiplied by 10^2.

serum. Thus, gamma globulin, present in relatively high concentrations as a result of transfer from the doe, was being made in extremely small amounts in the neonate and had a very low specific activity during the first 2 weeks of life. With the fall in serum gamma globulin concentration and a gradual increase in its production during the early weeks of life, the specific activity of this fraction increased. The early specific activities in the alpha and beta globulin fractions were about $\frac{1}{4}$ of those achieved in adults. The incorporation of labeled amino acids into albumin was slight during the 1st week of

life, indicating relatively little formation of this protein. On the basis of the data in Tables I and II, it is difficult to appreciate the relative amounts of synthesis of the four different protein fractions at various ages, since both the amount of synthesis and the concentration of protein, much of which may have been passively transferred from the mother, affect these figures. In order to get a more meaningful relative expression of serum protein synthesis per unit weight of subject, the serum protein concentrations were multiplied by their specific activities and plasma volumes; the resulting measures of synthesis are presented in Table III. Since the concentration of sulfur amino acids in the molecules of each of the protein fractions is probably different, these figures cannot be used to compare directly the synthesis of one protein

TABLE III
SERUM PROTEIN SYNTHESIS
S^{35}* counts in specific proteins

Age	S^{35} counts/ml. serum			
	Gamma globulin	Beta globulin	Alpha globulin	Albumin
days				
2	2	26	64	47
5	2	21	52	58
6	8	39	74	60
13	11	52	67	122
19	15	90	120	209
50	57	109	81	183
70	54	155	72	259
100	73	180	155	392

* All counts have been corrected for plasma volume changes. They should also be multiplied by 10^4.

fraction with another. However, since the amount of S^{35} injected per weight of rabbit was kept constant at all ages, the relative quantity of synthesis per unit of body weight of a given protein can be compared from one age to another. It can be seen from Table III that on a weight basis the neonatal rabbit synthesized only about $\frac{1}{36}$ of the gamma globulin, $\frac{1}{7}$ of the beta globulin, $\frac{1}{2}$ of the alpha globulin, and $\frac{1}{8}$ of the albumin that an adult synthesized. A graphic representation of the increasing rates of serum protein synthesis during the first 50 days of life is shown in Fig. 1.

2. *Plasma Volumes and Rates of Catabolism of Homologous Albumin and Gamma Globulin*

Table IV shows the plasma volumes of rabbits of different ages, expressed as milliliters of plasma per kilo body weight. The plasma volumes found with

RSA and RGG were similar and indicated a volume early in life 1 and ½ times as large as in adulthood. The plasma volume decreased gradually, reaching adult levels during the 3rd month of life.

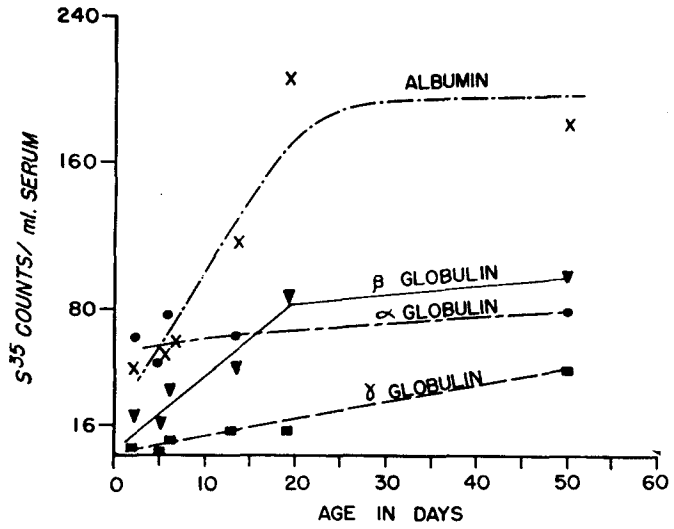

FIGURE 1. A graphic representation of the increasing rates of serum protein synthesis during the first 50 days of life. All S^{35} counts/ml. serum must be multiplied by 10^4.

TABLE IV
PLASMA VOLUMES

Age	Rabbit gamma globulin			Rabbit albumin		
	No. of rabbits	Av. wt.	Plasma volume/ kg. body weight	No. of rabbits	Av. wt.	Plasma volume/ kg. body weight
days		*gm.*	*ml.*		*gm.*	*ml.*
2–3	15	75	61	4	83	61
5	2	107	54	—	—	—
9–11	5	155	55	—	—	—
19–22	4	360	61	4	441	56
28–31	5	524	53	1	560	51
37–40	10	873	50	4	944	49
45–53	6	1311	46	4	1297	48
64–70	7	1809	40	6	1812	41

The results of the two methods used to measure catabolism of homologous albumin and gamma globulin were not in agreement. As shown in Table V, on the basis of loss of protein bound radioactivity from the circulation, the half-life of RSA in the 1st week or 10 days was about twice as long as the half-life found in rabbits 1 month old when adult values were attained. The loss of RGG from the circulation during the first 2 weeks appeared to be extremely

TABLE V

DISAPPEARANCE OF I*RSA AND I*RGG FROM THE CIRCULATION

Protein	No. of animals	Average half-lives of protein bound I^{131}									
		Age during measurement of labeled protein, *days*									
		3–6	6–10	8–14	14–22	22–30	33–40	40–47	54–61	83–87	94–111
		days	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*
RSA	5	11.8	8.5	5.5	6.0	5.1	5.3	5.2	4.7		
RSA	3			12.1	10.2	5.6	5.9				
RSA	4		14.0	6.8	5.2						
RGG	7	∞‡	32.6	19.0	11.5	11.5	12.6	9.9	9.9	7.4	6.0
RGG	3	∞‡		31.7	32.4	12.0	10.2				
RGG	3		15.0‡	36.3	13.2						

‡ Modal values.

TABLE VI

DISTRIBUTION OF PROTEIN AND NON-PROTEIN BOUND I^{131} FOLLOWING INJECTIONS OF I*RSA AND I*RGG INTO NEWBORN RABBITS

Age	Per cent of injected I^{131}										
	Skin		Carcass		Viscera		Stomach		Total		
	PB‡	NPB§	PB	NPB	PB	NPB	PB	NPB	PB	NPB	Total I^{131}‖
days											
RSA											
1/8	10	0	48	1	24	1	3	2	85	4	89
1	20	0	38	2	12	1	2	15	72	18	90
2	17	1	34	3	7	1	4	25	62	30	92
4	12	2	28	4	7	2	3	29	50	37	87
10	7	1	15	3	4	1	4	37	30	42	72
15	2	2	7	2	1	1	2	22	12	27	39
RGG											
1/8	8	0	44	0	26	1	1	2	79	3	82
1	19	1	44	1	15	0	1	11	79	13	92
2	15	2	36	2	11	1	4	11	66	16	82
4	17	1	35	3	12	0	2	21	66	25	91
10	11	2	25	2	8	1	4	35	48	40	88
15	6	2	14	2	6	1	4	40	30	45	75

‡ PB, protein bound I^{131}.

§ NPB, non-protein bound I^{131}.

‖ The difference between the total I^{131} found in the tissues and the amount injected may be accounted for by I^{131} in blood lost at autopsy and the I^{131} excreted.

slow; then the rate of loss increased for the next 2 to 3 months until adult values were attained.

The rate of breakdown of RSA and RGG in the neonates determined by measurement of the decrease in protein bound I^{131} and the accumulation of non-protein bound I^{131} in the entire animal (Table VI) was more rapid than

the rate determined by the loss of circulating protein bound I^{131}. If one accepts the appearance of non-protein bound I^{131} as evidence of catabolism of labeled protein, the half-life of RGG during the first 10 days of life was slightly more than 10 days and that of RSA slightly less than 10 days. The reasons for the disagreement between the results of these two methods are not apparent. However, since the latter method entails no assumption, it seems fairer to favor the values obtained by this method.

TABLE VII

SERUM PROTEIN CONCENTRATIONS AND AGGLUTININ TITRES TO SHEEP RBC AND *SHIGELLA*

Fraction	Group	No. of animals	Protein/ml. serum				
			Day* 7	Day 14	Day 21	Day 30	Does
			mg.	*mg.*	*mg.*	*mg.*	*mg.*
Gamma globulin	Controls	3	4.5	4.7	3.7	2.1	4.6
	Experimentals	5	5.6	5.0	5.6	4.3	
Beta globulin	Controls	3	8.2	7.5	9.7	7.0	6.3
	Experimentals	5	9.6	8.3	8.4	7.0	
Alpha globulin	Controls	3	7.8	8.4	6.6	6.5	7.4
	Experimentals	5	11.2	10.2	6.9	7.7	
Albumin	Controls	3	23.1	26.6	22.3	26.0	32.8
	Experimentals	5	16.6	22.2	19.1	22.8	
Total protein	Controls	3	43.6	47.2	42.3	41.5	51.1
	Experimentals	5	43.0	45.7	40.3	41.9	
			(Geometric mean titres)				
Titre sheep RBC	Controls	3	1.6	0	0	0	3
	Experimentals	5	1.2	83	96	332	
Titre *Shigella*	Controls	3	24	9.5	3	0	55
	Experimentals	5	7.4	36	664	1766	

* Day refers to age.

Both methods indicate slower catabolism of serum protein in early life than in adulthood and a slower turnover of RGG than RSA. These data also show that non-protein bound I^{131} presumably liberated by catabolism of labeled protein had a different fate in neonates than in adults. In adults non-protein bound I^{131} was rapidly eliminated by the kidneys, while in the first 10 to 14 days of life little excretion of the non-protein bound I^{131} was seen in spite of considerable urinary volumes. Instead, the non-protein bound I^{131} in the neonates was concentrated in the gastric contents, as observed by Humphrey (13). Assuming that the non-protein bound I^{131} was in the same chemical form in both neonates and adults (largely iodide) (14), it would appear that the neonatal kidney did not have the ability to eliminate non-protein bound I^{131} that the adult kidney had.

3. *Experimental Alteration of Serum Protein Metabolism*

Table VII shows changes of serum protein concentrations caused by injections of large amounts of the two particulate antigens. While only an occasional immunized animal at 2 weeks of age showed an increased gamma globulin or antibody response, by 3 to 4 weeks the immunized animals showed considerably more serum gamma globulin than the control litter mates. In normal animals the serum gamma globulin reached a low point at 3 to 4 weeks. However, immunized animals were making antibody at this time and showed little or no drop in the gamma globulin concentration present at birth. In the controls, the low titre to *Shigella* present at birth as a result of the gamma globulin

TABLE VIII

SERUM PROTEIN CONCENTRATIONS IN RABBITS RECEIVING RNA INJECTIONS*

Fraction	Group	No. of animals	Protein/ml. serum				
			Day 9	Day 20	Day 30	Day 46	Day 60
			mg.	*mg.*	*mg.*	*mg.*	*mg.*
Gamma globulin	Controls	5	3.5	2.3	1.4	6.0	11.6
	Experimentals	8	3.6	4.0	3.7	6.6	9.4
Beta globulin	Controls	5	5.5	11.5	6.8	7.5	5.9
	Experimentals	8	9.9	11.5	9.8	10.5	10.3
Alpha globulin	Controls	5	7.0	6.5	4.0	4.1	6.1
	Experimentals	8	10.3	8.2	5.9	7.4	9.1
Albumin	Controls	5	30.9	34.5	36.4	35.4	35.2
	Experimentals	8	19.7	25.9	35.5	34.2	30.2
Total protein	Controls	5	47.1	54.3	48.6	53.0	58.7
	Experimentals	8	43.6	49.5	54.8	58.8	59.1

* RNA was injected 6 times per week from the 1st or 2nd day of life until 60 days of age at a dose level of 5.0 ml. of a 5 per cent solution per kilo of body weight.

passively transferred from the doe disappeared by 3 to 4 weeks, coinciding with the decrease in gamma globulin.

The results of the RNA injections are listed in Table VIII. It seems that the RNA did have an enhancing effect on the gamma globulin synthesis during the 1st month of life but not thereafter. Gamma globulin levels in the RNA-treated animals 20 to 30 days of age were about double the levels in controls.

DISCUSSION

The serum protein concentrations present in the neonatal rabbit are determined by three factors: (*a*) the transfer of proteins from mother to offspring *in utero*, (*b*) the catabolism of the proteins by fetus and neonate, and (*c*) the

synthesis of the proteins by fetus and neonate. On the basis of observations at hand, it is impossible to evaluate each of these factors with any degree of certainty. It is possible, however, to say that the total effect of these factors resulted in widely differing concentration ratios between neonatal and maternal sera for the various serum proteins. The concentrations of the gamma and alpha globulins in the neonate were close to those seen in the maternal serum; however, the concentrations of beta globulin and albumin in the neonatal serum were only about ½ of those seen in the maternal serum. In the case of gamma globulin, if one assumed that the rates of synthesis and catabolism in the fetus were the same as those in the neonate, the gamma globulin levels in fetal and maternal circulations were in near equilibrium late in gestation. The roles of synthesis and degradation of alpha globulin in the neonate and fetus in achieving serum concentrations similar to those of the doe could not be determined. If the moderate rate of alpha globulin formation in the neonate applied to the fetus, it might be that part of this protein present at birth was made by the fetus. Possible explanations for the low levels of beta globulin and albumin at birth might be either a rapid catabolism of these proteins in the fetus or a poor transfer of these proteins from maternal to fetal circulation or both. The relatively greater catabolism than synthesis of albumin in the neonate might suggest a similar situation in the fetus which would tend to keep levels of circulating albumin low.

The status of serum protein synthesis from birth onward was more directly discernible from the observations of this study. Table III and Fig. 1 indicate little synthesis of albumin, beta globulin, and gamma globulin during the first days of life. The amount of albumin synthesis increased rapidly during the first 3 weeks after birth. Beta globulin synthesis increased somewhat more slowly during the same period while gamma globulin synthesis had a gradual increase over the first 2 to 3 months. The increase of alpha globulin synthesis during the first 2 months was less consistent and apparently slower than that of the other proteins. It must be remembered that the various globulin fractions are not homogeneous materials but that each fraction is composed of a number of molecular species and the observations made here represent only an average value for each group of molecules. It appears from these observations that the small amount of synthesis of gamma globulin at birth is not unique since beta globulin and albumin are also poorly synthesized by the neonate. The most marked difference between the synthesis of gamma globulin and of beta globulin and albumin was the rate at which these abilities were developed during the neonatal period. The ability to synthesize gamma globulin developed far more slowly than that for other proteins.

In view of the low concentration of serum protein in the neonate, it was of interest that the plasma volume per unit of body weight was approximately 1 and ½ times the adult value. Consequently, at birth and during the months

of development, the total circulating serum protein was approximately equal to the adult value. During this period the protein concentration gradually rose and the plasma volume declined to adult values. Attempts were made to determine the extravascular plasma protein pool in the young rabbits, but the slow elimination of non-protein bound iodine by the kidneys made this impossible.

Our two sets of observations on the rates of catabolism of homologous albumin and globulin in the young rabbits did not provide consistent information. The most reliable data in this area appear in Table VI and indicate an increase in non-protein bound radioactivity and a decrease in protein bound radioactivity during the first 2 weeks following injection of labeled protein. It was unformate that this type of observation could not be made serially on the same animal but rather that different animals had to be used for each point. In spite of this limitation, these figures suggest an approximate 10 day half-life of RGG and a somewhat shorter half-life of RSA in the first days after birth. An interesting by-product of this phase of the work was the observation originally made by Dr. Humphrey and confirmed here that non-protein bound I^{131} apparently liberated by degradation of labeled protein was not excreted in the urine of the neonate but rather concentrated in its gastric contents (13). This failure of renal excretion of non-protein bound iodine was found in the presence of considerable urinary volumes and persisted for the first 10 to 14 days of life. It is not apparent why there is no agreement between the rates of catabolism noted in Table VI and the rates of loss of labeled protein from the circulation given in Table V. The procedure used to obtain the information given in Table V was that commonly used in determining the half-lives of proteins in adult human and animal subjects. With this method repeated observations were made on the same animal. The apparent rates of degradation of RSA and RGG in the neonatal period, as shown in Table V, were considerably slower than the approximations arrived at from Table VI. With both methods, however, the rates of catabolism were slower in the neonatal period than they were in the adult period, and in both instances the rate of degradation of gamma globulin was slower than that of albumin. If one assumed that the observations made by both methods were correct, one would have to postulate a progressive shifting of serum protein from extravascular to intravascular pools during the neonatal period. Since we were unable to determine extravascular protein pools, we were unable to evaluate this possibility.

Our attempts to change the pattern of serum protein metabolism during the neonatal period induced significant changes, particularly in the synthesis of gamma globulin. Immunization of the neonates with large doses of particulate antigens as described by Sterzl and associates (15) produced a significant increase in gamma globulin levels and detectable antibody in the

neonates during the latter half of the first month of life. It would seem reasonably safe to assume that both the increased gamma globulin levels and the appearance of antibody represented increases in gamma globulin synthesis. The immunized neonates showed lower levels of circulating albumin than did their control litter mates, but the total protein of both groups was the same.

The enhancing effect of repeated RNA injections during the first months of life on the levels of circulating gamma globulin was much less than that seen in adult rabbits. Increases in circulating gamma globulin levels produced by RNA were detectable only during the latter part of the first month after which time both controls and RNA injected animals had similar levels.

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