Immunology, 1961, 4, 380.

The Metabolism of Homologous and Heterologous Serum Proteins in Baby Rabbits

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(*Received 28th December* 1960)

Summary. The catabolism of ^{131}I-labelled rabbit and human albumin and γ globulin has been studied in baby rabbits from the 1st to the 6th week after birth. The half-lives, based in plasma concentrations corrected for growth, were approximately constant throughout this period, and were as follows: rabbit albumin and human albumin, about 6 days; rabbit γ globulin and human γ globulin, 12–14 days. Until the age of about 3 weeks, renal excretion of iodide was very inefficient. Consequently free ^{131}I accumulated in the stomachs, and the whole body radioactivity greatly exceeded that due to retained labelled protein.

INTRODUCTION

In order to calculate how long heterologous proteins, which have been injected into newborn rabbits, are likely to persist in the circulation, it is necessary to know the metabolic half-life of these proteins in rabbits at various stages of development. Although there is plenty of published information relating to the turnover of rabbit or human albumin and γ globulin in adult rabbits, such information is lacking in respect of baby rabbits, apart from investigations carried out independently by Deichmiller and Dixon (1960), with whom the author has exchanged information. In so far as baby rabbits are born with low plasma levels of albumin and of γ globulin and the level of γ globulin continues to fall during the first 3 weeks of life, before the rate of synthesis is sufficient to counteract the combined effects of catabolism and of dilution due to expanding plasma volume, the catabolic rates during this early period have an additional interest.

The technique adopted in the experiments described below has been to follow the disappearance from the blood stream, and from the whole animals, of rabbit albumin (RSA) and γ globulin (RGG) and of human albumin (HSA) and γ globulin (HGG) labelled with ^{131}I. Provided that only trace amounts of iodine are introduced, and that the proteins are not denatured during preparation or labelling, their behaviour does not differ significantly from that of the native proteins (McFarlane, 1957; Freeman, Matthews, McFarlane, Bennhold and Kallee, 1958). Whereas adult animals can eliminate ^{131}I-labelled breakdown products as fast as they are formed and the radioactivity in the whole body is a good indication of the amount of protein remaining, it was found that baby rabbits retained free iodide and that erroneous results were obtained unless this was taken into account.

MATERIALS AND METHODS

ANIMALS. Sandylop rabbits, bred at the N.I.M.R., were used. They were fed on Bruce and Parkes pelleted diet 18 (1946). The does chosen were known to be good mothers and

were housed in a room set aside for breeding purposes. Their drinking water contained KI 5 mg./l. and NaCl 450 mg./l., or KI 0.5 mg. and NaCl 45 mg./l. The babies were weighed regularly.

HOMOLOGOUS AND HETEROLOGOUS PROTEINS. Rabbit serum albumin (RSA) was prepared by electrophoresis in a vertical column of treated cellulose (Gedin and Porath, 1957). Human serum albumin (HSA) was a crystallized, unheated preparation kindly provided by Professor Schulze (Behringwerke, Marburg-Lahn). Rabbit γ globulin (RGG) and human γ globulin (HGG) were prepared by chromatography of serum on DEAE cellulose (Sober, Gutter, Wyckoff and Peterson, 1956).

The proteins were examined by electrophoresis on paper in veronal buffer pH 8.6, and appeared to contain only a single component.

IODINATION. The proteins were labelled with ^{131}I, at an average of less than 1 atom/mol., by the ICl method of McFarlane (1958). After removal of unreacted iodide by passage through a column of Deacidite FF in the chloride form (Permutit Ltd., Gunnersbury), the proteins were mixed with 0.5–1.0 ml. normal rabbit serum and dialysed under pressure against physiological saline buffered at pH 7.

MEASUREMENT OF RADIOACTIVITY. The radioactivity in blood serum samples was measured in a well-type scintillation counter with efficiency 18 per cent, and counting was continued for long enough to give an accuracy of ± 3 per cent. The radioactivity in each animal, and in the various tissues and perfusion fluids was measured in the ring counter described by Campbell, Cuthbertson, Matthews and McFarlane (1956). Corrections were made for the variations in counting efficiency due to the geometry of the samples counted and for decay of radioactivity. The distribution of radioactivity in serum proteins was determined by subjecting them to paper electrophoresis and then scanning the paper strip with a GM tube through a collimated slit.

EXPERIMENTAL PROCEDURE. Injections of labelled or unlabelled proteins were given intracardially or intraperitoneally. Blood samples were taken from the ear, after cutting the marginal vein, and care was taken to avoid loss of fluid by evaporation before clotting had occurred. Serum was collected with a micropipette, diluted if necessary and mixed with a few mg. KI, and the protein precipitated by addition of an equal vol. 20 per cent trichloroacetic acid (TCA) followed by heating at 80° for 5 minutes. The precipitated protein was washed on the centrifuge with 10 per cent TCA, and dissolved in alkali. Aliquot samples of the first supernatant fluid and of the washed protein were used for radioactivity measurements.

Blood samples were taken, and whole body counts measured every 3 or 4 days. In order to determine the distribution of protein and non-protein radioactivity in the tissues of rabbits at various ages, some of the animals were given 1000 i.u. heparin i.v., anaesthetized with sodium amytal and perfused with Ringer's solution via the left auricle. The blood and perfusate were collected from the cut right auricle and perfusion was continued until no visible blood remained. The animals were then dissected, the tissues were finely minced and suspended in saline, and the protein precipitated with TCA for estimation of protein and non-protein radioactivity as described above. Care was taken to examine the thyroid and salivary glands.

RESULTS

Three separate experiments were made and were designed to test whether catabolism of heterologous proteins would be affected by injecting, on the day of birth, sufficient of

the amount of the protein to produce a state of immunological tolerance. Accordingly, all the babies (4–7) in two litters were given 30 mg. unlabelled HSA or HGG intraperitoneally within 24 hours of birth. About 5 mg. (50 μC) labelled RSA, RGG, HSA or HGG were administered by the same route 4–6 days later. In another experiment similar amounts of labelled RSA or RGG were given intracardially 1–2 days after birth and, in another, labelled HSA or HGG were given 6 days after birth.

Since baby rabbits are relatively 'juicy' compared with adults, it might be expected that the distribution of proteins between the intravascular and extravascular compartments would alter as the babies grew older and thereby complicate any calculations of catabolic rates. This was investigated by perfusion, as described above, of some of the babies which had received homologous proteins, and the main findings are given in

TABLE 1

DISTRIBUTION OF RADIOACTIVITY IN TISSUES OF BABY RABBITS AFTER INJECTION OF ^{131}I-LABELLED PROTEINS

The figures below give the percentage of the [^{131}I] protein present in different tissues of rabbits perfused at various ages and time intervals after giving the label. In parentheses are indicated the percentage of the total (protein+non-protein) ^{131}I.

	Age in days (days after injection in parentheses)		
	9(8)	*13(6)*	*20(19)*
[^{131}I] RGG in Blood+perfusate	36(27.3)	40(25.5)	41.3(31.4)
Carcase	40.5(33.6)	30.6(23.5)	38.2(28.7)
Skin	20.5(16)	18.5(12)	16.1(12.1)
Stomach and intestine	2.5(22)	9.5(38.8)	3.3(27.2)
Liver+spleen	0.5(0.4)	0.3(0.2)	0.9(0.7)
	11(6)		*20(19)*
[^{131}I] RSA in Blood+perfusate	33.4(23)		31.9(13.6)
Carcase	43 (29.7)		33.2(21)
Skin	19.5(13.5)		20.2(8.6)
Stomach and intestine	3.7(33.5)		14.5(56.7)
Liver+spleen	0.4(0.3)		0.2(0.1)

Table 1. It will be seen that at 9–19 days some 32–41 per cent of the protein radioactivity was recovered in the blood, and that the proportion of labelled protein in the skin and remainder of the carcase did not vary greatly and the variation was probably within the error of the experiment. In adult rabbits perfused in the same way 40–45 per cent of labelled protein is generally recovered in the blood and perfusate. This may indicate that a rather greater proportion was extravascular in the babies, but the difference is not sufficient greatly to affect calculations of catabolic rates. The thyroid glands in no case contained more than 2 per cent and the salivary glands more than 1 per cent of the total counts. The distribution of non-protein counts will be discussed below.

As a further check, to make sure that only the protein injected remained labelled, the distribution of radioactivity in the serum samples was measured on two occasions in each group of animals. Even 19 days after injection no radioactivity was detected in any serum protein fraction other than that which contained the injected material.

The rates of elimination based on serum protein radioactivities are shown in Fig. 1. The babies were growing rapidly throughout the 40 days of observation and the remaining

protein in the circulation was consequently being steadily diluted by newly formed plasma and lymph. As had been shown above, however, although the distribution of labelled protein in the tissues altered during development, the change was not very great. The measured specific activities in the serum samples (protein ct./ml.) were therefore corrected

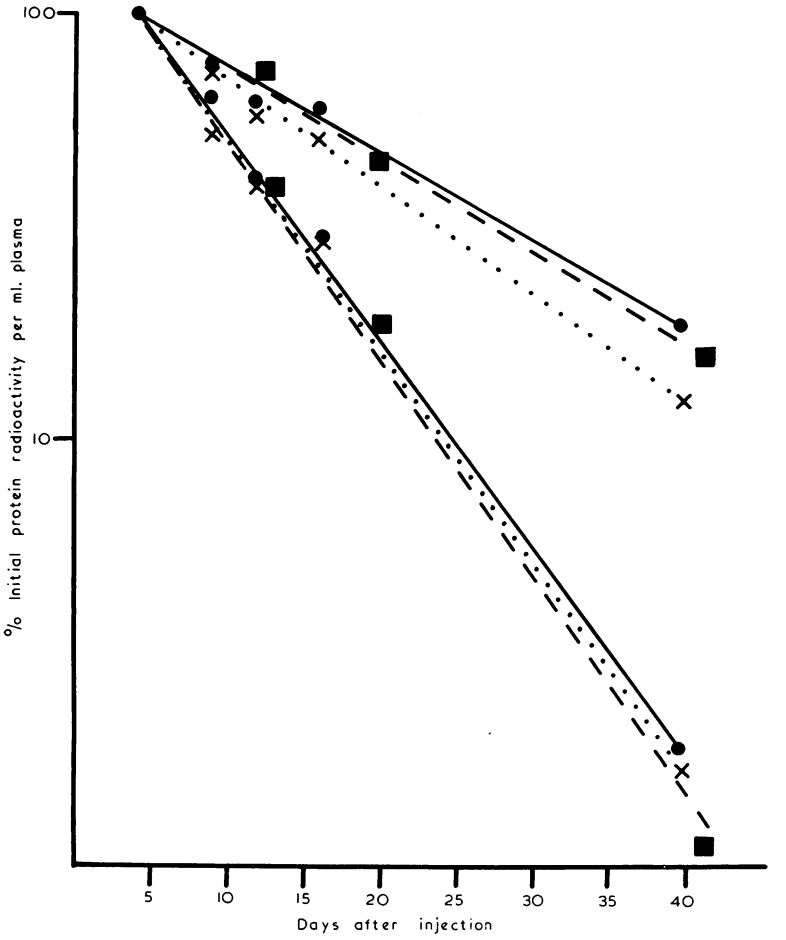

FIG. 1. *Elimination of proteins from blood plasma of baby rabbits (corrected for growth)*

●——● { [^{131}I] RGG (5 mg.) injected 6 days after birth (six rabbits)
[^{131}I] RSA (5 mg.) injected 4 days after birth (four rabbits)

■ - - - ■ { [^{131}I] HGG (5 mg.) injected 6 days after birth; 30 mg. HGG at birth (two rabbits)
[^{131}I] HSA (5 mg.) injected 4 days after birth; 30 mg. HSA at birth (two rabbits)

× - - - × { [^{131}I] HGG (3 mg.) injected 6 days after birth (three rabbits)
[^{131}I] HSA (4 mg.) injected 6 days after birth (three rabbits)

The upper curves relate to RGG and HGG and the lower curves to RSA and HSA.
The points represent mean values. In A and A′ the number of rabbits used diminished as individuals were sacrificed for determination of labelled protein distribution.

to a standard size for each baby by multiplying a factor = wt. at time of sampling/wt. at time of first sample. Zero time was taken as 2–3 days after intraperitoneal injection of the labelled protein, in order to allow complete absorption into the circulation and equilibration between blood and lymph.

It will be seen that the fractional rates of elimination from the whole plasma, when corrected in this way, were approximately constant from the 6th to the 40th days of life; that both rabbit and human γ globulins had half-lives of 12–14 days, while both rabbit and human serum albumins had half-lives of 5¼–6½ days; and that preliminary injection of 30 mg. heterologous protein to induce immunological tolerance did not obviously affect the elimination of a test dose administered soon afterwards. Because of the considerable assumptions involved in correcting the measured plasma concentrations of labelled protein, it was desirable to check the estimated half-lives by directly measuring the amount of radioactive protein remaining in the rabbits after different time intervals and comparing it with the amount administered. This was done in the case of those animals which were killed and perfused for determination of the distribution of protein-bound and free ^{131}I. The results (Table 2), although few, and variable from rabbit to rabbit, corroborate the conclusions based on plasma measurements. Thus the mean breakdown rate for RGG (5.5 per cent per day) corresponded to a half-life of 12.5 days, and that for RSA (8.9 per cent per day) to a half-life of 7.5 days.

TABLE 2

ELIMINATION OF RSA AND RGG MEASURED DIRECTLY

Material injected	*Age (days) at time of*		*% of injected protein remaining*	*Corresponding rate of breakdown (% per day)*
	Injection	*Death*		
[^{131}I] RGG	1	9	60	6
	2	10	71	4·5
	1	20	46	4·1
	6	12	64	7·5
[^{131}I] RSA	4	10	55·5	9·3
	1	20	18	8·5

ELIMINATION OF RADIOACTIVITY MEASURED BY WHOLE BODY COUNTING

In adult animals non-protein ^{131}I (largely inorganic iodine) resulting from catabolism of protein is eliminated in the urine so fast that measurement of the whole body radioactivity remaining in the animals gives a reasonably close approximation to the amount of intact radioactive protein. Similar measurements in baby rabbits gave a wholly misleading picture (Fig. 2). As judged by whole body counting, the half-life of RGG was 49 days and of RSA 22 days during the first 20 days, but at about the 22nd day of life the half-lives decreased abruptly. The explanation lay in the fact that during the first 3 weeks of life baby rabbits excrete iodide through the kidney very inefficiently, whereas their salivary glands excrete iodide well. Thus the intestinal tract was found to contain, at various times, 22 to 57 per cent of the total radioactivity in the body, at least nine-tenths of which was in the form of inorganic iodide. By far the greater part of the radioactivity was in the stomach, the ^{131}I content (protein and non-protein) of the duodenum, jejunum and ileum being little greater than would be expected from the presence of lymph together with traces of blood remaining after perfusion. Inorganic iodide excreted in the saliva or by the gastric mucosa appears therefore to collect in the stomach and then promptly to be reabsorbed into the blood stream from the small intestine, only to be re-excreted by the same route — while a much smaller portion is excreted by the kidneys. The very poor capacity of the kidneys of new-born infants to excrete chloride has been described by McCance (1948).

DISCUSSION

Although the experiments had been expected to distinguish between the elimination rates uf HSA and HGG in tolerant and non-tolerant baby rabbits, the fact that immune elimination had not occurred after 40 days in the animals which received only 5 mg. heterologous protein 6 days after birth, instead of 30 mg. within 24 hours, suggests that this dose had sufficed to render them also tolerant. From the work of Smith and Bridges

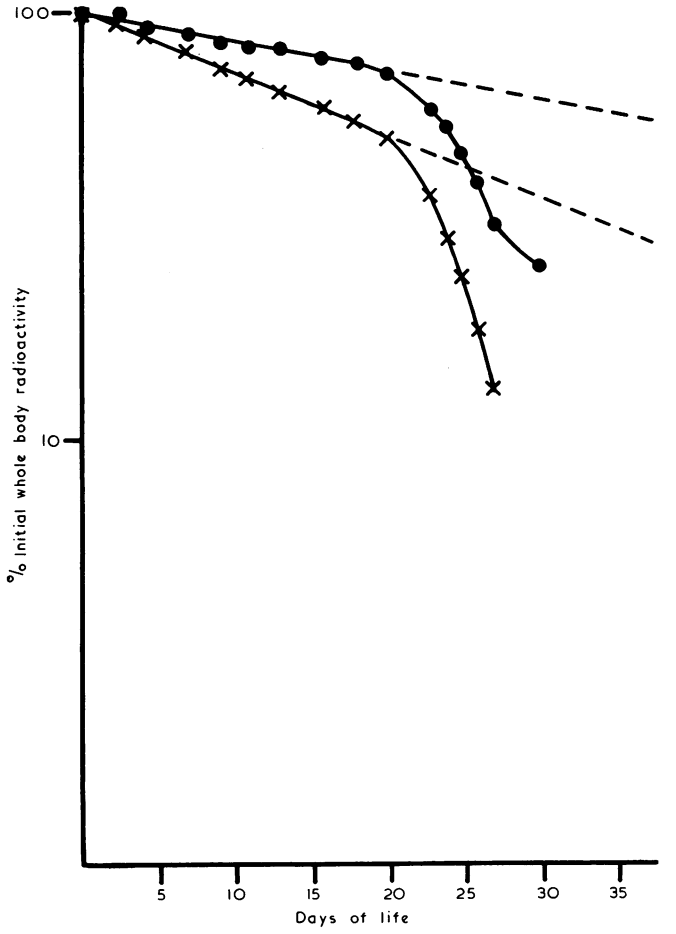

FIG. 2. *Elimination of total radioactivity from baby rabbits after* ^{131}I*-labelled proteins*

●——● Per cent of initial whole body counts remaining after [^{131}I] RGG (3 mg. on day after birth)

X——X Per cent of initial whole body counts remaining after [^{131}I] RSA (3 mg. on day after birth)

(1958) which appeared after this work was completed, this result would have been expected.

As already mentioned, it was not possible to deduce the amount of radioactive protein remaining in the animals from their whole body counts, because of the failure to excrete iodide quantitatively. Since the babies were growing rapidly, the concentration of labelled protein in the plasma was falling owing to the increase in volume of the plasma and lymph

pool, irrespective of catabolism. A correction was made for this, on the assumption that the pool remained proportional to the weight of the animals. Although this assumption was shown by the perfusion experiments to be approximately correct, a possible inaccuracy is nevertheless introduced into the calculations of the rate of disappearance of labelled protein from the plasma. The differences between the observed half-lives of albumin and γ globulin are, however, far too large to be due to inaccuracies from this source. Furthermore, the data given by Deichmiller and Dixon (1960) relating to the plasma volumes of newborn rabbits indicate that these bear an approximately constant relationship to body weight at least for the first 3 weeks of life, although they are proportionately about one and a half times larger than the plasma volumes of adult rabbits.

From the end of the 1st week until the 6th week the half-lives of RGG deduced from the experiments described above are about 13 days, and of RSA or HSA about 6 days. Deichmiller and Dixon (1960) also estimated the half-lives of RGG and RSA labelled with ^{131}I. Their estimates varied according to the method of calculation used. When based on the loss of protein radioactivity from the circulation the half-life of RGG was too long to measure at 3–6 days, was 15–33 days at 6–10 days and fell to about 12 days by the 22nd day after birth; that of RSA was 12 days, 8.5–14 days and about 5.6 days at corresponding ages. However, when calculated from the overall loss of protein ^{131}I from the body, the half-life of RGG during the first 10 days of life was slightly over 10 days and that of RSA somewhat under 10 days. In a complicated situation in which catabolism of ^{131}I-labelled proteins is being measured in growing animals whose plasma and lymph protein pools are changing both because of growth and of varying rates of synthesis, corrections for these factors are difficult to apply. The second method of calculation used by Deichmiller and Dixon is similar to that used in Table 2, and entails no assumptions, so that these authors are inclined to give it greater weight. The results so obtained are closer to those reported here. The latter are also subject to assumptions about pool size, although they were directly checked in some instances by measurement of the loss of protein-bound ^{131}I from the bodies of individual baby rabbits, and reasonable agreement was obtained.

Although it is difficult to give accurate values to the half-lives, there is no doubt that RGG and HGG are catabolized in newborn rabbits at a rate substantially slower than that in older animals, whereas the half-lives of RSA and HSA are much the same at any age. It is interesting to consider why this should be so. Inasmuch as plasma protein catabolism appears to occur predominantly in close relation to the blood stream, any differences between the distributions of albumin or globulin between blood and lymph will affect their respective catabolic rates. Although there were differences between babies and adults, they were too small to provide an explanation of the different behaviours of albumin and γ globulin. The only obvious difference between the two types of protein in baby, as compared with adult rabbits is that the concentration of γ globulin in babies falls to about one-tenth that in adults, whereas that of albumin does not go below about two-thirds. There is evidence in man that low concentrations of γ globulin, in hypogammaglobulinaemia, are associated with a relatively long half-life for this protein (about 34 days, instead of the normal adult value of about 20 days — Martin, Gordon, Felts and McCullough, 1957), and that in new-born children the half-life of maternal diphtheria antitoxin is 35 days (Barr, Glenny and Randall, 1949) and of incomplete rhesus antibodies 30 days (Wiener, 1951). Furthermore, in a subject lacking serum albumin the half-life of homologous albumin was 56 days instead of the usual 24 days (Freeman, Matthews, McFarlane, Bennhold and Kallee, 1959). However, in none of the instances cited were

the half-lives of both albumin and γ globulin compared, and it is not certain whether the catabolism of both would have been slow. Furthermore, in the baby rabbits the half-life was prolonged throughout the whole of the period of observation, despite the fact that the γ-globulin level fell and then rose during this time.

Incidental observations in other species suggest that the occurrence of large differences between infants and adults in the rates of breakdown of homologous or heterologous γ globulins is by no means the rule. Thus Humphrey and Turk (1961) mention that the half-life of HGG in baby guinea pigs was 7–8 days, similar to that in adults; and Bangham and Terry (1957) in suckling rats found half-lives of about 5.2 and 4.7–8.0 days for rat and monkey γ globulin respectively, which again are not greatly different from those found in adult rats of the same strain.

The explanation will probably have to wait until more is known about the sites and mechanism of plasma protein breakdown.

ACKNOWLEDGMENT

I would like to express my thanks to Mrs. R. de Rossi for her help with these experiments.

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