J. Physiol. (1969), **205**, *pp.* 435–451
With 7 text-figures
Printed in Great Britain

THE BREAK-DOWN OF [^{131}I]γ-GLOBULIN IN THE DIGESTIVE TRACT OF THE NEW-BORN PIG

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(*Received* 2 *July* 1969)

SUMMARY

1. The intestinal absorption of [^{131}I]porcine and bovine serum γ-globulin after oral administration has been investigated in conscious pigs less than 20 hr old. Absorption was measured by the concentration of ^{131}I in venous blood during the 6 hr after feeding and also by the distribution of ^{131}I between homogenates of the alimentary tract and the rest of the animal at the end of the experiment.

2. The concentration of ^{131}I in the blood was always low after feeding [^{131}I]γ-globulin, although a large proportion of the isotope fed was found to have left the alimentary tract. This indicated that much of the [^{131}I]-γ-globulin had been hydrolysed into fragments of low mol.wt. which were not retained in the plasma. There were no significant differences between results obtained with homologous and heterologous γ-globulin.

3. Examination by gel-filtration confirmed that, after feeding [^{131}I]-serum γ-globulin, much of the ^{131}I in the plasma was associated with material of mol.wt. less than 12,400 and demonstrated that the break-down of bovine γ-globulin was comparable with that of homologous γ-globulin.

4. Comparison of the absorption of [^{131}I]serum γ-globulin from colostrum with that from a chloride solution with a similar Na^+ and K^+ concentration showed that, although the blood concentration remained low, colostrum reduced the hydrolysis of the labelled protein.

5. This effect of colostrum could be simulated by the addition to the chloride solution of either the synthetic trypsin inhibitor Trasylol or a higher concentration of unlabelled protein.

6. Gel-filtration of samples of the contents of the stomach, duodenum and terminal ileum after feeding [^{131}I]serum γ-globulin showed that proteolysis occurred at all these sites.

INTRODUCTION

During the first few weeks after birth the survival of the young pig is dependent upon the possession of maternal antibodies. These are obtained from the colostrum, certain high mol.wt. components of which can pass, without change, across the epithelial cells of the small intestine in the suckling animal during the first 24–48 hr after birth. Within this period the amount of immune globulin which reaches the circulation is dependent upon the volume and immune globulin content of the colostrum ingested, upon the proportion of this protein which escapes enzymic break-down within the stomach and intestine and finally upon the rate of passage of the unchanged γ-globulin from the intestinal lumen into the circulation.

The investigations of the absorption of [^{131}I]serum γ-globulin reported in this paper provide some indication of the quantitative significance of proteolysis to the transfer of immune globulin across the small intestine of the young pig. A preliminary report of certain of these experiments has been published previously (Hardy, 1965).

METHODS

Pigs. Animals were obtained from local farms and were removed from the sow before suckling occurred. Their subsequent care has been described in detail previously (Hardy, 1969*b*).

Feeding technique. Bovine serum γ-globulin (Fraction II: Armour) and porcine serum γ-globulin (Fraction II: Calbiochem) were labelled with ^{131}I by the method as described for albumin by Veall (1954). After passage through Deacidite F.F. (Permutit), less than 1% of the radioactivity remained in the supernatant after precipitation with 6% TCA (w/v) and more than 95% of the ^{131}I labelled material showed the characteristics of unchanged γ-globulin during starch-gel electrophoresis. Furthermore the results of gel-filtration on Sephadex G-100 (Pharmacia) confirmed that at least 95% of the total radioactivity fed was associated with material of mol.wt. exceeding 150,000 (see below).

Colostrum was obtained from Jersey cows within 24 hr of calving, was defatted by centrifugation and when necessary stored at $-20°$ C until required (see Hardy, 1969*a*). The composition of the chloride solution used in certain experiments (NaCl 56·7 mM, KCl 44·8 mM) approximates to the concentration of these ions in cow colostrum whey (Balfour & Comline, 1962).

Experimental solutions comprised unlabelled γ-globulin in 2% (w/v) solution in the solvent (e.g. cow colostrum) and 5–10 μc labelled protein. Each animal was fed a total of 45 ml. by stomach tube in three 15 ml. doses at hourly intervals (Hardy, 1969*b*).

Measurement of absorption. The techniques involved here have previously been described in detail (Hardy, 1969*b*). To summarize, two indices of absorption have been examined: first, the appearance of ^{131}I labelled material in the blood, and secondly, the distribution of labelled material between homogenates of the alimentary tract and homogenates of the carcass. The blood ^{131}I concentration has been expressed as a percentage of the ^{131}I concentration of the solution fed, 'blood per-

centage radioactivity'. The recovery of ^{131}I from the alimentary tract has been expressed as a percentage of the total recovery.

Gel-filtration. Gel-filtration was performed on a standard commercial column (Sephadex, type K 25/45, Pharmacia) using Sephadex Medium G-100 at 4° C with phosphate-buffered saline (0·01 M sodium phosphate buffer pH 7·4, 0·1 M-NaCl). Samples for analysis comprised 2 ml. containing 5 mg cytochrome *c* (from horse heart: Koch-Light). The effluent from the column was collected in 4·4 ml. fractions.

The radio-iodine concentration of each fraction was determined with a well-type scintillation counter (Panax type 100 *c*). The elution volume for cytochrome *c*, which could be approximately estimated by inspection, was accurately determined by measuring the optical density of ten to twelve relevant fractions at 410 mμ with a Uvispek Photoelectric Spectrophotometer Type H 700 (Hilger & Watts). In certain sample runs, and in calibration of the gel column, the void volume was determined by estimation of the elution volume of Blue Dextran 2000 from its optical density at 630 mμ.

Radio-iodinated serum γ-globulin was completely excluded from the gel particles and consequently its elution volume was equal to that of Blue Dextran 2000 and equivalent to the void volume of the column. Radio-iodinated fragments after break-down of [^{131}I]serum γ-globulin were eluted in plasma in a single relatively homogeneous peak after that of cytochrome *c*.

Since the intact globulin and the digested fragments were eluted on either side of cytochrome *c*, the relative extent of proteolysis could be represented as the percentage of the total ^{131}I which was eluted after cytochrome *c*.

Samples for gel-filtration. Samples of 5 ml. blood were taken from the jugular vein and placed in a heparinized centrifuge tube. After centrifugation at 3000 rev/min for 10 min, 2 ml. plasma was removed and placed in a polyethylene storage tube at $-20°$ C to await analysis. Samples of 2 ml. urine were obtained after removal of the bladder and were stored at $-20°$ C until required. Samples of the fluid contents of the stomach were obtained in certain pigs by removal of the stomach and expulsion of the contents into a small beaker. Two samples of intestinal contents were obtained, from the 10–20 cm immediately distal to the pylorus, and from the terminal 10–20 cm of the ileum, by gentle massage. It was often not possible to obtain sufficient material (at least 2 ml.) by this method, in which case the attempt was abandoned, as it was considered that undue mechanical abuse of the gut segments would result in the presence of large numbers of epithelial cells within the sample.

Samples of the contents of the alimentary tract were diluted to a final volume of 10 ml. with isotonic saline solution, thoroughly mixed and placed in a polyethylene centrifuge tube. After centrifugation for 15 min at 12,000 rev/min, samples of 2 ml. of supernatant fluid were removed and stored at $-20°$ C to await gel-filtration.

RESULTS

The measurement of absorption

In the experiments to be described, the absorption of [^{131}I]serum γ-globulin has been measured in two ways: the relative concentration of ^{131}I in the blood, and the disappearance of ^{131}I from the alimentary tract. The relationship between these two parameters for the absorption of [^{131}I]-polyvinyl pyrrolidone (PVP) has previously been reported (Hardy, 1969*b*); significant regressions could be calculated for the two mol.wt. fractions employed, indicating that [^{131}I]PVP K.60 (mean mol.wt. 160,000) was

largely retained within the plasma after absorption, while [^{131}I]PVP K.30 (mean mol.wt. 40,000) passed out of the circulation in much greater quantities.

In the present experiments no significant regression existed between the blood concentration of ^{131}I and the recovery of ^{131}I from the gut for animals fed [^{131}I]serum γ-globulin. Results from these animals are shown in Fig. 1

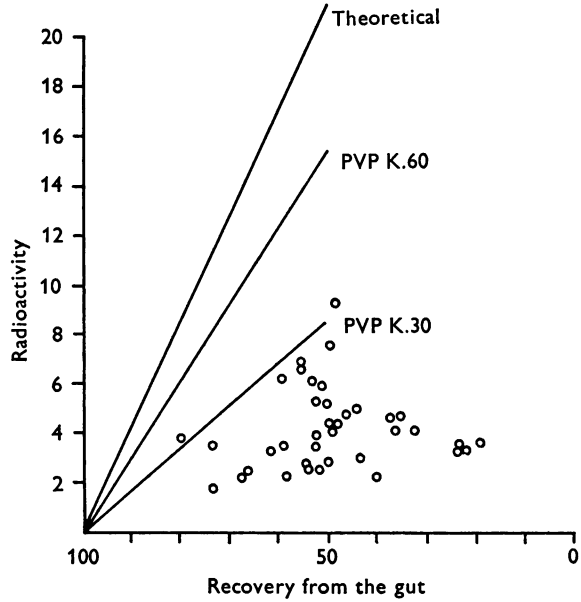

Fig. 1. Comparison of the relation between blood percentage radioactivity and recovery from the gut for pigs fed [^{131}I]serum γ-globulin (○). Regression calculated for [^{131}]PVP K.60 and [^{131}I]PVP K.30, corrected to pass through the origin, and theoretical regression for a 1200 g pig are also shown (see Hardy, 1969*b*). Ordinate, blood percentage radioactivity 6 hr after feeding; abscissa, percentage of the ^{131}I recovered, found in the alimentary tract.

and may be compared with the regressions for [^{131}I]PVP K.60, [^{131}I]PVP K.30 and the theoretical regression for a 1200 g pig assuming that all the solute absorbed from the gut remained in the plasma.

It is apparent from Fig. 1 that the amount of ^{131}I present in the blood of those animals fed [^{131}I]serum γ-globulin was in fact much less than the theoretical value. A large proportion of the ^{131}I labelled material absorbed had therefore not been retained within the circulation. This rapid loss of ^{131}I labelled material from the circulation when [^{131}I]serum γ-globulin was fed implied that a large proportion of the [^{131}I]serum γ-globulin had been degraded before or during absorption and that it was the low mol.wt.

^{131}I labelled fragments which were lost from the plasma. In contrast, in the young calf protein break-down was less marked (Hardy, 1969*c*) and in consequence the blood percentage radioactivity observed when [^{131}I]-bovine serum γ-globulin was fed was higher than that seen in pigs (Fig. 2).

The demonstration of break-down products of [^{131}*I*]*serum* γ*-globulin*

If extensive proteolysis did occur before or during absorption in the pig, it would be expected that ^{131}I labelled fragments from the digested protein would be present in the plasma and urine. The technique of gel-filtration has been used to detect and measure ^{131}I labelled products of

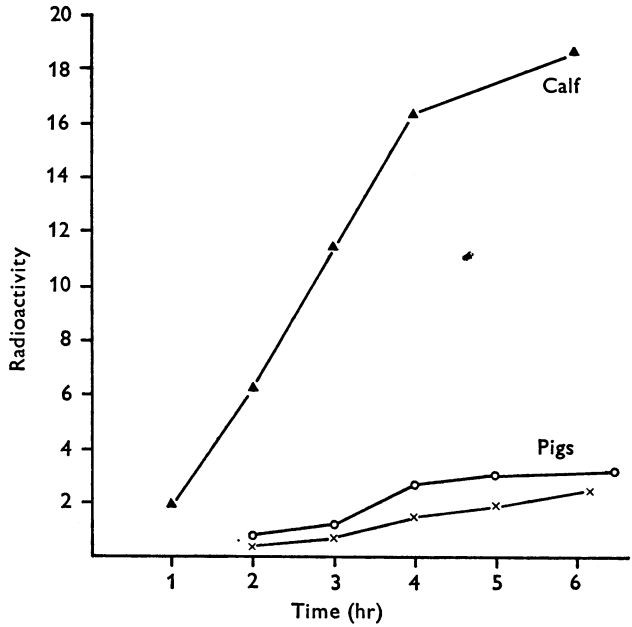

Fig. 2. Comparison between the radioactivity in venous blood of a calf and two pigs fed [^{131}I]bovine serum γ-globulin in cow colostrum (40 ml./kg body wt.). Ages when fed: calf, 4 hr; pigs, 10 hr. Ordinate: as Fig. 1. Abscissa: time after first feed (hr).

protein hydrolysis in plasma and urine. It was found that the ^{131}I in plasma was eluted in two distinct peaks (Fig. 3). The first peak corresponded with the elution volume of the [^{131}I]serum γ-globulin in the colostrum fed and also with the void volume of the column, and therefore was associated with substantially undegraded protein of mol.wt. exceeding 150,000. The second peak, which was eluted after the marker substance cytochrome *c*, represented digested protein fragments of mol.wt. less than 12,400 since the mol.wt. of cytochrome *c* is reported to be 12,400 (Margoliash, 1962). The clear separation of the two labelled peaks has allowed an

index of proteolysis to be obtained from the percentage of the total ^{131}I in a sample which was eluted after cytochrome *c*.

Almost all the ^{131}I present in the samples of labelled γ-globulin fed to the experimental animals was combined with undegraded protein, since it was eluted before cytochrome *c*, whereas samples of plasma and urine invariably contained a high percentage of low mol.wt. labelled fragments. This can be seen from Fig. 3, which demonstrates the distribution of radioactivity

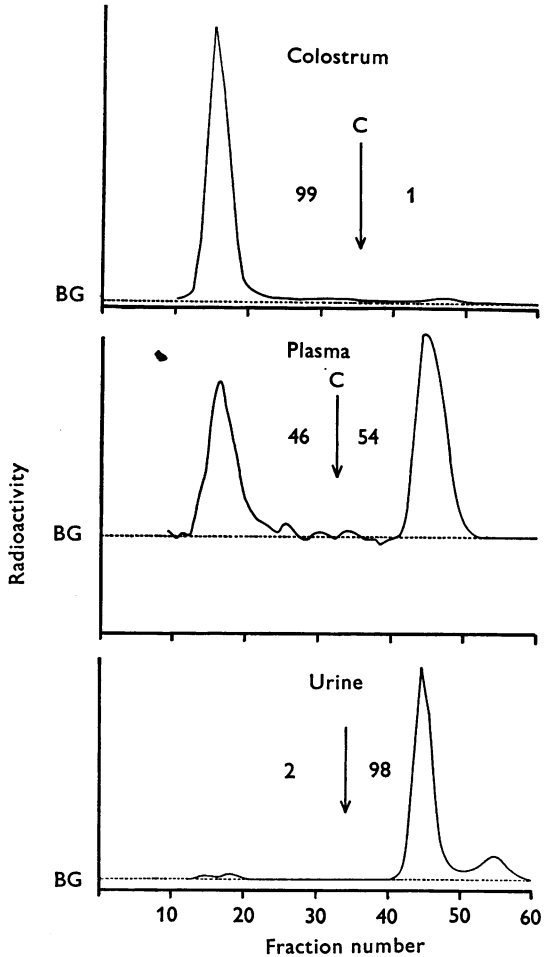

Fig. 3. Gel-filtration of the samples of the colostrum fed and plasma and urine taken 6 hr after feeding from a 10 hr old pig fed 45 ml. cow colostrum containing [^{131}I]bovine serum γ-globulin. Ordinate: radioactivity of individual fractions related to background (BG). Abscissa: fraction number (volume of eluate). Elution volume of cytochrome *c* shown by arrow, percentage of net radioactivity eluted before and after cytochrome *c* indicated on the appropriate side of the arrow.

after gel-filtration of samples of the colostrum, plasma and urine taken 6 hr after feeding cow colostrum containing [^{131}I]bovine serum γ-globulin.

The effect of age and the species of origin of the protein on the degree of proteolysis

Since it has been shown previously that the absorption of [^{131}I]PVP K.60 from the intestine of the unsuckled pig declined with age (Hardy, 1969*b*), it was of interest to determine the effect of age on the break-down of labelled protein. For this purpose pigs were divided into three age groups and representatives of each age group were fed either bovine or porcine [^{131}I]γ-globulin in cow colostrum: the results of these experiments are summarized in Table 1.

TABLE 1. Absorption and break-down of [^{131}I]bovine and porcine γ-globulins fed in cow colostrum to pigs at different ages

Age of pig when fed (hr)	Blood % radio-activity 6 hr after feeding	% total ^{131}I eluted after cytochrome *c*			Globulin species
		Colostrum	Plasma	Urine	
0–5	4·2	1	25	98	Porcine
	5·6	1	17	98	Porcine
	5·0	1	19	99	Porcine
	4·7	2	23	98	Bovine
	4·9	2	15	—	Bovine
6–14	5·5	2	19	99	Porcine
	3·3	1	54	98	Bovine
	6·2	1	12	—	Bovine
15–20	2·6	6	60	98	Porcine
	2·4	6	46	—	Porcine
	2·8	2	45	—	Bovine
	1·9	2	58	100	Bovine

Proteolysis, as indicated by the presence of ^{131}I labelled fragments of low mol.wt. in the plasma, was seen in pigs less than 5 hr after birth and had increased in severity by 15–20 hr after birth. There was little difference between the results obtained with homologous γ-globulin and those with bovine γ-globulin.

Solvent factors and the absorption of [^{131}I]serum γ-globulin

The existence of proteolysis during the absorption of [^{131}I]serum γ-globulin complicated the assessment of the influence of age and solvent factors upon absorption, since it has been shown that the ^{131}I measured in the blood did not exclusively represent [^{131}I]serum γ-globulin.

A comparison of the blood percentage radioactivity and the recovery

from the gut of pigs fed [^{131}I]bovine serum γ-globulin in either cow colostrum or chloride solution is shown in Table 2. There was no significant difference between the mean blood percentage radioactivity of animals 6–14 hr old when fed the protein in either cow colostrum or chloride solution, but in the latter case significantly less of the radioactivity fed was recovered from the gut ($P < 0{\cdot}01$).

The extensive proteolysis apparent when [^{131}I]serum γ-globulin was fed in chloride solution has been analysed in more detail by some further

TABLE 2. The absorption of [^{131}I]bovine serum γ-glubulin from either cow colostrum or from NaCl 56·7 mM, KCl 44·8 mM

	Cow colostrum		NaCl 56·7 mM, KCl 44·8 mM				
					% total ^{131}I eluted after cytochrome *c*		
Age of pig when fed (hr)	Blood % radio-activity	Recovery from the gut	Blood % radio-activity	Recovery from the gut	Soln. fed	Plasma	Urine
6–14	2·5	54	4·2	35	—	—	—
	5·2	52	4·1	36	—	—	—
	3·7	—	3·3	22	—	—	—
	6·0	60	3·8	—	1	95	99
	2·5	52	3·4	23	—	—	—
	3·3	—	3·4	—	1	88	100
	6·2	—	2·9	—	1	93	98
	—	—	3·5	19	—	—	—
	2·3	—	4·1	32	—	—	—
Mean and s.e. of mean	4·0 ± 0·3	54 ± 2	3·6 ± 0·2	28 ± 3	—	92	99
15–20	2·4	66	3·5	23	—	—	—
	2·1	47	3·5	23	—	—	—
	2·2	58	2·9	—	3	94	—
	2·8	—	2·7	—	3	93	—
	1·9	—	—	—	—	—	—
Mean and s.e. of mean	2·3 ± 0·1	57	3·1 ± 0·2	23	—	93	—

experiments. The chloride solution employed differed from cow colostrum in many respects, but it seemed likely that the greater proteolysis in chloride solution might be the result of two principal factors: the higher total protein concentration in colostrum or the presence there of substances which would inhibit proteolytic enzymes.

The effect of increasing the concentration of carrier γ-globulin while feeding a constant amount of radio-iodinated γ-globulin in chloride solution can be seen in Fig. 4. Since the amount of radio-iodinated protein fed was the same in all pigs, it might be expected that the disappearance of

65% from the gut of pigs fed 2% γ-globulin solution would result in higher blood percentage radioactivities than those in pigs fed 4 and 6% protein solution in which 53 and 51% respectively of the labelled protein had left the gut.

The fact that the blood percentage radioactivities in all six pigs were comparable indicated that a larger proportion of the labelled material absorbed from the gut of those pigs fed 2% carrier protein was lost from

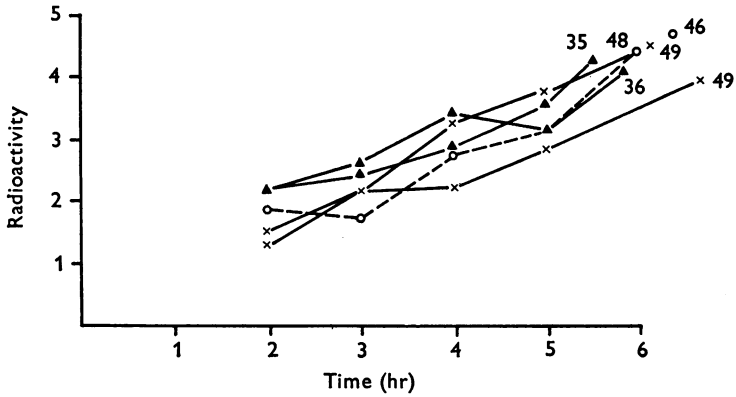

Fig. 4. The absorption of [^{131}I]bovine serum γ-globulin from chloride solution (NaCl 56·7 mM, KCl 44·8 mM) by 12 hr old pigs in the presence of various concentrations of carrier γ-globulin. Pigs fed carrier protein, 2 g/100 ml. (▲–▲), 4 g/100 ml. (○–○) and 6 g/100 ml. (×–×). Percentages refer to amount of isotope recovered from the alimentary tract. Ordinate: blood percentage radioactivity; abscissa: time after first feed (hr).

the plasma. This demonstrated that the hydrolysis of labelled protein by pigs fed 2% carrier protein was greater than that in the other animals and implied that the provision of alternative substrate for proteolytic enzymes decreased the break-down of radio-iodinated protein.

The alternative possibility, that the decreased proteolysis when colostrum was fed could be attributed, in part, to the inhibition of proteolytic enzymes, was also explored, since both cow and sow colostrum are known to contain trypsin inhibitors. The effect of the synthetic trypsin inhibitor Trasylol (*F.B.A.*) on the absorption of [^{131}I]bovine serum γ-globulin from chloride solution containing 2 g carrier γ-globulin/100 ml. solution is shown in Fig. 5. It can be seen that, although the blood percentage radioactivities of control pigs were little different from those of pigs fed Trasylol, the recovery of ^{131}I from the gut of the latter animals was considerably greater, thus demonstrating the decreased loss of degraded protein from the gut.

The effect of proteolytic break-down upon the relation between blood

percentage radioactivity and the recovery of ^{131}I from the gut of pigs fed [^{131}I]serum γ-globulin is summarized in Fig. 6. The theoretical relation and the regressions calculated for PVP K.60 and PVP K.30 (Hardy, 1969*b*) can be compared with the results obtained when [^{131}I]serum γ-globulin was fed in cow colostrum and in chloride solution with Trasylol or with differing amounts of carrier protein.

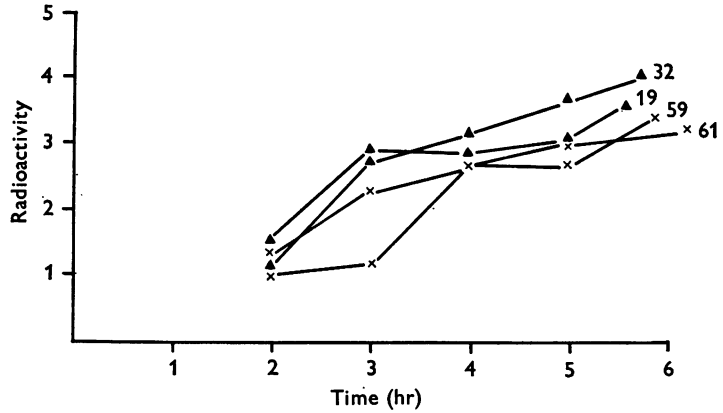

Fig. 5. Effect of Trasylol on the absorption of [^{131}I]bovine serum γ-globulin from chloride solution containing carrier γ-globulin, 2 g/100 ml. Pigs fed 12,500 k.i.u. Trasylol (×–×), control pigs (▲–▲). Animals 8 hr old when fed. Percentages refer to the amount of isotope recovered from the alimentary tract. Ordinate and abscissa as Fig. 4.

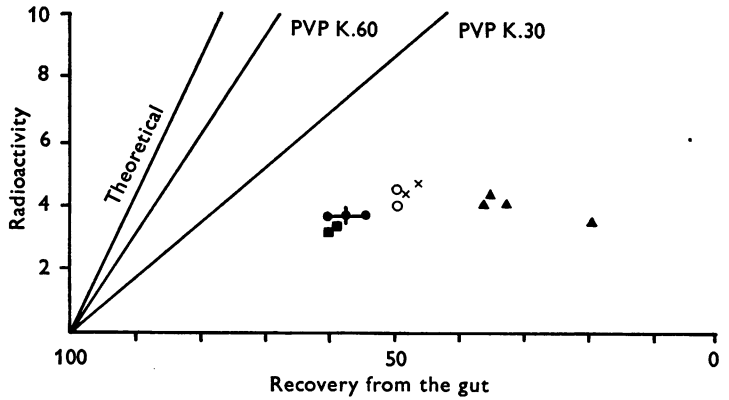

Fig. 6. The relation between blood percentage radioactivity and recovery from the gut for pigs fed [^{131}I]serum γ-globulin. Solvents: NaCl, KCl, carrier protein, 2 g/100 ml. ▲; carrier protein, 4 g/100 ml. ×; carrier protein, 6 g/100 ml. ○; carrier protein, 2 g/100 ml. + 12,500 k.i.u. Trasylol, ■. Cow colostrum + carrier protein, 2 g/100 ml. (mean and S.E. of mean). Regressions for PVP K.60, PVP K.30 and the theoretical regression (see Fig. 1) are included for comparison. Ordinate and abscissa as Fig. 1.

The sites of proteolysis

Some evidence has been obtained by gel-filtration to indicate the degree of proteolysis at various points along the alimentary tract, but the elution of these samples was not entirely satisfactory.

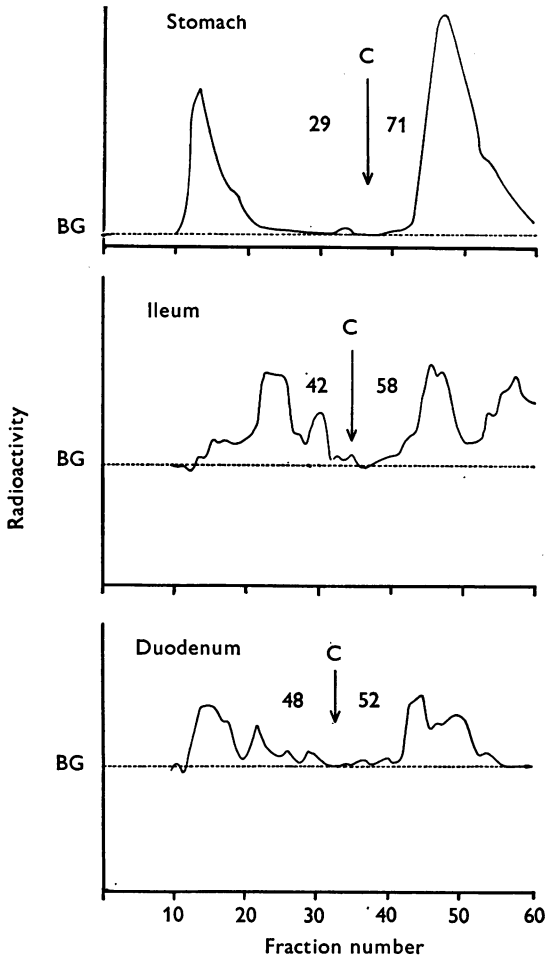

Fig. 7. Gel-filtration of samples of stomach, duodenal and ileal contents taken from a 21-hr-old pig fed [^{131}I]bovine serum γ-globulin in cow colostrum. Ordinate and abscissa as Fig. 4. BG, background.

A typical result is illustrated in Fig. 7 which shows gel-filtration of the contents of the stomach, the duodenum, and the ileum from a pig 21 hr old when fed cow colostrum containing [^{131}I]bovine serum γ-globulin. It can be seen that 71 % of the total ^{131}I in the stomach contents of this animal was associated with the products of hydrolysis. The sample from

the duodenum showed 52% and that from the ileum showed 58% respectively of the total ^{131}I associated with material of mol.wt. less than that of cytochrome *c*.

These results indicate that considerable hydrolysis of [^{131}I]serum γ-globulin takes place in the stomach of the new-born pig. Results obtained from samples of intestinal contents using this technique are rather unsatisfactory, but nonetheless indicate that the contents of the duodenum and the ileum contain a high proportion of ^{131}I labelled fragments.

DISCUSSION

In many species, including the pig, the transfer of immune globulins to the circulation of the new-born during normal suckling is restricted to the first 24–48 hr after birth. It has been suggested that this period may be curtailed by the onset of protein digestion (Mason, Dalling & Gordon, 1930; Hill, 1956; Deutsch & Smith, 1957; Chamberlain, Perry & Jones, 1965), but it has been demonstrated that the absorption of polyvinyl pyrrolidone by the young pig (Lecce, Matrone & Morgan, 1961; Hardy, 1965, 1969*b*), by the calf (Hardy, 1969*a*) and by the young rat (Clarke & Hardy,1969) ceases at the same time as the absorption of intact protein. Thus, since PVP is not broken down within the gut, protein digestion cannot be the factor which limits the absorption of antibodies. It is obvious, however, that proteolytic activity within the stomach and small intestine of a new-born animal will prejudice the absorption of antibody protein during the restricted period when this can take place. For this reason it is useful to be able to determine the relative degree of protein break-down in experimental animals.

The techniques described in this paper provide two indices of the hydrolysis of isotopically labelled large proteins: first, the relation between their disappearance from the gut and their retention in the plasma, and secondly, the measurement of labelled break-down products in the plasma after absorption. The latter measurement, of course, suffers from the disadvantage that the low mol.wt. fragments tend to be lost from the plasma much more rapidly than undegraded protein, so that the absolute percentage of labelled fragments determined by gel-filtration is an underestimate, although comparisons between different animals have proved of value.

These techniques have demonstrated that proteolytic break-down of labelled γ-globulin takes place in pigs less than 5 hr after birth, and that there is an increase in the severity of this process in animals tested 15–20 hr after birth.

During these experiments, there was a close quantitative similarity

between the behaviour of homologous serum γ-globulin and bovine serum γ-globulin with respect to the time course of absorption, the amount absorbed from the gut and also the proportion of low mol.wt. ^{131}I labelled digestion products in the plasma. It therefore seems unlikely that any specific mechanisms for the preferential absorption of homologous serum γ-globulin were operative under the experimental conditions which prevailed. This confirms the observations of Olsson (1959*a*), who demonstrated comparable absorption of porcine and bovine colostrum γ-globulins and also showed a close similarity between the absorption of porcine and equine serum γ-globulins (Olsson, 1959*b*). The 6·6 S globulins of porcine, equine and ovine origin have also been shown to be absorbed in comparable amounts (Kaeberle & Segre, 1964; Locke, Segre & Myers, 1964). The observations of Pierce & Smith (1967*a*) agree with previous work and that reported here, in that the absorption of bovine colostrum IgG and that of porcine colostrum IgG was comparable when either was fed in dialysed colostrum to very young unsuckled pigs. In contrast, however, when a mixture containing equal proportions of both these proteins was fed, porcine IgG appeared to be absorbed about twice as readily as bovine IgG. The interpretation of these results is hampered by the fact that the quantitative immuno-diffusion test used to measure the transfer of IgG could not distinguish between undegraded protein and immunologically precipitable digestion products.

Although the weight of evidence argues against an overt difference in the absorption of IgG derived from different species *in vivo*, it is apparent that under *in vitro* conditions preferential transfer of certain protein species can be demonstrated (Pierce & Smith, 1967*b*; Brown, Smith & Witty, 1968). These more subtle differences seen *in vitro* may well be obscured by the more vigorous proteolytic activity encountered in the intact animal.

Factors in colostrum have been shown to accelerate the rate of absorption of [^{131}I]PVP K.60 by the pig, above the low levels seen when this solute is fed in NaCl 56·7 mM, KCl 44·8 mM solution (Hardy, 1969*b*). It was therefore of interest to see whether the absorption of [^{131}I]serum γ-globulin showed a similar dependence upon solvent factors. The result of this investigation was complicated by the concurrent influence of colostrum on the degree of break-down of the labelled protein. Thus, although gel-filtration showed little intact [^{131}I]γ-globulin in the plasma of animals fed the protein in chloride solution, it remains uncertain how much of this could be attributed to the absence of solvent factors necessary for macromolecular absorption and how much to the destruction of the labelled protein before it entered the circulation.

The break-down of [^{131}I]serum γ-globulin when fed in chloride solution

could be reduced by the addition of unlabelled γ-globulin, which presumably afforded alternative substrate for the proteolytic enzymes. If this was indeed the case, it can be related to the high concentration of immune globulin and total protein in sow colostrum taken immediately after parturition (Norbring, 1957; Payne & Marsh, 1962). Protein break-down in the digestive tract of the new-born calf is less intensive than that in the new-born pig (Hardy, 1969*c*) and the immune globulin and total protein concentrations in bovine colostrum are correspondingly less than those in sow colostrum.

The discovery of a trypsin inhibitor in cow colostrum by Laskowski & Laskowski (1951) and much higher concentrations of pepsin-resistant trypsin inhibitor in sow colostrum by Laskowski, Kassell & Hagerty (1957) first suggested that pancreatic enzymes might destroy immune globulin in the new-born animal, but experimental evidence to support this suggestion was not obtained until the work of Norbring & Olsson (1958*a*, *b*). During these investigations, the addition of bovine colostrum trypsin inhibitor to sow colostrum was shown to result in a significantly increased absorption of paratyphoid H agglutinins and colostrum γ-globulin by pigs 24 and 36 hr old when fed, although in animals fed 48 and 72 hr after birth, the inhibitor had little or no effect on absorption. This was attributed by the authors to digestion of the inhibitor by pepsin in the older animals. They also found that the addition of bovine colostrum trypsin inhibitor to pig serum caused an increase, 'probably significantly', in the absorption of γ-globulin from the serum when fed at birth and at 26 hr of age. The amounts of inhibitor used in these experiments were extremely large, however, since each animal was fed sufficient to inhibit 70 g trypsin, which was equivalent to the inhibitor present in 35 l. sow colostrum.

In the more recent studies of Chamberlain *et al.* (1965) smaller amounts of sow colostrum trypsin inhibitor were used, but the results are not directly comparable with those of the present experiments since the animals were 80 hr old when the labelled γ-globulin was fed.

The experiments reported here have shown that the break-down of [^{131}I]serum γ-globulin when fed in chloride solution was decreased by the addition of relatively small amounts of the synthetic trypsin inhibitor Trasylol to the solvent. It is claimed by the manufacturer that 50 kallikrein inhibiting units (k.i.u.) will inhibit the proteolytic activity of 100 μg crystalline trypsin by 95%, so that in the experimental pigs fed 12,500 k.i.u. it would be expected that the maximum trypsin inactivation would be equivalent to 95% inhibition of 25 mg crystalline trypsin, assuming that Trasylol itself was not significantly inactivated by other digestive enzymes. This would approximately equal the trypsin inhibitor content of 12 ml. sow colostrum or 40 ml. cow colostrum: see Laskowski *et al.* (1957).

The ability of both natural and synthetic trypsin inhibitors to decrease the break-down of γ-globulins within the new-born pig gut implied that a component of the over-all proteolysis was derived from pancreatic juice, and there is some additional evidence to suggest that the pancreas of the new-born pig contains considerable amounts of trypsinogen. It has been reported that the trypsin content of dried pancreas was 'relatively high' and that the increase of trypsin activity with age was largely due to an increase in the size of the gland rather than to an increased trypsin concentration in the gland (Lewis, Hartman, Liu, Baker & Catron, 1957). Although there have been no direct studies of the secretion of trypsin by the pancreas of the new-born pig, there is some indirect evidence to suggest that the trypsin measured in extracts of the gland may be secreted into the small intestine, as Hartman, Hays, Baker, Neagle & Catron (1961) have reported proteolytic activity in samples of gut contents taken in the new-born pig from both the duodenum and terminal ileum with pH 6·5 and 6·8 respectively.

Although results obtained with the trypsin inhibitor and from gel-filtration of the contents of the duodenum and terminal ileum implied that protein digestion occurred in the small intestine, gel-filtration of gastric contents showed that proteolysis also took place within the stomach. The new-born pig can secrete acid into the stomach, as stomach contents of pH < 2 have been noted in the unsuckled animal (Hardy, 1966). The pH of stomach contents from suckled animals is somewhat higher, however, since values of between 4·0 and 4·3 were reported in pigs less than 24 hr old by Hartman *et al.* (1961). This disparity can be attributed in part to the buffering capacity of colostrum, since colostrum whey is an efficient buffer to the addition of HCl down to at least pH 4 (R. N. Hardy, unpublished observations). The ability of colostrum to buffer against the addition of acid would of course have some physiological significance if it contributed to the reduction of gastric proteolysis.

The *in vitro* break-down of various protein substrates by enzymes from the stomach of the new-born pig has been described by other workers. The proteolytic activity of dried stomachs taken from pigs at birth was assessed with a skim-milk and agar substrate by Lewis *et al.* (1957), who showed that there was some activity at this time although there was a threefold increase in activity within the first week after birth. However, Hartman *et al.* (1961), using a casein substrate, reported that gastric proteinase activity was more or less constant from birth to 3 weeks of age.

The information at present available therefore indicates that there is some proteolytic activity in the stomach of the new-born pig and that HCl secretion at this time is sufficient to reduce the gastric pH to below pH 4·5, even in suckled animals. The identity of the enzyme responsible for the

proteolytic activity within the stomach has not been finally established, but, since there have been no reports of rennin in this species, it seems likely, as has been assumed by other workers, that pepsin is responsible for the break-down of protein previously observed *in vitro*, and demonstrated *in vivo* in this investigation.

I would like to thank Dr R. S. Comline for his advice and encouragement and the Agricultural Research Council for financial assistance.

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