The Survival of Globulins Absorbed from the Gut in Suckling Rats

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During an investigation on the absorption of ^{131}I -labelled serum proteins fed orally to young rats (Bangham & Terry, 1957), we found that suckling rats appeared to absorb about 7.5% of the fed homologous globulins but less than 0.2% of the albumin. Heterologous globulins were also absorbed but, in general, to a lesser extent than homologous globulins. The purpose of this present investigation was to compare the rate of breakdown of the absorbed ^{131}I -labelled proteins with that of serum proteins injected intraperitoneally into suckling rats.

EXPERIMENTAL

Hooded-strain (N.I.M.R.) rats were used in all these experiments: heterologous serum was obtained from a rhesus monkey. All sera were iodinated by the jet method of McFarlane (1955) with not more than 0.5 atom of ^{131}I /molecule of protein. Batches of 8–10 young rats, 7, 14 and 21 days' old, were used in these studies. Their mothers were given NaI (0.02%) as carrier in their drinking water for 3 days before the start of the experiment and until its end. The litters were kept with their mothers in metabolism cages; the young rats were not washed before their radioactivity was measured.

In each experiment, half of the rats in each age group received a dose of 4.25 mg. of labelled protein (15–30 μC) by mouth (Bangham & Terry, 1957); the other half of the group received an equivalent dose by intraperitoneal injection. Immediately after dosing, the total body radiation was measured in a ring counter whose background was approximately 500 counts/min. (Campbell, Cuthbertson, Matthews & McFarlane, 1956). Initially, counts were made on individual rats in order to investigate the uniformity of the dosing; thereafter the counts (minimum 4000 counts) were made on each batch of rats daily or every second day. The counts, corrected for background and decay, were plotted on semilogarithmic paper as a percentage of the initial count rate.

During the period of the experiment, the young rats increased in weight and it was thought possible that increasing self-absorption might significantly affect the count rate. There was, however, no significant self-absorption (change in count rate) of a standard dose in a volume of saline equivalent to the weight of the smallest rats at the beginning, or the largest rats at the end, of the experiment.

In order to determine how the residual radioactivity was distributed at the end of the period of observation, the 7- and 14-day-old rats that had been fed or injected with homologous serum were killed after 32 days, and the activity contained in the thyroids, skins, hair and carcasses was

measured. The skin was removed completely, except for the paws, and pinned out on a board. The hair was removed by clipping and gentle treatment with a sulphide depilating powder. The hair, skins, thyroids and carcasses were then separately dissolved in x-NaOH and the count of a sample of the resulting solution was compared with that of a sample of the original dose. A well-type scintillation counter (background 400 counts/min.) was used and each sample counted for at least 4000 counts.

RESULTS

Uniformity of the dose. Measurements of total body radioactivity, which were made on individual rats immediately after dosing, did not vary by more than 1% for the rats in any one group. The count rate for the 7-day-old rats which had received serum intraperitoneally was the lowest in each experiment. This was due to slight leakage of the labelled serum at the site of injection. Nevertheless, measurements between groups varied by less than 5%.

Homologous serum. The curves of the logarithm of the total body radioactivity plotted against time for those groups of rats receiving ^{131}I -labelled homologous rat serum intraperitoneally are shown in Fig. 1. Over the first 10–11 days the curve approximates to a straight line. As the dose consists of a number of different serum proteins, each having different half-lives, the decrease in activity with time would not be expected to be exactly exponential. The estimated half-life of this mixture is, however, about 3.8 days for both 7- and 14-day-old rats and 3.2 days for the 21-day-old rats.

The curves for the rats receiving the same labelled serum by mouth (Fig. 2) show a steep fall during the first 5 days. This is due to the rapid clearance of ^{131}I , which is removed from proteins apparently broken down in the gut. From 5 to 15 days the rate of decrease in total body radioactivity is approximately exponential for rats in all three age groups. The estimated half-life in the 7- and 14-day-old rats is about 5.2 days. This represents the half-life of selectively absorbed adult γ -globulin, and is approximately the same as that found in adult animals. The curve for the 21-day-old rats has a half-life of about 4.2 days, and represents the turnover of a very small amount of both globulin and albumin.

Heterologous serum. The curves of total body radioactivity for those batches of rats receiving labelled monkey serum intraperitoneally and by mouth are shown in Figs. 3 and 4 respectively. Fig. 3 shows an exponential decrease over the first 10 days for each batch, although the half-life for the 7- and 14-day-old rats is about 2.7 days, and for the 21-day-old rats about 1.9 days. From 15 to 27 days the curves, although now separated from one another, run roughly parallel in straight lines.

Fig. 4 shows that after the preliminary drop due to the clearance of iodide, there is again a period of from 4 to 10 days when the slope is approximately

exponential. The half-life of monkey γ -globulin in 7-day-old rats is 4.7 days, which is reasonably close to that for homologous γ -globulin. In 14-day-old rats the half-life is 8.0 days, a figure greater than any other previously obtained. While there may be several interpretations for this difference in half-life in 7- and 14-day-old rats, it is at least evident that the heterologous globulin is not catabolized more rapidly in the orally fed animals than in the injected ones. The reason for the longer half-life remains to be further investigated.

The 21-day-old rats either did not absorb a measurable amount of protein, or if they did it was catabolized almost at once.

Fig. 1

Fig. 2

Fig. 1. Curves of total body radioactivity of batches of rats receiving ^{131}I -labelled homologous serum by intraperitoneal injection. $\blacktriangle$, 7-day-old rats (group of five); $\bullet$, 14-day-old rats (group of five); $\blacksquare$, 21-day-old rats (group of four).

Fig. 2. Curves of total body radioactivity of batches of rats fed orally with ^{131}I -labelled homologous serum. $\blacktriangle$, 7-day-old rats (group of five); $\bullet$, 14-day-old rats (group of five); $\blacksquare$, 21-day-old rats (group of four).

Fig. 3

Fig. 4

Fig. 3. Curves of total body radioactivity of batches of rats receiving ^{131}I -labelled monkey serum by intraperitoneal injection. $\blacktriangle$, 7-day-old rats (group of five); $\bullet$, 14-day-old rats (group of five); $\blacksquare$, 21-day-old rats (group of four).

Fig. 4. Curves of total body radioactivity of batches of rats fed orally with ^{131}I -labelled monkey serum. $\blacktriangle$, 7-day-old rats (group of five); $\bullet$, 14-day-old rats (group of five); $\blacksquare$, 21-day-old rats (group of four).

Table 1. *Distribution of radioactivity remaining after 32 days in rats 7 and 14 days' old which had been fed or injected with homologous whole adult ^{131}I -labelled serum*

Each value was obtained from the pooled material from a group of four rats. Some of the activity found in the skin fraction may represent a small residue of hair left on the skins. No explanation can be offered for the higher percentage of the dose remaining in the skinned carcass in the injected rats. 'Carcass' indicates whole body minus thyroid, skin and hair.

Age when dosed (days)	Route of dose	Percentage of dose remaining	Percentage of remainder of dose in			
			Skinned carcass	Skin	Hair	Thyroid
7	Oral	3.9	5.0	9.8	77.7	7.5
14	Oral	4.1	7.3	5.7	71.3	15.7
7	Intraperitoneal	3.3	21.6	10.3	62.8	5.3
14	Intraperitoneal	3.7	15.5	5.7	73.9	4.9

Residual activity. Although sodium iodide was given as carrier in all these experiments, the curves of retained radioactivity all flatten out after 12–15 days to a slope with a much longer half-life. Table 1 shows the percentage of the original dose present in the hair, skin, thyroid and the rest of the carcass 32 days after dosing with homologous proteins. The only difference in distribution between the group fed with serum and that receiving serum by injection is in the higher activity found in the rest of the carcasses of the latter. The hair contained about 70% of the total residual activity, which was, on average, nine times as much as that in the thyroid. The rest of the carcass contained on average 12 times as much as the thyroid.

DISCUSSION

Campbell *et al.* (1956) have shown that the total body radioactivity, as measured with a ring counter, of a normal adult rat which has been given a single undenatured homologous serum protein labelled with ^{131}I may be used to study the rate of breakdown of the protein. The curve for total body radioactivity plotted against time showed a true exponential rate of decline. They found that the half-lives of homologous albumin and γ -globulin obtained in this way were about 3.3 days and about 6.6 days respectively in their rats.

We have used this method to study the rate of catabolism of the γ -globulin which is absorbed by the gut of suckling rats. After an initial steep drop during the first 5 days the total body radioactivity of these suckling rats showed an exponential rate of decline with a half-life of 5.2 days (Fig. 2). When a mixture of normal homologous ^{131}I -labelled proteins was injected into these rats the curve was no longer exponential, owing to the different rates of catabolism of albumin and the globulins, but the slope was steeper than that for the rats which had absorbed globulin. Furthermore, the heterologous (monkey) γ -globulin, which was similarly selectively absorbed from the gut, appeared to survive at

least as long as, or longer than, the homologous γ -globulin. The similar survival of monkey-serum proteins injected intraperitoneally into 7- and 14-day-old rats provides another example of the known inability of very young animals to form antibody to the foreign protein administered. Monkey-serum proteins were rapidly eliminated when injected into the 21-day-old rats which were then old enough to become immunized to them.

With this technique, there are several factors that confuse the curves for the orally fed rats: (1) Owing to the digestion or denaturation of the unabsorbed proteins, there is a large excess of inorganic iodide, the excretion of which in the first 5 days accounts for the steep drop in the curve during that time. (2) There was a large variation in growth rate within groups and between groups of rats. In such rapidly growing animals there are presumably rapid changes in the relative and absolute size of the intra- and extra-vascular and lymph spaces of these proteins, all of which may affect their rate of breakdown. (3) The mother ingests some urine and faeces when tending her young, and she then excretes inorganic iodide in the milk. (4) Adult serum proteins may be metabolized at a rate different from that in the adult animal. (5) The slow excretion rate from the 15th to the 27th day represents ^{131}I retained in the hair (see Table 1). This fraction is a small absolute percentage of the dose administered but, on account of the slow rate of loss, by the end of the month it represents 70% of the total activity remaining, and has by then completely changed the slope of the curve. Leblond (1954) investigated the distribution of radioactivity after the injection of [^{131}I]iodide in rats. He found similar retention of activity in the hair, of which about 80% was present as iodide, and none as any of the thyroid hormones. We have found that iodide accounted for about 75% of this activity. Contamination of the hair with ^{131}I excreted as iodide in the urine may account for some of this activity, although there was very little left in the 21-day-old rats (Fig. 2). These older

rats, however, do live a more independent life than the younger rats, which are continually tended by their mother. It is of interest to note the small amount of activity present in the thyroid.

Despite these complicating factors, however, the middle parts of the curves (Fig. 2, 5–15 days) are exponential and, we believe, represent a constant rate of breakdown of the absorbed protein over this time. Little is known of the behaviour of normal, or denatured or heterologous proteins in rats of this age, and the degradation of denatured protein may normally proceed at a rate quite different from that in the adult rat. Nevertheless, we believe that the cumulative evidence of the demonstration of immunologically active antibodies absorbed from the gut (Halliday, 1955; Halliday & Brambell, 1956), together with the demonstration in the serum of ^{131}I -labelled protein which moves electrophoretically in the position of γ -globulin (Bangham & Terry, 1957), suggests that what we have been observing in these animals is the catabolism of this absorbed antibody γ -globulin. This γ -globulin, which has been absorbed selectively by the gut of these suckling rats, has then preserved its immunological, electrophoretic and metabolic properties as a protein.

SUMMARY

1. The survival of homologous and heterologous whole adult ^{131}I -labelled serum proteins which have

been absorbed from the gut or injected intraperitoneally in rats 7, 14 and 21 days' old has been studied by measuring the total body radioactivity with a ring counter.

2. The survival time of the homologous protein absorbed (60–90 % γ -globulin) in 7- and 14-day-old rats is approximately the same as that of γ -globulin injected into adult rats.

3. The survival time of heterologous (monkey) serum protein absorbed in 7- and 14-day-old rats is at least as long as or longer than that of homologous serum protein absorbed.

4. It is inferred from these results that some of the γ -globulin is not denatured during its absorption from the gut into the blood stream.

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REFERENCES

- Bangham, D. R. & Terry, R. J. (1957). *Biochem. J.* **66**, 579.
Campbell, R. M., Cuthbertson, D. P., Matthews, C. M. & McFarlane, A. S. (1956). *Int. J. appl. Radiat. Isotopes*, **1**, 66.
Halliday, R. (1955). *Proc. Roy. Soc. B*, **143**, 408.
Halliday, R. & Brambell, F. W. R. (1956). *Proc. Roy. Soc. B*, **145**, 170.
Leblond, C. P. (1954). *Endocrinology*, **54**, 104.
McFarlane, A. S. (1955). *Biochem. J.* **62**, 135.

Studies on the Portal Blood of Sheep

1. ABSORPTION OF AMMONIA FROM THE RUMEN OF THE SHEEP

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During recent years there has been considerable interest in the nitrogen metabolism of ruminants, and in the role of rumen micro-organisms in protein digestion. It has become increasingly evident during investigations on various aspects of protein breakdown and synthesis that the microbial activities in the rumen are not always advantageous to the host animal. In particular, the breakdown of food protein to ammonia in the rumen may lead to an appreciable loss of nitrogen to the animal. McDonald (1948*a*) focused attention on this problem when he showed the importance of ammonia production in protein degradation and the ready absorption of ammonia from the rumen of the anaesthetized sheep. Chalmers, Cuthbertson & Synge (1954) have confirmed this finding, again on anaesthetized sheep. However, little attention has been given to

the quantitative relationship between the ammonia concentration in the rumen and in portal blood or to the measurement of the loss of dietary nitrogen in the form of ammonia.

In the experiments to be described it was shown that there was a correlation between rumen and portal-blood ammonia over a wide range of rumen-ammonia concentrations. Leakage of ammonia through the liver into the systemic circulation occurred at relatively low portal concentrations. At higher rumen-ammonia levels it was possible to correlate rumen-, portal- and peripheral-blood ammonia and to relate the levels to the onset and development of toxic symptoms.

A preliminary account of some of this work has been published (Annison, Hill & Lewis, 1955).