The Absorption of ^{131}I -Labelled Homologous and Heterologous Serum Proteins fed Orally to Young Rats

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The transference of passive immunity from mother to offspring has been shown to take place in ungulates almost exclusively by absorption of antibodies from the colostrum and milk; in man, rabbit and guinea pig almost entirely *in utero*, and in dog, rat and mouse by both means (see Brambell, Hemmings & Henderson, 1951).

Young rats suckled by hyperimmune mothers have been shown to be resistant to infection with *Trypanosoma lewisi* (Culbertson, 1938) and with *Plasmodium berghei* (Bruce-Chwatt & Gibson, 1956; Terry, 1956). With immunological methods, Halliday (1955) demonstrated homologous agglutinating and precipitating antibodies in the blood of suckling rats which had been given immune sera by stomach tube. The inference is that these globulin molecules have been absorbed whole and unchanged by the gut. The present paper describes a quantitative and qualitative investigation into the absorption of ^{131}I -labelled serum proteins by young rats of various ages.

EXPERIMENTAL

All rats used in these experiments were of the N.I.M.R. hooded strain. The heterologous sera were obtained from a Himalayan rabbit and a rhesus monkey. The various sera were iodinated by the jet method of McFarlane (1955) with not more than 0.5 atom of ^{131}I /molecule. Batches of 4–10 young rats, 7, 14 and 21 days' old, were used in the feeding experiments; wherever possible, all rats in any one batch were litter mates. The mothers were given NaI (0.02%) as carrier in their drinking water for 3 or 4 days before the start of the experiment and until its end. The young rats were removed from their mothers and weighed 1 hr. before feeding.

Feeding and collection of blood. Each young rat received a dose containing 4.25 mg. of total iodinated serum proteins (15–30 μC) in 0.15 ml. given by stomach tube. There was no difficulty in feeding the 14- and 21-day-old rats and, with practice, about 80% of the feeds to 7-day-old rats were successful. When the tube passed into the trachea instead of the oesophagus the fault was at once apparent; the young rat turned pale and collapsed. The animals were rejected where there was any doubt of the dose not having passed directly into the stomach. After feeding, the rats were returned to their mothers. In most experiments 3 hr. later, and in one series 24 hr. later, the rats were anaesthetized and their blood was collected. Especial care was taken to collect blood only by clean heart puncture and also to avoid

haemolysis. The blood from each batch of rats was pooled and allowed to clot.

Separation and assay of proteins. The sera were dialysed against distilled water at a pressure of 60 mm. Hg at 2° for 24 hr. and then loaded on to an electrophoretic column (Flodin & Porath, 1954). The column (43 cm. $\times$ 3 cm.; cellulose treated with acetyl chloride and ethanol, in phosphate-borate buffer, pH 8.4, I 0.1) was developed for 48 hr. at 30 ma (approx. 500v). After 32 hr. a dialysing sac was attached to the lower end of the column to collect the albumin, which was allowed to migrate off the column into the sac. The globulins were eluted in 3.3 ml. fractions at a rate of approx. 10 ml./hr. These fractions were assayed for optical density at 280 m μ in a Hilger Uvispek spectrophotometer, and for radioactivity for a minimum of 2000 counts in a well-type scintillation counter whose background was 400 counts/min. The albumin recovered from the sac was made up to 25 ml., and 3 ml. of this was counted. The dose serum was assayed for radioactivity by counting 3 ml. of a 1% dilution.

Calculation of results. The counts, corrected for background, for the albumins and globulins were added up and adjusted for the total theoretical blood volumes of the rats in the batch. The relation between body weight and blood volume [blood volume = 0.099(body weight) $^{0.8}$] was obtained from Chisholm (1911) and was calculated for each individual rat. The total activity contained in the dose serum was divided into that due to the albumin and that due to the globulins. The ratio albumin activity:globulin activity was obtained by fractionating the dose serum (see below) and was found to be 4.1:1. This ratio was used in all subsequent calculations. Thus the percentages of the albumin and total globulins in the dose serum which were absorbed by the young rats could be calculated for each batch.

Possible sources of error. The uniformity of the dosing was investigated in a series of twenty-four rats by measuring the total body radiation of the individual animals in a ring counter. The count rate varied by less than 1% in the series. The introduction of the dose anywhere except into the stomach would enable the albumin to be absorbed readily into the blood stream; that this did not occur is shown by the extremely low activity in the recovered albumin fractions (Table 1). Further errors of approximately 5–10% may have occurred in the measurement, handling and dialysis of the serum. A quantitative elution from the column is assumed since the cellulose had already been equilibrated with young and adult rat serum during previous electrophoretic separations. It should be noted that there is a small constant amount of radioactivity in the globulin fraction of the curve for the 21-day-old rats (Fig. 2a). We do not know the significance of these counts and we have

not taken them into account in the calculation of the results. We have been unable to find figures relating body weight to blood volume in the young rat other than the formula of Chisholm (1911) mentioned above. These figures may well need modification in the light of modern methods of determination of blood volume.

RESULTS

Iodination of the dose serum proteins

Before undertaking the feeding experiments it was necessary to investigate the uniformity of the iodination of the serum proteins which constituted the dose. Accordingly, 3 ml. of iodinated whole adult rat serum was fractionated on the column under the conditions stated above. The albumin was collected in bulk and a 3 ml. sample assayed for radioactivity. The eluate containing the globulin fractions was assayed for optical density and radioactivity; the curves for these properties are shown in Fig. 1. The high degree of correspondence between the curves indicates that the iodination of the globulins has been uniform. The albumin had a total count rate of 46.8×10^6 counts/min., and the pooled globulins one of 11.4×10^6 counts/min. This ratio of albumin activity to globulin activity of 4.1:1 was used in all subsequent calculations.

Feeding of homologous serum proteins

The results of assaying the globulin fractions of the sera of rats 7, 14 and 21 days' old, fed with iodinated whole adult rat serum and bled 3 hr. later, are shown in Fig. 2. The optical-density curves for all sera of suckling rats show that these sera contain a large amount of β -globulin with little α - or γ -globulin; this is in contrast with the curve

for the adult serum (Fig. 1). The radioactivity curve for the serum of the 21-day-old rats (Fig. 2a) did not correspond to any portion of the curve for the adult serum, but was a straight line just above background. This result indicates that no significant amount of any adult globulin fraction had been absorbed at this age. In contrast, the radioactivity curves for the sera of 7- and 14-day-old rats show well-defined peaks corresponding to the γ -globulin fraction of the adult serum (Fig. 2b, c). The calculated percentage absorption of albumin and total globulins for each age group is shown in Table 1. Apparently little albumin is absorbed at any of the ages studied, and the globulin absorbed by 7- and 14-day-old rats is almost exclusively γ -globulin.

In a second series of experiments, bleeding was deferred until 24 hr. after feeding with homologous serum. Because of the close correspondence between the results obtained previously for 7- and 14-day-old animals, only 14- and 21-day-old rats were used in this series. The results were similar to those obtained when blood was taken 3 hr. after feeding: a definite peak of radioactivity in the γ -globulin fraction of the 14-day-old rats and a straight line, not significantly above background, for the 21-day-old rats. However, the average percentage of labelled globulins found in the serum was less than a third of that found at the end of 3 hr. (Table 1). This result is probably explained by the equilibration of the absorbed globulins between the plasma and the extravascular pool. In the adult rat the extravascular pool has been shown to contain about one-and-a-half times as much globulin as the plasma (Campbell, Cuthbertson, Matthews & McFarlane, 1956).

Fig. 1. Column-electrophoresis eluate of whole adult rat serum, labelled with ^{131}I by the jet method (McFarlane, 1955), which was used for the dose material. The dose correspondence of radioactivity with protein content indicates uniform labelling throughout the range of globulins. The albumin migrated off the column and was collected and measured separately. —, Optical density; ●—●, radioactivity.

Fig. 2. Column electrophoresis eluate of serum obtained from suckling rats 3 hr. after a dose of the labelled serum shown in Fig. 1. (a) Pooled blood from four rats 21 days' old. There is a small constant amount of radioactivity which does not correspond with any protein peak. (b) Pooled blood from six rats 14 days' old; (c) pooled blood from ten rats 7 days' old. It can be seen that 60–70% of the labelled protein present is γ -globulin. Fig. 2 (b) shows the commonest pattern. It should be noted how little γ -globulin is present in the serum of these young rats. —, Optical density; ●—●, radioactivity.

Table 1. Amount of labelled albumin and globulin found in the blood of 7-, 14- and 21-day-old rats, 3 and 24 hr. after the animals had been fed with whole adult homologous and heterologous ^{131}I -labelled sera

Age of recipient rats (days)	No. of rats in group	Species of labelled serum given	Time of bleeding (hr.)	Amount of fed albumin found in the blood (%) (A)	Amount of fed globulins found in the blood (%) (B)	B/A
7	10	Rat	3	1.79*	8.80	4.91
			3	0.13	3.90	29.6
			3	0.12	6.42	52.6
		Rabbit	3	0.06	1.93	31.5
		Monkey	3	0.06	2.77	45.1
			3	0.07	4.82	71.3
14	6	Rat†	3	0.28	12.6	45.4
			3	0.07	8.85	131.0
			24	0.08	3.26	40.7
			24	0.05	1.51	30.2
		Rabbit	3	0.16	2.74	17.5
		Monkey	3	0.05	0.32	6.65
			3	0.06	6.34	105.2
21	4	Rat	3	0.04	<0.5	—
			3	0.07	<0.5	—
			3	0.08	<0.5	—
			24	0.17	<0.5	—
		Rabbit	3	0.04	<0.5	—
		Monkey	3	0.02	<0.5	—
			3			

* In view of the constantly low figures for the percentage of albumin absorbed, this figure may have been high owing to an erroneous misplacing of the dose.

† The difference in the average amount of protein found in the blood of 14-day-old rats at 3 hr. (A, 0.14%; B, 8.8%) and 24 hr. (A, 0.07%; B, 2.39%) is probably due to equilibration of these proteins between the intra- and extra-vascular spaces.

Feeding of heterologous serum proteins

When ^{131}I -labelled rabbit and monkey sera were fed to suckling rats, the results obtained were qualitatively the same as when homologous serum was fed. In no case was much albumin absorbed and the 21-day-old rats did not absorb significant quantities of globulins. The globulins absorbed by 7- and 14-day-old rats again showed a peak in the γ -globulin fraction, but the calculated percentage absorption was, in general, less than that for homologous protein (Table 1, rabbit and monkey). A considerable variation was found between different litters.

DISCUSSION

The interpretation of our results rests on the assumption that these iodinated proteins behave physiologically as normal unlabelled proteins. This question has been studied in detail by McFarlane (1955) and by Cohen, Holloway, Matthews & McFarlane (1955), who found that plasma proteins iodinated by this technique behaved exactly like ^{14}C -labelled proteins as regards their rate of elimination and equilibration between the plasma and the extravascular pool; in experiments where

iodinated plasma proteins were fed to adult rabbits, no iodinated product of protein digestion was incorporated to any detectable extent into plasma protein synthesized within 40–60 hr. of isotope administration. On this evidence we believe that the iodinated protein recovered from the blood of suckling rats represents at least a minimum of the undenatured protein absorbed. It is conceivable that a certain proportion of absorbed proteins becomes partially denatured during their absorption. However, we have carried out experiments to determine the rate of breakdown of absorbed protein and we find (Bangham & Terry, 1957) that, at least 4 days after feeding, there is an exponential elimination comparable with that of the same proteins in the adult rat (Campbell *et al.* 1956).

Our finding that ^{131}I -labelled globulin is absorbed unchanged from the gut of the suckling rat up to the age of 21 days (an age when they are normally weaned) is in agreement with the result obtained previously by immunological means (Halliday, 1955). The use of isotopically labelled proteins has made this study quantitative. An average of 7.5% of the homologous globulins fed to the 7- and 14-day-old rats was absorbed within 3 hr. It can be seen from the curves of radioactivity in Fig. 2b

and *c* that γ -globulin may be 60–90 % of the total absorbed globulin. With a dose of 4.25 mg. of total adult serum proteins this represents the passage of a considerable quantity of γ -globulin across the gut. Unless the ratio of the extravascular to the intravascular pool in these young rats is markedly different from that in adult rats, there is little or no further absorption of globulins after 3 hr. Our results also confirm Halliday's observation that homologous antibody was absorbed more readily than heterologous antibodies, although our results showed a wide variation. The preferential transference of homologous (as opposed to heterologous) antibody from mother to offspring has also been shown to occur *in utero* in the rabbit (Brambell, Hemmings, Henderson & Rowlands, 1950; Cohen, 1950).

The curves shown in Fig. 2*b* and *c* indicate that the over-all effect of feeding a mixture of serum proteins to 7- and 14-day-old rats is that the γ -globulin is selectively absorbed unchanged into the blood stream. This may be due to the susceptibility of the other serum proteins to the pH and proteolytic conditions present in the gut. In this context the work of Hill (1956) is of interest. He showed that the stomach of the suckling rat retains an embryonic type of mucosal lining, which is composed of low cuboidal cells; that the pH of the stomach contents is constantly about 7, and that no pepsin is secreted. When the rat is 18–21 days' old the stomach mucosa, pH and pepsin secretion rapidly change to that found in the normal adult animal. On the other hand, the selection of γ -globulin may represent a real specific absorption of antibody. The only organ which has previously been shown to be capable of this discrimination is the mammary gland of ungulates (Askonas, Campbell, Humphrey & Work, 1954). These workers demonstrated that the immune globulins in the colostrum of goats were derived from the serum globulins and that these proteins were present in the colostrum in several times their concentration in the serum. In order to study the mechanism of the absorption by the gut of whole unchanged protein molecules, it would be of interest to compare our findings with a study of the absorption of the more stable of the milk proteins, such as the lactoglobulins.

SUMMARY

1. Three hours after feeding with homologous ^{131}I -labelled whole adult rat serum, the blood of

suckling rats 7 and 14 days' old contained significant amounts of labelled globulins, amounting to about 7.5 % of the globulins contained in the dose serum.

2. When bleeding was deferred until 24 hr. after feeding, about 2.4 % of the globulins in the dose serum could be detected in the blood. This lower figure was probably due to the equilibration of the absorbed globulins between the plasma and the extravascular pool.

3. Rats which were 21 days' old at the time of feeding (an age when they are normally weaned) absorbed less than 0.5 % of the globulins contained in the dose serum.

4. In rats of all ages very little labelled albumin (0.2 %) could be detected in the blood 3 and 24 hr. after feeding.

5. Similarly, 7- and 14-day-old rats when fed with labelled whole adult rabbit or monkey serum, absorbed significant but smaller amounts of globulin and very little albumin.

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