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PROTEOLYTIC ACTIVITY DURING THE ABSORPTION OF [^{131}I]γ-GLOBULIN IN THE NEW-BORN CALF

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SUMMARY

1. Proteolytic activity within the small intestine of unsuckled calves less than 20 hr of age, anaesthetized with sodium pentobarbitone, has been assessed from the break-down of [^{131}I]bovine serum γ-globulin infused into the duodenum.

2. Absorption of [^{131}I]γ-globulin was measured by analysis of venous blood, the levels of radioactivity attained in which were comparable with those when [^{131}I]PVP K.60 (mean mol.wt. 160,000) was administered. When lymph collected from the thoracic duct during the absorption of [^{131}I]γ-globulin was injected into the femoral vein, the levels of radioactivity in the blood were close to those expected if the labelled material in the lymph had been retained within the plasma. These observations suggested that [^{131}I]γ-globulin was absorbed into the circulation of the anaesthetized young calf without significant break-down.

3. Gel-filtration of lymph and plasma from calves fed [^{131}I]γ-globulin has confirmed that proteolysis before and during absorption was slight, since little ^{131}I labelled material of low mol.wt. was found.

4. Gel-filtration of the contents of the alimentary tract from calves fed [^{131}I]γ-globulin showed that some hydrolysis occurred in the abomasum and duodenum and that this was reduced by barbiturate anaesthesia. Protein break-down in the terminal ileum was slight both in the conscious animal and in animals anaesthetized with sodium pentobarbitone.

INTRODUCTION

The small intestine of the new-born of many species possesses a unique permeability to substances of high mol.wt. In the young calf immune globulins from the colostrum can pass across the small intestine and into the lacteals during the first 24–36 hr after birth and these proteins convey to the animal the passive immunity essential for its survival. It has pre-

viously been reported that there is extensive proteolytic break-down within the alimentary tract of the young pig of [^{131}I]γ-globulins fed in colostrum (Hardy, 1969*c*) and it was the purpose of the experiments to be reported in this paper to determine whether comparable break-down of γ-globulin occurred in the young calf.

It will be shown that digestive function in this species is less well developed than that in the young pig during the first 20 hr after birth, with the consequence that relatively little break-down of [^{131}I]serum γ-globulin could be demonstrated.

METHODS

The general experimental procedures used in this work have been reported in detail elsewhere (Hardy, 1969*a*): calves were anaesthetized with sodium pentobarbitone and cannulae were placed in the trachea and right femoral vein. Experimental solutions were introduced into the duodenum via a glass cannula (100 ml./10 min) and lymph was collected from a polyethylene cannula placed in the thoracic or main intestinal lymph duct. In control calves, which remained conscious during the experiment, experimental solutions were administered with a feeding bottle and blood samples were taken from the jugular vein via a polyethylene cannula inserted under local anaesthesia. The technique of gel-filtration (Sephadex G. 100) has also been described previously (Hardy, 1969*c*).

Lymph re-circulation experiments. Consecutive 10 min lymph samples were taken throughout the experiment. At the end of each collection period, 3 ml. lymph was removed for scintillation counting and, after the volume had been noted, the remaining lymph was immediately injected into the femoral vein and washed through the cannula with 3 ml. heparin-saline. Blood samples were taken every 20 min from the femoral vein.

In order to calculate the theoretical blood ^{131}I concentration at any point of time it was necessary to know the blood volume of the animal and the amount of isotope introduced into the femoral vein up to that point.

Blood volume was taken to approximate to 8% of body weight (plasma volume 4·9% body wt. (Pierce, 1959); haematocrit: 35–40%) (R. N. Hardy, unpublished).

The amount of ^{131}I contained in the lymph introduced into the femoral vein at the end of each collection period (A) could be calculated from: $V/3 \times C$ where V = volume injected and C = net counts/min for a 3 ml. aliquot.

The cumulative amount of ^{131}I injected into the femoral vein was represented by the sum of the amount injected at the end of each collection period (ΣA).

The concentration of ^{131}I expected in the blood at any time could be calculated from $\Sigma A/B$ where B = blood volume.

The concentration of ^{131}I in blood or lymph has been expressed as a percentage of the ^{131}I concentration in the experimental solution infused into the duodenum after Balfour & Comline (1962) thus:

Theoretical blood percentage radioactivity = $100\,(\Sigma A/B)/M$ where M = net counts/min per 3 ml. colostrum administered.

Actual blood percentage radioactivity = $P/M \times 100$ where P = net counts/min per 3 ml. blood.

Samples for gel-filtration

Blood. At the end of the experiment, 10 ml. blood was taken from the femoral vein, mixed with a little heparin and spun at 3000 rev/min for 10 min. The plasma was then removed and stored in a polyethylene bottle at −20° C to await gel-filtration.

Lymph. Samples of lymph were taken from the intestinal lymph duct into a 5 ml. syringe by direct puncture with a no. 1 hypodermic needle. The lymph, together with a small quantity of heparin, was discharged into a polyethylene bottle and stored at −20° C until required.

Contents of the alimentary tract. Fluid from the abomasum was readily obtained and could be taken directly into a syringe through a wide-bore needle. To obtain samples of duodenal fluid, the proximal 2 m of the small intestine were removed after ligation of each end. The external surface of the intestinal segment was washed briefly in warm saline and its fluid contents were then allowed to drain into a beaker from the cut ends while it was suspended by its mid-point. In this way it was usually possible to collect at least 10 ml. fluid within a few minutes. The contents of the terminal 2 m of the ileum were obtained in a similar manner. Samples of gut contents were placed in polyethylene centrifuge tubes and spun for 15 min at 12,000 rev/min. The supernatant was stored at −20° C until gel-filtration.

RESULTS

When [^{131}I]serum γ-globulin was fed to new-born pigs, the levels of radioactivity measured in the blood were much lower than those measured in pigs fed [^{131}I]polyvinyl pyrrolidone (PVP) K.60 (mean mol.wt. 160,000). It has been shown that this discrepancy could be explained by the hydrolysis of a proportion of the [^{131}I]serum γ-globulin before absorption, so that labelled fragments were produced which were sufficiently small to escape rapidly from the plasma. During this work preliminary results suggested that the break-down of [^{131}I]serum γ-globulin was much less vigorous in the young conscious calf (Hardy, 1969*c*, Fig. 2).

The behaviour of ^{131}I labelled material appearing in the circulation after the absorption of [^{131}I]bovine serum γ-globulin

*Comparison with [^{131}I]PVP K.*60. The properties of the capillary membranes are such that serum γ-globulin and substances of comparable mol.wt., such as PVP K.60, can only pass into the extracellular fluid or urine extremely slowly (Wasserman, Loeb & Mayerson, 1955), whereas low mol.wt. labelled digestion products of [^{131}I]serum γ-globulin rapidly escape from the plasma. It has been shown that [^{131}I]serum γ-globulin and [^{131}I]PVP K.60 are absorbed from the small intestine into the lymph of the anaesthetized, unsuckled calf in closely comparable amounts (Hardy, 1969*a*). Therefore, since [^{131}I]PVP K.60 is not affected by enzymes within the gut (Hardy, 1969*b*), the rate of accumulation of ^{131}I in the blood after feeding this solute provides a standard of comparison for the behaviour of

labelled material after feeding [^{131}I]serum γ-globulin. That is to say, that if little of the protein has been hydrolysed into small fragments, blood levels of ^{131}I after administration of [^{131}I]serum γ-globulin should be comparable with those seen when [^{131}I]PVP K.60 is used. Such is indeed the case in the calf, as can be seen from Figs. 1 and 2, which contrast with results reported previously in the young pig (Hardy, 1969*c*).

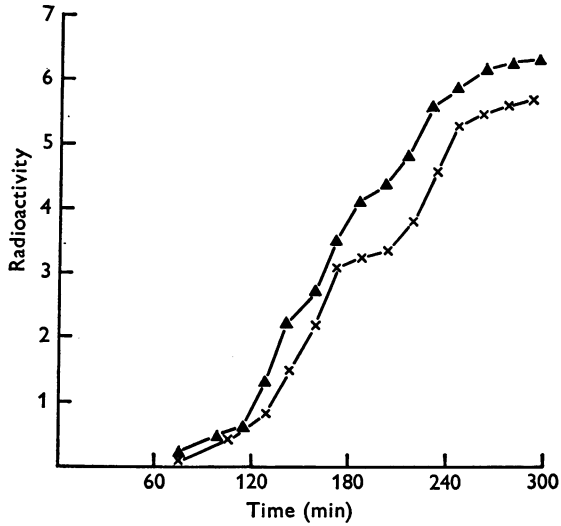

Fig. 1. Absorption of [^{131}I]PVP K.60 from cow colostrum measured by venous blood samples. Blood percentage radioactivity in Calf 52, 15 hr old (×—×) and Calf 54, 7 hr old (▲—▲). Ordinate indicates blood radioactivity as a percentage of the radioactivity of the solution infused into the duodenum (blood % radioactivity): abscissa, time (min).

Lymph re-circulation experiments. It was not possible to demonstrate from the previous experiments that all the labelled material which had been absorbed from the intestine had remained in the circulation. In order to examine this point further, therefore, experiments were performed in which lymph taken from the thoracic duct during the absorption of [^{131}I]serum γ-globulin was monitored for ^{131}I and then returned to the animal by intravenous injection. The consequent radio-iodine concentrations in the blood were then compared with values calculated on the assumption that all the ^{131}I labelled material contained in the reinfused lymph had been retained within the circulation. The technique and calculations employed have been described previously on page 454.

The result of one such experiment is illustrated in Figs. 3 and 4. The lymph percentage radioactivity, lymph flow and cumulative amount of ^{131}I injected into the femoral vein (ΣA) are shown in Fig. 3, while the

actual and theoretical blood percentage radioactivities are plotted in Fig. 4. It is apparent from these results that the concentration of ^{131}I in the blood of this animal corresponded well with the theoretical level, which suggests that little of the [^{131}I]serum γ-globulin had been hydrolysed into small fragments.

Evidence from gel-filtration

The technique of gel-filtration on Sephadex G. 100 provides a method for the determination of the approximate mol.wt. of radio-iodinated material in solution. It has been possible therefore to measure the proportion of low mol.wt. ^{131}I labelled material present in the lymph and

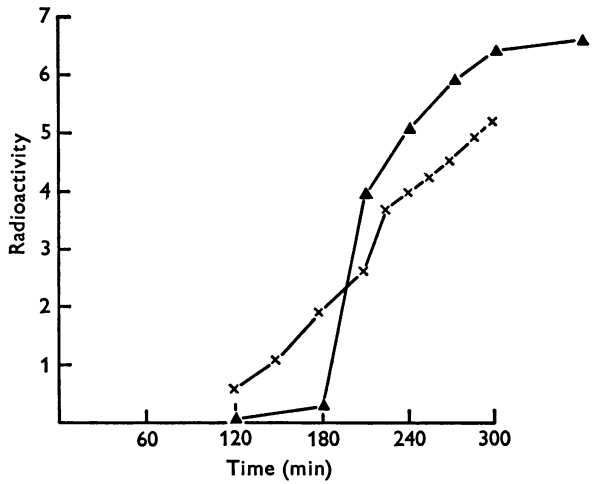

Fig. 2. Absorption of [^{131}I]bovine serum γ-globulin from cow colostrum, measured by venous blood samples. Blood % radioactivity in Calf 68, 7 hr old (×—×) and Calf 74, 10 hr old (▲—▲). Ordinate and abscissa as Fig. 1.

plasma of experimental calves, and thus to assess the degree of proteolytic break-down during absorption. The technique of gel-filtration was identical with that used on samples from young pigs and has previously been described in detail (Hardy, 1969*c*).

The results obtained from Calf 66, aged 3 hr, are shown in Fig. 5. In a 2 ml. sample of the colostrum administered, 1 % of the ^{131}I measured was associated with low mol.wt. material and the remaining ^{131}I was found in a single peak corresponding with the void volume of the column, and therefore with material of mol.wt. at least 150,000.

The almost complete absence of proteolytic break-down of γ-globulin during absorption in this anaesthetized animal was shown by the distribution of ^{131}I labelled material after gel-filtration of plasma and lymph.

In the lymph 99% and in the plasma 100% of the ^{131}I measured was found in a single, apparently homogeneous, peak with an elution volume corresponding with that of the intact [^{131}I]γ-globulin in the colostrum.

Similar experiments in other anaesthetized calves have suggested that there is a tendency for proteolysis to become more apparent in older animals as indicated by the presence of more ^{131}I labelled material of low mol.wt. in the plasma and lymph of these animals. This is illustrated in

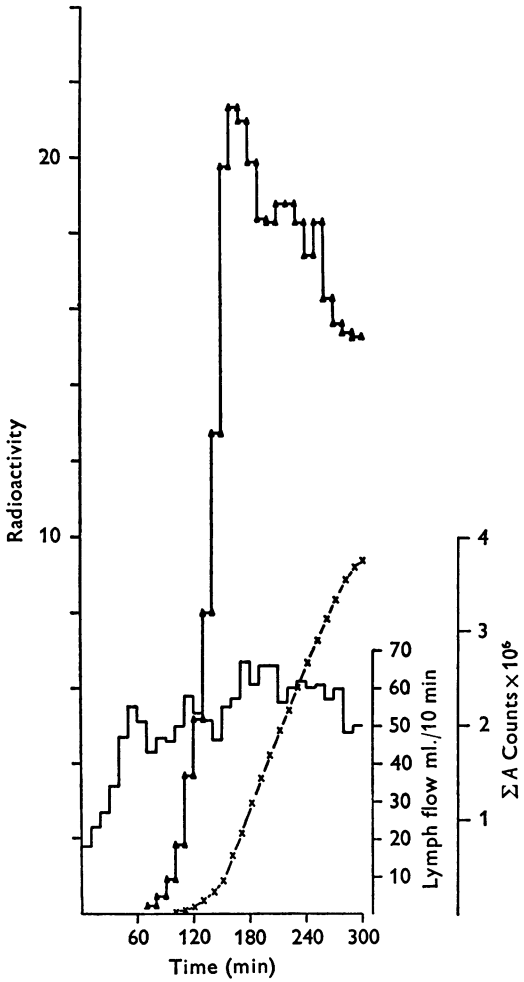

Fig. 3. Infusion of lymph into the femoral vein. Calf 56, 24 hr old. [^{131}I]-bovine serum γ-globulin in cow colostrum introduced into the duodenum. Ordinates: lymph percentage radioactivity (▲—▲), lymph flow (——) and cumulative amount of isotope injected into the femoral vein (×—×). Abscissa: time.

Fig. 6, which shows the distribution of ^{131}I labelled material in the lymph and plasma of a 7-hr-old calf (Calf 68, see Fig. 2).

It would be expected by analogy with the pig, that the degree of proteolytic break-down of γ-globulin in the colostrum during absorption would increase with the age of the calf and indeed the evidence produced from animals of 3 and 7 hr of age would support this view. It was, therefore, surprising to find in a 21-hr-old animal that the proportion of ^{131}I labelled material in the plasma with a mol.wt. less than 12,400 was less

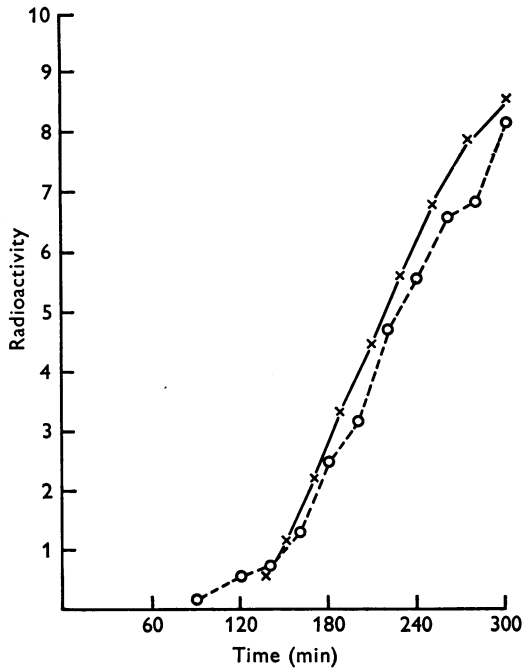

Fig. 4. Calf 56. Percentage radioactivity measured in samples of femoral venous blood (○ - - - ○) and the theoretical blood % radioactivity (× — ×) as calculated p. 454. Ordinate and abscissa as Fig. 1.

than 1 % of the total. It is possible that this animal was exceptional, but it serves to emphasize further the great contrast between the proteolytic activity of the young calf gut and that of the pig.

It might be thought that the discrepancy between the results obtained in the pig (Hardy, 1969*c*) and those reported here from the young calf could be explained by the fact that the pigs remained conscious throughout the experiment while the calves had been anaesthetized with sodium pentobarbitone. It became clear early in these investigations, however, that proteolytic break-down remained slight even in control calves which had not been anaesthetized: the results from one such animal are shown in Fig. 7.

Site of protein break-down

One difference between orally fed control calves and anaesthetized calves fed by duodenal infusion was revealed during gel-filtration analysis of contents of the alimentary tract.

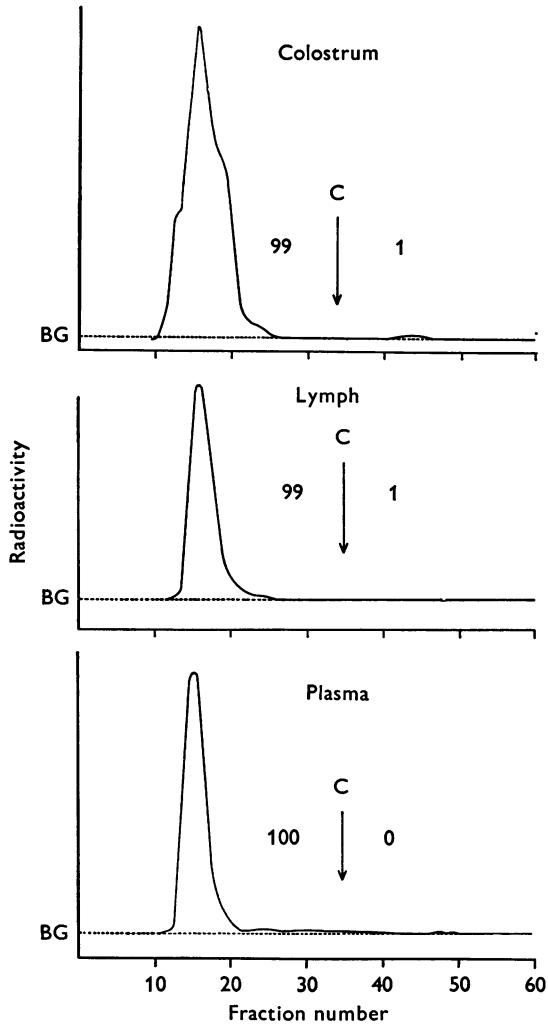

Fig. 5. Gel-filtration of samples of the colostrum fed and lymph and plasma, taken 6 hr after feeding, from a 3-hr-old calf fed [^{131}I]bovine serum γ-globulin in cow colostrum. Elution volume of cytochrome *c* shown at C, percentage of net radioactivity eluted before and after cytochrome *c* shown on the appropriate side of C. Ordinate: radioactivity of individual fractions relative to background (BG). Abscissa: fraction number (volume of eluate).

Gel-filtration of samples of gut contents from a control animal (Fig. 8) showed that 83% of the total ^{131}I found after gel-filtration of abomasal contents was eluted with material of mol.wt. less than that of cytochrome *c* and although the corresponding value for duodenal contents was 85%,

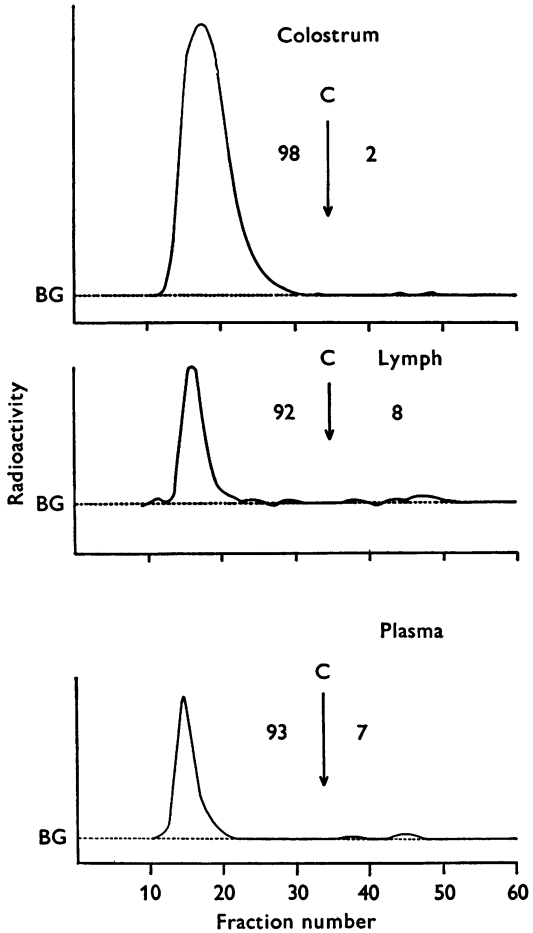

Fig. 6. Gel-filtration of samples of the colostrum fed and lymph and plasma taken 5 hr after feeding from Calf 68, 7 hr old. Cow colostrum containing [^{131}I]bovine serum γ-globulin was introduced into the duodenum. Annotation as Fig. 5.

only 18% of the ^{131}I in the ileal contents was associated with low mol.wt. material. This can be compared with results obtained from an animal anaesthetized with sodium pentobarbitone. This calf was given 500 ml. cow colostrum with [^{131}I]bovine γ-globulin by duodenal infusion and absorption, which was followed by analysis of femoral venous blood, has

been previously illustrated in Fig. 2. A glass cannula was tied into a relatively avascular part of the abomasum and 200 ml. colostrum, similar to that infused into the duodenum, were placed in the abomasum 15 min after the duodenal infusion was started. Samples of abomasal fluid were

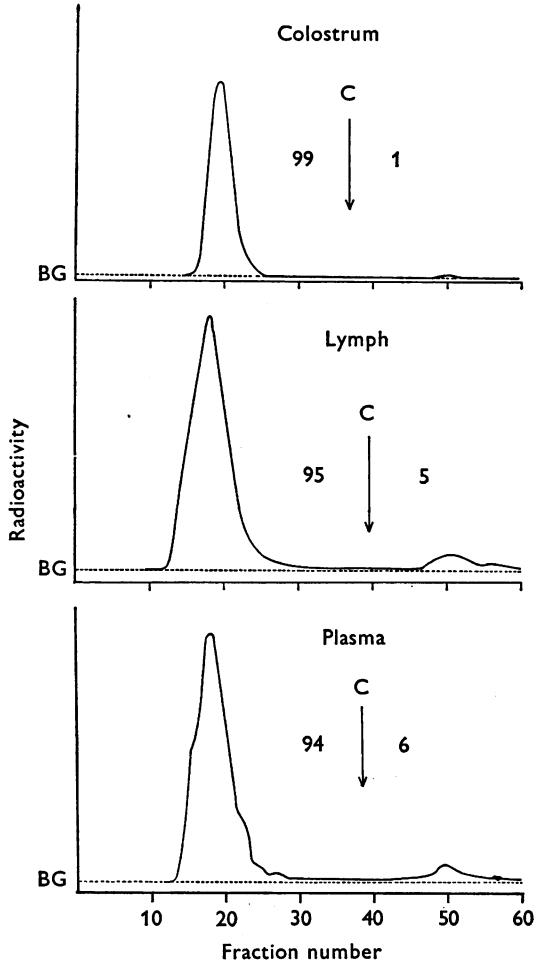

Fig. 7. Gel-filtration of the colostrum fed and lymph and plasma taken from Calf 70, a 4 hr-old-calf, fed 1 l. cow colostrum containing [^{131}I]bovine serum γ-globulin by mouth. The animal remained conscious throughout the experiment. Annotation as Fig. 5.

taken every 2 hr by passing a polyethylene tube through the glass cannula and the pH of these samples was measured under liquid paraffin after they had reached room temperature (Table 1).

A sample of abomasal fluid taken at 6 hr was examined by gel-filtration,

and it will be seen from Fig. 9 that only 15% of the ^{131}I present was associated with material of mol.wt. less than 12,400. The corresponding values for duodenal and ileal contents in this animal were 19 and 16% respectively.

These results indicate that in the conscious animal with patent pylorus, considerable proteolysis occurred within the abomasum and, possibly as a consequence of this, low mol.wt. fragments were seen also in the duodenum.

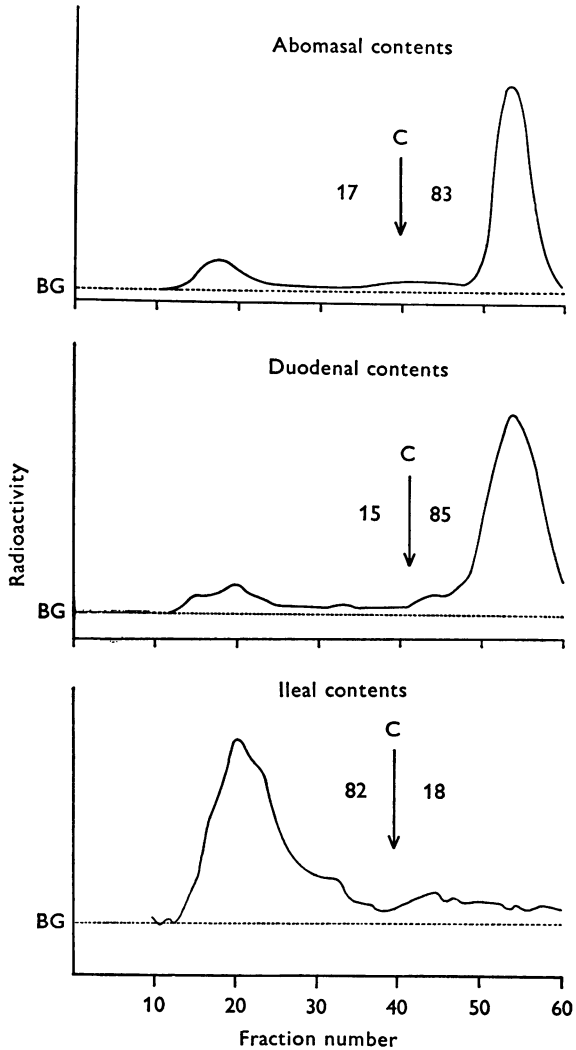

Fig. 8. Gel-filtration of samples of abomasal, duodenal and ileal contents taken 6 hr after feeding 1 l. cow colostrum containing [^{131}I]bovine serum γ-globulin to Calf 70, 4 hr old. Annotation as Fig. 5.

TABLE 1. The pH changes observed in samples of whey taken from the abomasum of a 10-hr-old calf, anaesthetized with sodium pentobarbitone. Cow colostrum containing [^{131}I]bovine serum γ-globulin was incubated within the abomasum

Time in abomasum (hr)	pH
Original colostrum 0	6·60
2	6·95
4	7·00
6	7·15

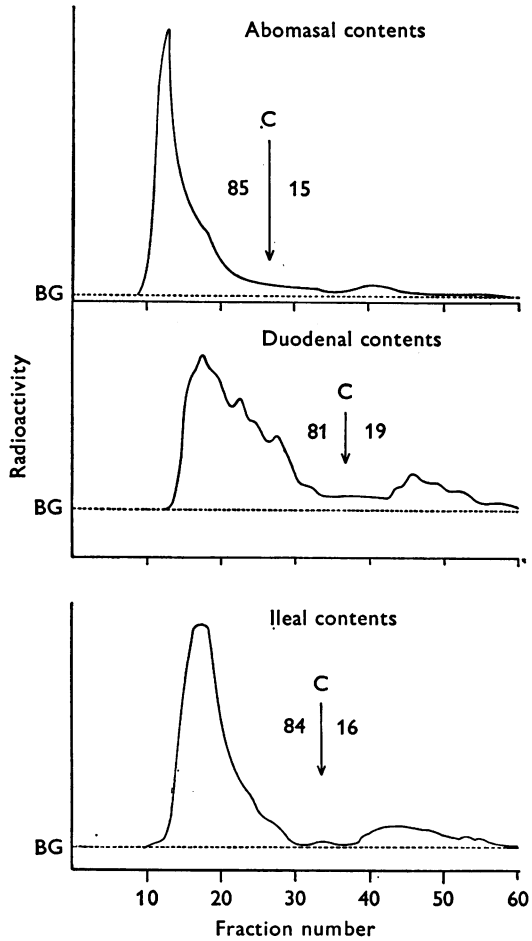

Fig. 9. Gel-filtration of samples of abomasal contents, after incubation of 200 ml. cow colostrum containing [^{131}I]serum γ-globulin in the abomasum for 6 hr, and duodenal and ileal contents 6 hr after the duodenal infusion of 500 ml. of a solution similar to that placed in the abomasum. Calf 74, 10-hr-old anaesthetized with sodium pentobarbitone. Annotation as Fig. 5.

The relatively small proportion (18 %) of low mol.wt. ^{131}I labelled material in the terminal ileum suggested that little proteolysis was occurring at this level and that during passage through the small intestine the low mol.wt. ^{131}I labelled material present in the duodenum had been absorbed more rapidly than the intact protein. In contrast, in the calf anaesthetized with sodium pentobarbitone and with the pylorus occluded, acid secretion in the abomasum was insufficient to lower the pH of the contents and little proteolysis occurred in either the abomasum or the small intestine.

DISCUSSION

In the young pig [^{131}I]PVP K.60 tends to remain in the plasma after absorption from the intestine, whereas much of the ^{131}I entering the plasma in animals fed [^{131}I]serum γ-globulin is rapidly lost from the plasma by virtue of its association with low mol.wt. fragments from protein digestion. In consequence of this, in the pig, much higher blood radioiodine concentrations were seen in animals fed [^{131}I]PVP K.60 than in those fed [^{131}I]γ-globulin (Hardy, 1969*c*).

In this paper comparable experiments performed on a number of anaesthetized calves showed that the terminal blood % radioactivities in animals fed PVP K.60 (5·7 and 6·3) were similar to those seen in calves fed serum γ-globulin (5·3 and 6·5). Since these two solutes are absorbed in comparable amounts from the intestine (Hardy, 1969*a*) the results suggested that, having entered the circulation, both PVP K.60 and serum γ-globulin were lost from the plasma at a similar, relatively slow, rate.

The large size of calves prevented any attempt to relate the blood radioactivity to a direct estimate of absorption obtained from the recoveries from gut and carcass, as can be done in the pig. In the calf, however, an estimate of the amount of the original dose which had been absorbed was obtained from the product of the volume of lymph produced and its radioiodine content, and an assessment of the fate within the circulation of ^{131}I labelled material absorbed was made in two calves, by the intravenous injection of the lymph collected. The close correspondence between the theoretical blood radioactivity and the observed values in these animals implied that most of the ^{131}I labelled material absorbed into the lymph was of a molecular size sufficient to prevent significant loss from the circulation during the 5 hr experiment.

It is known that low mol.wt. protein components from colostrum, particularly β-lactoglobulin, appear in the urine during the immediate postnatal period when the calf can absorb undegraded protein. However, proteins with the sedimentation of γ-globulin are not seen in the urine despite the high concentrations of this component in the plasma after

suckling (Pierce & Johnson, 1960). The elimination of β-lactoglobulin from the blood after absorption from the colostrum appears to be simply a consequence of its low mol.wt., since it has been shown that gelatine of similar mol.wt. (*ca.* 40,000) is cleared in a comparable manner (Pierce, 1961). In fact the threshold for glomerular filtration in the new-born calf seems to lie within the mol.wt. range 40,000–50,000 since only small amounts of fetuin with the latter mol.wt. are found in the urine (Pierce, 1959). Since little of the radio-iodinated material present in the re-infused lymph in the present experiments escaped from the circulation, it can be assumed that it was not filtered through the glomerular or other capillary membranes and consequently that it had a mol.wt. exceeding 50,000. In fact there were negligible amounts of ^{131}I labelled material in the urine of experimental calves.

The comparison of the blood radio-iodine concentrations of calves fed [^{131}I]serum γ-globulin or [^{131}I]PVP mol.wt. 160,000 and the results of the lymph re-circulation experiments, gave rise to two possibilities: either very little proteolytic break-down occurred during γ-globulin absorption, or, if proteolysis did occur, only a small proportion of the radio-iodinated fragments produced were of a mol.wt. less than 50,000. The latter possibility seemed to be unlikely in view of the evidence obtained in the young pig (Hardy, 1969*c*), which indicated that if significant proteolysis did take place during absorption, a large proportion of the ^{131}I labelled fragments were sufficiently small to be rapidly lost from the plasma, with the consequence that blood ^{131}I concentrations remained low, and considerable radioactivity was found in the carcass and urine.

It has been confirmed by the use of gel-filtration techniques that the amount of proteolysis during γ-globulin absorption in the calf is very small relative to that seen in the pig. The major portion of the ^{131}I in samples of lymph and plasma was eluted in a single homogeneous peak corresponding with the mol.wt. of the radio-iodinated protein fed. The proportion of labelled fragments with a mol.wt. less than 12,400 measured in these experiments was, of course, a minimum estimate, since these fragments would be able to escape from the plasma. Nevertheless, the results can be compared directly with those obtained in the young pig.

The results are in accord with the demonstration by Balfour & Comline (1962) that the radio-iodinated material in the lymph of experimental calves given [^{131}I]γ-globulin *per duodenum* did not pass through a Cellophane membrane permeable to material of mol.wt. less than 10,000, and in addition that 92% of the ^{131}I in the dialysed lymph had a mobility characteristic of serum γ-globulin during column electrophoresis. On the other hand, evidence that modifications in the structure of a proportion of the γ-globulin may occur during absorption was provided by the detec-

tion within the urine of suckled calves of material with the antibody activity of the colostrum γ-globulin fed, but without the sedimentation characteristics of γ-globulin (Pierce & Johnson, 1960). It is not known whether this material corresponds with the low mol.wt. labelled fragments of [^{131}I]serum γ-globulin detected in the plasma by gel-filtration during the present experiments.

The site of proteolytic break-down was investigated by the gel-filtration of samples of abomasal, duodenal and ileal contents after feeding [^{131}I]-serum γ-globulin in cow colostrum, but the results obtained must be treated with certain reservations. In the abomasum and duodenum, while no radio-iodinated material of high mol.wt. would be absorbed from the lumen, it is likely that components of relatively low mol.wt. would be absorbed (see e.g. Karel, 1948; Wiseman, 1964), in which case the percentage of low mol.wt. material reported in these regions would constitute a minimum estimate. In the terminal ileum both high and low mol.wt. components might be expected to be absorbed, which makes the interpretation of the results of gel-filtration of ileal contents extremely difficult. Despite these criticisms and the unsatisfactory elution of samples of gut contents during gel-filtration, the results obtained are of some value in delineating the site of protein break-down.

Gel-filtration of a sample of abomasal contents from a 4-hr-old conscious calf fed [^{131}I]bovine serum γ-globulin in cow colostrum showed that more than 80 % of the total ^{131}I was associated with fragments of digested protein. This result, however, probably exaggerated the normal proteolytic break-down at this site, since the sample analysed was taken from the whey which remained in the abomasum 6 hr after feeding and, as such, was obviously not representative of the greater part of the whey, which would have passed into the small intestine much more rapidly.

It is clear from this result, however, that proteolytic enzymes do hydrolyse γ-globulin within the abomasum of the new-born calf. The activity of this organ is a subject which has been virtually ignored in recent years, since the currently accepted method of analysing abomasal activity involves the construction of a fistula, a process which precludes investigations during the immediately post-operative period (Ash, 1964). Furthermore, interpretation of the older literature is difficult since it was often not appreciated that enzymes other than rennin could clot milk and that rennin itself could have other protein substrates. In addition, much of the published work referred to *in vitro* analyses of the proteolytic activity of abomasal homogenates at low pH, which may have little relevance to the actual conditions of secretion into the abomasum at birth (see e.g. Huber, Jacobsen, Allen & Hartman, 1961).

Despite the general absence of relevant evidence in the literature, two reports suggest that little, if any, pepsin occurs in the abomasum of the new-born calf. Berridge, Davis, Kon, Kon & Spratling (1943) concluded that the production of pepsin was a consequence of weaning, and Herschel, Hill & Porter (1961), although unable to show that pepsin activity was initiated by feeding roughage, confirmed the absence of pepsin in the abomasum of the very young calf, since this enzyme was first detected at 1–2 weeks of age.

If pepsin is not present in the abomasal secretion of the new-born calf, the proteolytic break-down of γ-globulin seen in these experiments must in all probability be attributed to rennin.

Rennin will clot milk in neutral solutions (Sewall, 1878; Berridge, 1954; Taylor, 1962) but the optimal pH for this action is 3·8 (Berridge, 1954). There have been no reports of the effect of pH on the proteolysis of γ-globulin by rennin, but it may be that the pH of the abomasal contents is potentially a limiting factor in determining the proteolytic activity of any rennin which may be secreted. In addition, pH may limit the conversion of the inactive precursor pro-rennin into the enzyme itself, since little activation occurs above pH 5·3 (Berridge, 1954).

The pH of the gut contents of the unsuckled calf was investigated by Parrish & Fountaine (1952) who reported that combined omasal and abomasal contents were of average pH 4·4 with a range between pH 4·0 and 4·8. Colostrum has considerable buffering activity and can neutralize large amounts of HCl and would probably increase the ambient pH to the range pH 6–7 in the abomasum of the suckled animal. This would comply with Pierce (1962), who reported that the pH of the abomasal contents of newly born suckling calves was 'slightly on the acid side of neutrality', and was thus 'not sufficiently low for effective peptic proteolysis'.

From the information available it can be concluded that the proteolytic activity in the abomasum of the new-born calf is not due to pepsin and that it can probably be attributed to the action of rennin. The pH of the abomasum in the unsuckled calf would permit the activation of this enzyme from its precursor but, after suckling, further activation would be limited, due to the buffering action of colostrum which would raise the pH and also therefore restrict the extent of proteolysis. In older calves the pH of the abomasal contents is considerably lower, since acid secretion is more vigorous and can exceed the buffering capacity of the milk. Ash (1964) found that, in a series of unweaned calves more than 20 days old, the contents of innervated abomasal pouches were invariably less than pH 2 before feeding. Immediately after feeding the pH rose to 3·05–5·83 and then decreased slowly until the next feed. Inhibition of proteolytic activity within the abomasum by barbiturate anaesthesia was demon-

strated in the present investigation by the results of gel-filtration of samples of abomasal contents.

Gel-filtration of fluid contents from the duodenum after feeding [^{131}I]-serum γ-globulin in colostrum showed that more than 80 % of the total ^{131}I was associated with digested protein in the conscious animal. In contrast, less than 20 % of the labelled protein was broken down in an animal, anaesthetized with sodium pentobarbitone, in which the pylorus had been occluded.

It was not possible from these results to determine the proportion of the protein break-down due to abomasal activity or proteolytic enzymes secreted into the duodenum, but there is some other evidence to suggest that the pancreas is relatively inactive in the new-born calf. The flow of juice is scant and it is of low enzyme content (R. S. Comline, unpublished observations). It is also reported that the proteolytic activity of pancreatic homogenates in 24-hr-old calves is low, increasing fourfold during the first week (Huber *et al.* 1961).

If the secretions of the pancreas indeed contribute little to proteolysis in the new-born calf it is difficult to reconcile the widely held view that the trypsin inhibitor in cow colostrum is important, as such, to the calf in permitting the absorption of immune protein which would otherwise be digested.

The results of gel-filtration have shown that the proportion of digested fragments of [^{131}I]serum γ-globulin is small in samples taken from the ileum of both conscious calves and calves anaesthetized with sodium pentobarbitone. Therefore little proteolysis would seem to occur in this, the region of the small intestine where the absorption of undigested protein takes place.

The results of this investigation have shown that the break-down of γ-globulin within the alimentary tract of the calf is small relative to that seen in the pig, and it seems unlikely that the break-down of a small proportion of the immune globulins of the colostrum, whether within the abomasum or the duodenum, would seriously prejudice the survival of the calf. In a series of experiments in which the survival of unsuckled calves fed various volumes of colostrum was measured, Aschaffenberg, Bartlett, Kon & Walker (1949) showed that while five-sixths of calves deprived of colostrum died, all six of a group fed only 80 ml. colostrum, survived. If this is the case, the volume of colostrum ingested by normal suckling during the absorptive period would seem to provide a very great safety factor.

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