J. Physiol. (1967), **190**, *pp.* 1–18
With 10 *text-figures*
Printed in Great Britain

THE INTESTINAL ABSORPTION OF PIG AND BOVINE IMMUNE LACTOGLOBULIN AND HUMAN SERUM ALBUMIN BY THE NEW-BORN PIG

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(*Received* 21 *March* 1966)

SUMMARY

1. Homologous and heterologous colostral immune globulins and human serum albumin were fed to new-born pigs and an attempt was made to estimate the amounts appearing subsequently in serum.

2. All three proteins, fed separately in large amounts to different pigs, appeared in the serum in low concentration about 45 min after feeding, and then rose quickly to a high level. No difference could be detected between the amounts absorbed when equal amounts had been fed but there was a wide variation between pigs. Previous dialysis of pig colostrum against bicarbonate saline did not affect the rate or amount of pig immune globulin absorbed after feeding.

3. When pig and bovine colostral IgG were fed together at equal concentrations in bovine colostrum, the absorption of pig IgG was greater than that of bovine IgG. Human serum albumin, added to bovine colostral IgG in bovine colostrum, was absorbed readily and this did not interfere significantly with the absorption of bovine colostral IgG.

4. The efficiency with which the pig intestine absorbed bovine colostral IgG depended on the dose and/or concentration fed, increasing as the dose fed was increased to 2 g and remaining constant for higher doses.

5. Some of the absorbed immune globulin was shown to exist in a partly degraded form.

6. The process of protein transfer across the intestine of the new-born pig may select, to a limited degree, between different proteins, but the digestion of protein shown to take place and the large variation between individual pigs makes interpretation of these results uncertain.

INTRODUCTION

The absorption of undegraded protein across biological membranes can readily be studied using the new-born of several species of domestic animals. In the ungulate, relatively large amounts of ingested immune lactoglobulin are absorbed, in an essentially unchanged form, across the mucosa of the small intestine immediately *post partum*. It is believed that a micropinocytotic process takes protein into the cell (Clark, 1959) and that subsequently the immune globulin moves out of the cell and drains into the mesenteric lymphatics, passing through the thoracic duct into the general circulation (Comline, Roberts & Titchen, 1951).

Many observations, on different species, have emphasized the complexity of this process. While it has been stated that the pig intestine immediately after birth can absorb both immune globulins and other macromolecules (Lecce, Matrone & Morgan, 1961; Hardy, 1965), the rat and mouse intestine absorb immune globulins (Halliday, 1957; Hemmings & Morris, 1959), but not the homologous serum albumin (Bangham & Terry, 1957).

It has recently been recognized that difficulties may arise in the interpretation of experiments where an estimate of the amount of absorbed immune globulin is based on antibody activity. Morris (1965) showed in the rat that specific agglutinins were modified during absorption so that they lost most of their agglutinating activity and also that the 19*S* immune globulin, which on a molecular basis has been shown to be 750 times more efficient than 7*S* globulin at agglutinating red cells (Greenbury, Moore & Nunn, 1963), is not absorbed. Direct serological agglutination results are therefore misleading as a quantitative measure of immune globulin absorption, when the location of the agglutinins within the immune globulin complex fed is not known, or when serological tests designed to reveal incomplete antibody are not used (see Halliday, 1957; Morris, 1957; Brambell, Halliday & Morris, 1958; Hemmings & Morris, 1959). Because of these considerations a further study of the process of protein absorption was undertaken in the new-born pig using a quantitative immunodiffusion assay to estimate the amounts of absorbed immune globulins. This assay was an improvement over serological agglutination tests, but it proved impossible to distinguish between the intact globulins and their degradation fragments. This drawback was overcome in subsequent work when the whole process of transfer was studied *in vitro* (Pierce & Smith, 1967). Under these circumstances no digestion of transferred protein could be detected.

METHODS

Pigs. Large White or Landrace new-born pigs were removed from the sow immediately after birth before they could suck the sow. That part of the litter required for the experiments, six to eight animals, was kept separate from the sow under infra-red lamps until required. The new-born pigs were used as quickly as possible, usually within 2 hr of birth and always within 8 hr. This difference in time between birth and the start of experiments did not noticeably change the amount of protein absorbed subsequently.

Experimental procedure. New-born pigs were first weighed and a control 2 ml. sample of blood removed from the superior vena cava using a fine needle and 5 ml. syringe. Protein solutions were given by stomach tube (2·1 ml./100 g body weight) and the pig then allowed to move about freely in a heated box. At fixed times after administration of the protein further 2 ml. samples of venous blood were removed from the superior vena cava. Up to eight samples of blood were taken in this way over a 5 hr period and the pigs then killed by intravenous injection of Nembutal.

Proteins. Colostrum was collected from pigs or cattle at parturition and about 50 % of the total protein was present as immune lactoglobulin (colostral IgG). This colostrum was centrifuged at 755 *g* for 15 min to remove most of the free fat and was then stored at −10° C. The analytical ultracentrifugal pattern for pig colostrum showed two poorly defined components contributing approximately 15% protein and sedimenting at s_{20w} = 9·4 and 11·0 (Fig. 1*d*; where s_{20w} is the sedimentation coefficient). The main component, colostral IgG, sedimented at s_{20w} = 6·4. At present the nature of the faster globulins is not known and they were not included with the IgG component at analysis.

Pig serum IgG was precipitated at 10 g Na_2SO_4/100 ml. adult pig serum. The precipitate was washed 3 times in 10% (w/v) Na_2SO_4, dissolved and dialysed against phosphate–saline buffer. Ultracentrifugal examination of this protein at a concentration of 0·75 % showed essentially one peak sedimenting at s_{20w} = 6·3 and only one component when examined electrophoretically and immunoelectrophoretically with rabbit anti-whole pig serum (Fig. 1*a*).

Bovine serum IgG examined at 0·75% protein concentration was from Armour Pharmaceutical Co. Ltd. Five per cent of aggregated IgG was detected on ultracentrifugal analysis and this was included as IgG for quantitative estimations. The main peak sedimented at s_{20w} = 6·6. Electrophoresis showed a single component but a weak β protein contaminant was shown by immunoelectrophoresis (Fig. 1*b*).

Human serum albumin (HSA) was kindly supplied by the Blood Products Unit, Lister Institute, Elstree, Herts., as a time-expired preparation and therefore unfit for human use. The HSA had been fractionated by the method of Kekwick & Mackay (1954) from pools of human plasma. Analytical ultracentrifugation, electrophoresis and immunoelectrophoresis of the HSA fraction revealed the presence of minor α and β globulin contaminants (Fig. 1*c*).

Rabbit antisera. Rabbits were immunized by repeated intramuscular injections of bovine serum and pig immune globulin in Freund's complete adjuvant. Specific anti-bovine serum IgG (anti-bovine IgG) was obtained by repeated absorption of rabbit anti-bovine serum with freeze-dried precolostral calf serum which had been examined previously by immunoelectrophoresis and shown to be agammaglobulinaemic. Finally, the anti-serum was absorbed with adult pig serum to remove any cross reaction with pig IgG. Specific anti-pig serum IgG (anti-pig IgG) was obtained by the absorption of rabbit anti-pig IgG serum with precolostral pig serum selected for very low levels of IgG by previous immunoelectrophoretic analysis. The anti-serum was then absorbed with adult bovine serum to remove any cross reaction with bovine IgG. The rabbit anti-human serum used to detect HSA was made specific by absorption with bovine and pig sera. Reactions with other human serum proteins did not complicate reading the quantitative immunodiffusion test (see below), since there were only insignificant amounts of human serum proteins other than HSA present.

Gel filtration. Proteins were dialysed against a buffer containing 0·05 M-Tris and 0·5 M-NaCl adjusted to pH 8·0 with HCl. NaN_3 was added to a concentration of 0·05 % (w/v). The Sephadex G 200 column measured 40 × 2·5 cm and had a void volume of approximately 70 ml. Fractions of 3 ml. were collected every 10 min and the optical density monitored at a wave-length of 280 mμ in a Unicam SP 500 Spectrophotometer. The position of undegraded immune globulin was estimated by running an Armour preparation of serum immune globulin down the same column under the same conditions. The various fragments of IgG were characterized by their behaviour by immunodiffusion and by immunoelectrophoresis. The column of Sephadex G 100 measured 20 × 2·1 cm and was prepared and run in the same way as the column of G 200 Sephadex.

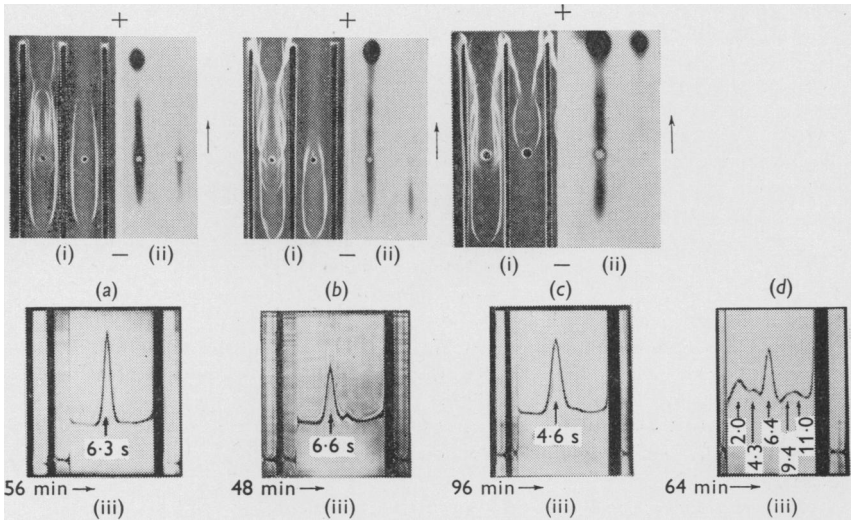

Fig. 1. (i) Immunoelectrophoretic and (ii) electrophoretic patterns for (*a*) pig serum and pig serum IgG, (*b*) bovine serum and bovine serum IgG, and (*c*) human serum and human serum albumin. All immunoelectrophoretic patterns were developed with antisera prepared against the whole serum. Ultracentrifuge patterns *a* (iii) and *b* (iii) are pig and bovine serum IgG respectively; *c* (iii) human serum albumin and *d* (iii) pig colostrum. All sedimentation coefficients are given at 20° C in water (s_{20w}) and time of sedimentation is shown in minutes.

Agar-gel precipitin tests. The double-diffusion immune precipitin method of Ouchterlony (1958) was used to identify and to establish immunological relations between the serum and colostrum IgG proteins and degradation products. Ion agar was used at 1 % (w/v) in phosphate buffer, pH 7·5, containing 0·05 M-NaCl and 0·1 % (w/v) NaN_3. The sizes and positions of the wells were varied according to the characteristics of the reactants and the amount of material available.

Immunoelectrophoresis. A modification of the technique of Grabar & Burtin (1960) was employed using a gel containing 1·5 % (w/v) agar, 0·1 % (w/v) NaN_3 and veronal buffer pH 8·6, $I = 0·025$. The same buffer at $I = 0·05$ was used for the electrode vessels. A field strength of 7 V/cm for approximately 2 hr was applied across the agar.

In some instances the identity of certain precipitin arcs was confirmed by the diffusion of an identified protein from a second trough cut parallel to the antiserum trough, but on the opposite side of the electrophoretically separated protein antigens. A line of precipitate

parallel to and between the two troughs fuses partially or completely with the electrophoretically separated protein sharing some or all of the antigenic determinants (Osserman, 1960).

Ultracentrifugation. Routine analyses were made using the Spinco model E analytical ultracentrifuge. The material was sedimented at 59,780 rev/min in phosphate saline buffer, pH 7·8 $I = 0{\cdot}1$. The temperature was controlled and the sedimentation coefficients (s_{20w}) corrected by the normal procedure to the viscosity and density of water at 20° C. The Schlieren patterns were analysed geometrically (Longsworth, 1942). The areas of the different components were then measured planimetrically and the percentage values corrected for radial dilution.

Movable partition cell ultracentrifugation using the normal ultracentrifuge buffer was carried out using the Yphantis–Waugh cell (Y–W cell) (Yphantis & Waugh, 1956). This is a separation cell in which, at any point during sedimentation, the contents of the cell can be isolated into centrifugal and centripetal compartments from which they can be subsequently recovered. Very effective isolation of components, sedimented beyond the separation position of the plate (i.e. into the centrifugal compartment), can be achieved. An average supernatant concentration contaminating the centripetal compartment after sedimentation of the protein into the centrifugal compartment is about 1 % of the original concentration (Yphantis, 1963). In the present experiments, bovine serum IgG at 516 mg/ml. was sedimented to just below the separation position. Measurements of the centripetal compartment contents using the Zeiss interferometer showed 3·6 % of the original protein as a contaminant. This figure must be taken as a maximum since the IgG preparation (Armour) was not homogeneous and proteins at a concentration below the resolving power of the Schlieren optics could be sedimenting more slowly and therefore be present in small amounts, in the centripetal compartment when the run was terminated.

Protein estimations. Protein concentrations were calculated from semimicro-Kjeldahl determinations on duplicate samples of bovine colostrum, pig serum IgG fractions and HSA, assuming a protein/nitrogen ratio of 6·25. All preparations were previously dialysed against phosphate saline buffer (KH_2PO_4, 1·4 mM; Na_2HPO_4, 16·2 mM; NaCl, 50 mM), $I = 0{\cdot}1$, at pH 7·8, to remove low molecular weight substances which might have contained nitrogen and because of this no allowance was made for non-protein nitrogen.

Quantitative immunodiffusion test (QID test). Estimations of IgG in serum and other fluids were made using the method of Gell (1957). The precipitin patterns in agar gel (prepared as for immunodiffusion) developed between known concentrations of protein and a series of different dilutions of specific antisera were compared about 18 hr later with two or three different dilutions of the protein to be estimated. The usual antiserum dilutions were 1/5, 3/20, 1/10, 3/40, 1/20 and 3/80. The standard antigen dilutions were prepared from concentrations of protein starting at about 40 mg/100 ml., serially diluted to a final concentration of 0·28 mg/100 ml. The antisera gave readily detectable precipitates down to concentrations of 1·1 mg/100 ml. and usually down to 0·56 mg/100 ml. The differences between patterns could be read to within half a dilution. Standard bovine IgG solutions were prepared from Armour IgG and were based on a nitrogen determination and ultracentrifugal analysis for this component which sediments at $s_{20w} \approx 7$ (7*S* component). Some faster sedimenting protein in the Armour IgG (see Fig. 1*b*), thought to be dimerized IgG, has been included with the IgG at analysis. Concentrations of bovine and pig colostral IgG were derived from the percentage analysis of the 7*S* component of the patterns, derived from ultracentrifugal analysis and a nitrogen determination. All values were checked by the QID test and the two estimates agreed within the error of the latter method so that ultracentrifugal analysis was taken to give the actual concentration for determinations of colostral IgG and HSA for feeding experiments.

Although the method of quantitative immunodiffusion gave satisfactory estimates of intact IgG, it was not able to distinguish between the intact molecule and degradation pro-

ducts of IgG which had retained their ability to contribute to the immune precipitate. The presence of these fragments was not always evident since undegraded IgG unifies the precipitin line. In this dilemma we have been reluctantly forced to represent amounts of immune globulin in pig sera in terms of *IgG equivalents*.

Colostrum fed. Colostrum was given by stomach tube, 2·1 ml./100 g body weight. In some experiments colostrum was fed without dialysis, in others after dialysis against bicarbonate saline (Krebs & Henseleit, 1932). When colostrum required diluting in order to adjust the IgG concentration without alteration in the ionic composition, distilled water was used which had been dialysed previously against large volumes of fresh colostrum.

RESULTS

The absorption of homologous and heterologous IgG and HSA

Dialysed and native pig colostrum and dialysed bovine colostrum, each containing 6·4 g IgG/100 ml. were given to new-born pigs by stomach tube. Figure 2*a* shows that similar concentrations for each protein were reached in the pig serum 4½ hr later, each point being the mean of estimates on

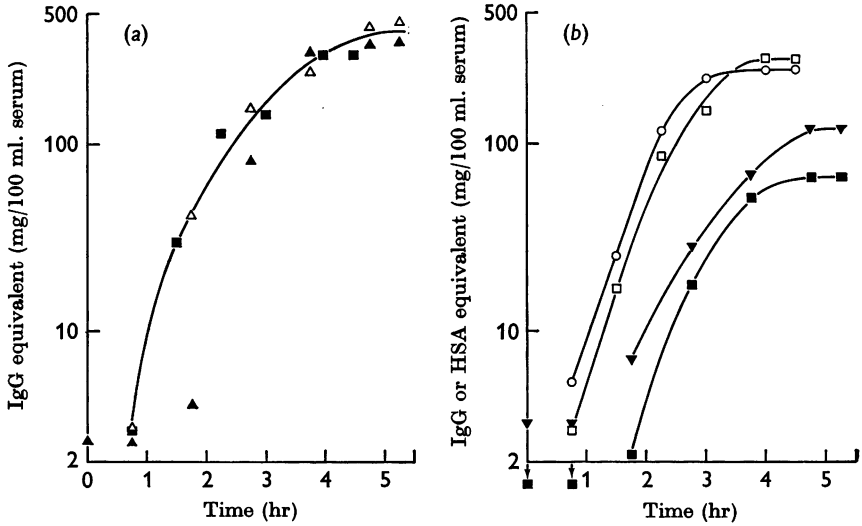

Fig. 2 (*a*). Bovine –■– and pig –▲– IgG equivalents in the sera of new-born pigs taken after oral administration of bovine or pig colostrum previously dialysed against bicarbonate saline. Each colostrum contained 6·4 g IgG/100 ml.; volume given was 2·1 ml./100 g body weight. Pig colostral IgG equivalent –△– measured after feeding undialysed pig colostrum to new-born pigs in the same dose and at the same concentration.

(*b*). Bovine –■– and pig –▲– IgG equivalents in the sera of new-born pigs after administration of dialysed bovine colostrum containing 6·4 g pig serum IgG and 6·4 g bovine colostral IgG/100 ml. Bovine colostral IgG –□– and human serum albumin –○– equivalents in sera of new-born pigs after giving dialysed bovine colostrum containing 6·4 g bovine colostral IgG and 6·3 g HSA/100 ml. Each point is the mean of estimates on three different pigs.

three different pigs. However, there were large differences between individual pigs, 258–517 mg pig IgG and 163–400 mg bovine IgG equivalent/100 ml. pig serum. These differences were even greater in subsequent experiments (see Fig. 9) and possible reasons for this variation are discussed later. In all the following feeding experiments protein solutions were dialysed against bicarbonate saline before administration.

Freeze-dried HSA was added to bovine colostrum and the protein concentrations adjusted after dialysis against bicarbonate saline to give 6·3 % (w/v) HSA and 6·4 % (w/v) bovine colostral IgG. When this solution was given to new-born pigs (2·1 ml./100 g body weight) the mean serum concentration attained by each protein was the same 4–5 hr later (Fig. 2*b*).

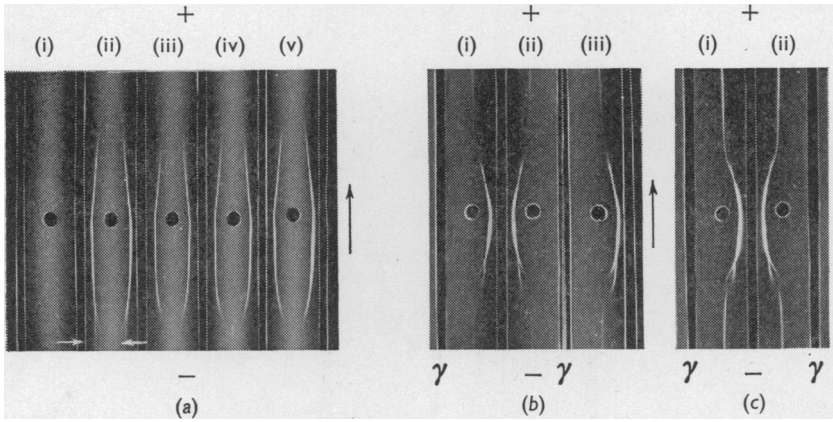

Fig. 3. Immunoelectrophoretic patterns developed with anti-bovine IgG of: (*a*) (i) adult pig serum showing the absence of any cross-reaction with pig serum proteins; (*a*) (ii) new-born pig serum 3 hr after feeding bovine colostrum. Note the appearance of an addition arc (→ ←) partially cross-reacting with the main IgG arc. (*a*) (iii–v) 1/50, 1/25 and 1/10 dilutions respectively of the bovine colostrum fed. (*b*) and (*c*) Early and late immunoelectrophoretic patterns respectively of new-born pig sera (i) 3½ hr and (ii) 4 hr after feeding bovine colostrum. The patterns show a cross-section between undegraded bovine serum IgG (γ) and both the immune precipitate arcs (iii) shows a similar immunoelectrophoretic pattern developed by gut lumen contents taken from a new-born pig 1½ hr after feeding.

In a further experiment pig serum IgG was added to dialysed bovine colostrum so that both pig serum and bovine colostral IgG were present, each at a concentration of 6·4 g/100 ml. This solution was given to new-born pigs at the same rate as in the previous two experiments. The homologous IgG reached a higher serum concentration than the heterologous (Fig. 2*b*), the amount of pig IgG absorbed being nearly twice that of bovine (64 and 36 % of the total absorbed protein). This result was consistent between all pigs in the group and because blood concentrations of both globulins were

measured in the same pig, the difference in the final concentrations attained is considered to be significant.

Immunoelectrophoresis of piglet serum after feeding bovine colostral IgG showed a precipitin arc not previously seen in the precipitin pattern for colostral IgG tested before feeding (Fig. 3). This arc was cathodic to the main IgG arc and partially cross-reacted with it when specific antibovine IgG was used to develop the pattern. A similar double arc was shown when

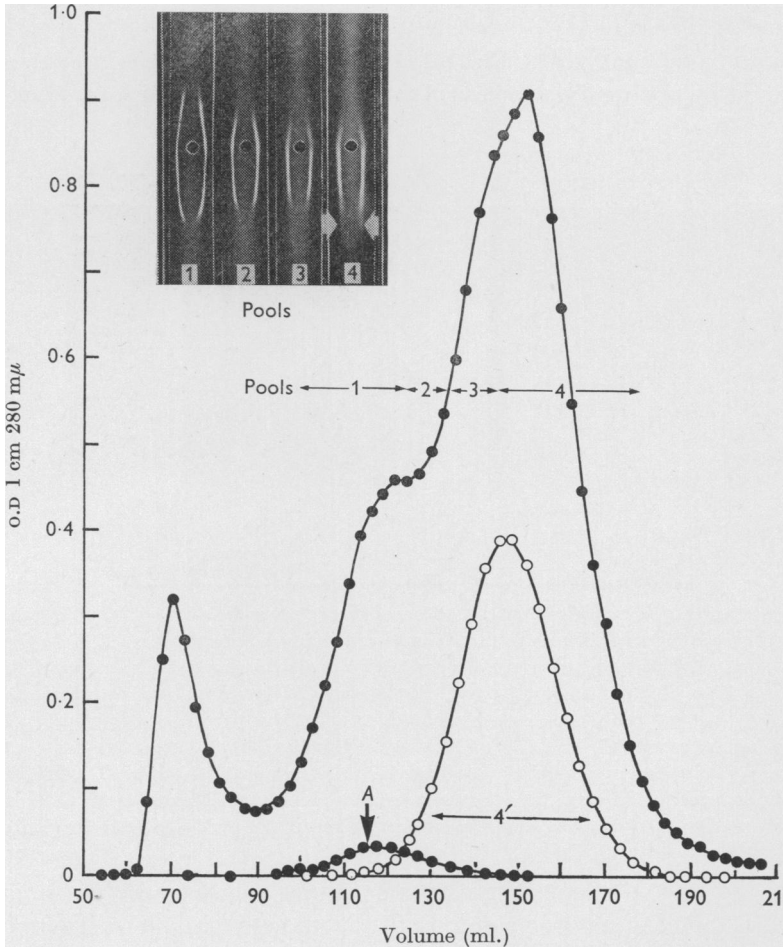

Fig. 4. The elution pattern for the Sephadex G 200 fractionation of new-born pig serum after feeding dialysed bovine colostrum –●–, together with pooling data. Pool 4 was refractionated –○– and pooled (4′). The elution position for undegraded serum IgG is shown at *A* –●–. The electrophoretic patterns for pools 1–4 developed with anti-bovine IgG are shown. White arrows show the extra cathodic arc fractionated in pool 4. All patterns were developed with anti-bovine IgG serum.

the contents of the first third of the small intestine were flushed out with saline 1½ hr after feeding and then tested by immunoelectrophoresis. Although no splitting of the immune globulin precipitin line was observed in the QID test, the immunoelectrophoretic pattern could be interpreted most readily as being evidence for the presence of degradation product(s) of bovine colostral IgG. Results are therefore plotted in terms of IgG equivalents, the meaning of which has been defined earlier.

The examination of post-feeding pig serum for digest fragments of IgG

Sephadex fractionation. Sera were pooled from pigs, 4–5 hr after feeding bovine colostral IgG and this was subsequently fractionated by gel

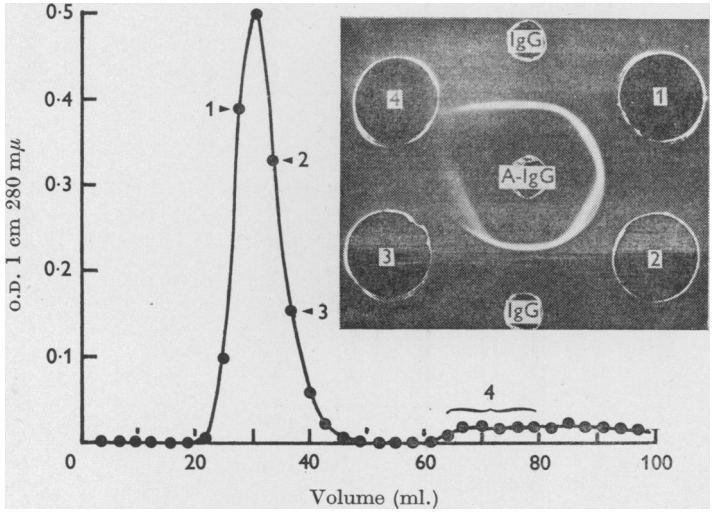

Fig. 5. Sephadex G 100 fractionation of pool 4′ (see Fig. 4) and double immunodiffusion patterns developed with anti-bovine IgG (A-IgG) against tubes 1–3 (× 10 concentrated), pool 4 (× 100 concentrated) and bovine colostrum containing 0·01 % IgG.

filtration using Sephadex G200. The elution pattern and the origin of fractions (*F*) 1–4 is shown in Fig. 4. The initial peak of U.V. absorbing material coincided with the exclusion volume of the column and the material therefore has a molecular weight in excess of 200,000. Aggregated globulin or macroglobulin would be eluted in this position but the identity of this peak was not checked. The position in which IgG was eluted is shown at *A* in Fig. 4. *F* 1–4 were concentrated about 10 times and then examined immunoelectrophoretically (Fig. 6). *F* 4 shows evidence of the double precipitin arc previously associated with digest fragments. *F* 4 was again fractionated down a Sephadex G 200 column and the pool 4′ indi-

cated in Fig. 4 further fractionated down a Sephadex G 100 column (Fig. 5). Individual tube samples 1–3 and pool 4 were concentrated and examined by gel diffusion. The results, particularly those shown by fraction 3 in Fig. 5, confirm the presence of IgG fragments all cross-reacting with undegraded IgG. The position of IgG immunologically reactive protein in the elution pattern following the molecular sieving down Sephadex G 200 and

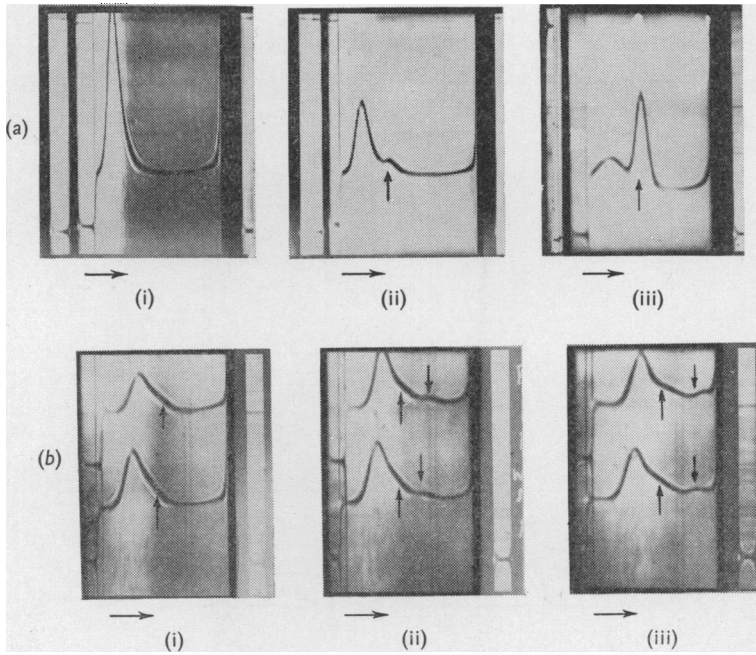

Fig. 6. Ultracentrifuge patterns (*a*) after 48 min at 59,780 rev/min for (i) pre-feeding new-born pig serum examined at 3·5 % protein concentration and showing no $\approx$ 7*S* component; (ii) serum from the same pig examined at 1 % protein concentration after feeding bovine colostrum showing a well defined $\approx$ 7*S* component (↑) (actual sedimentation coeff. $s_{20w} = 6{\cdot}6$); (iii) bovine colostrum showing the main $\approx$ 7*S* component (↑) (actual sedimentation coeff. $s_{20w} = 6{\cdot}3$) and slower sedimenting protein.

(*b*) (i,ii) after 80 min and (iii) 104 min at 59,780 rev/min for (i) pre-feeding and (ii) post-feeding (bovine colostrum) pools of pig sera showing the appearance of $\approx$ 7*S* component (↓) in post-feeding pools and the presence of a shoulder (↑) on the leading edge of the main slow peak for all patterns, (iii) included to emphasize this feature.

G 100, together with their gel diffusion and immunoelectrophoretic patterns, confirms the earlier immunoelectrophoretic results showing the presence of both intact IgG and digest fragments in the post-feeding serum of the new-born piglet. These results do not show the extent to

which IgG frgaments contribute to the QID estimations of IgG in post-feeding pig serum and further experiments were carried out in an attempt to assess the amount degraded.

Ultracentrifugal fractionation. Myers & Segre (1963) have shown in the pig that maternal antibody can be transferred from mother to offspring *in utero* and this was confirmed by the QID test results on pre-feeding pig serum. However, the amount of protein sedimenting as immune globulin in pre-suckling pig serum was extremely small and could not be analysed even using 3·5% (w/v) total serum protein concentrations (Fig. 6*a*(i)). In post-feeding sera a well developed IgG component was detected by ultracentrifugal analysis at total protein concentration of 1% (w/v) (Fig. 6*a*(ii))

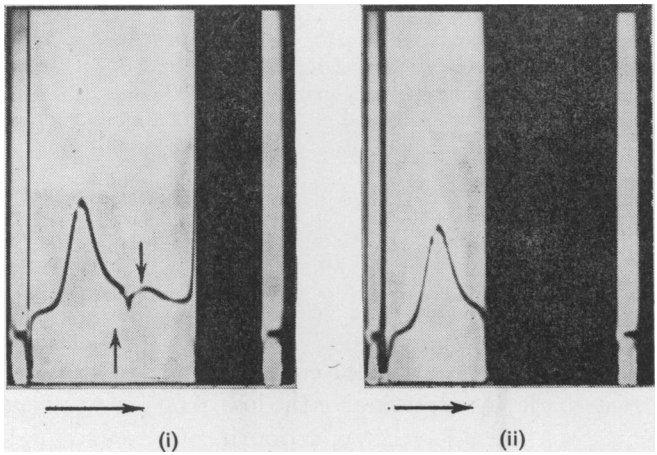

Fig. 7. Ultracentrifuge patterns for new-born pig serum, after feeding dialysed bovine colostrum, shown during deceleration at the end of a Y-W cell run. (i) at 10,000 rev/min, the ≈ 7*S* IgG component (↓) has sedimented into the centrifugal compartment. The small blip (↑) is obtained by placing a small opaque spot on the cell window at the point where the moving platform comes to rest. This is shown by the pattern at (ii) obtained after further deceleration to 2000 rev/min when the plate had returned to the starting position.

and this may be compared with the IgG component in the pig colostrum (Fig. 6*a*(iii)). This observation was confirmed by further analysis of post-feeding piglet serum (Fig. 6*b*(ii), (iii)). A feature of the ultracentrifugal pattern was the shoulder on the leading edge of the main slow component (see Fig. 6*b*(i)–(iii)). These two components had sedimentation coefficients s_{20w} = 3·7 and 4·3 respectively. Neither should be confused with the presence of IgG fragments as they were a consistent feature in all sera from new-born pigs and were present before and after suckling.

The absence of a demonstrable IgG component in pre-suckling pig

serum showed that ultracentrifugal analysis of sera from the same pigs after suckling could be used to measure the amount of undegraded IgG (i.e. the 7*S* fraction) which had been absorbed.

Four post-suckling serum pools were sedimented at 59,680 rev/min in the Y–W cell until the undegraded IgG just sedimented into the centrifugal compartment. The run was then terminated and the positions of the boundaries photographed at about 10,000 rev/min before partition, and again at 2000 rev/min after partition (Fig. 7). QID tests were carried out on the contents of the centripetal compartment.

Table 1 shows that much of the discrepancy between the ultracentrifuge determination, in terms of 7*S* globulin, and the QID estimation, could be accounted for by fragments sedimenting more slowly and therefore

TABLE 1. QID estimations of bovine colostral IgG and fragments in four pools of pig serum taken 4–5 hr after feeding bovine colostrum. Values are mg IgG or IgG equivalent/100 ml. serum. 1: IgG estimated by ultracentrifuge analysis. 2: IgG fragment(s) estimated by QID test of fractions separated by the Y-W cell. 3: IgG and IgG fragment(s) estimated by the QID test

Serum pool	Total protein (g/100 ml.)	1	2	1+2	3
1	2·18	165	67	232	277
2	2·36	170	33	203	213
3	2·29	252	94	346	415
4	2·20	200	148	348	415

partially retained in the centripetal compartment. In these analyses the assumption was made, in correcting for the loss of fragments from the centripetal compartment through sedimentation into the centrifugal compartment, that the position of the main slow peak was coincidental with that of the fragments. Thus, the discrepancy between column 1, the ultracentrifugal analysis of the 7*S* component in the serum pools, and column 3, the QID estimation of total IgG equivalent, is corrected within the limits of experimental error, by the inclusion of the QID estimate for the IgG fragments (column 2). The results (Table 1) show that up to 50% of the protein detected by the QID test could be due to fragments and confirm that the QID test can only be interpreted quantitatively as a measure of IgG equivalent.

Digest fragments in the urine of the new-born pig

Since digest fragments of bovine colostral IgG were shown in the general circulation, the urine was examined for fragments sufficiently small to be excreted by the kidney. Using anti-bovine IgG, a precipitin line was detected which showed a reaction of partial identity with undegraded IgG. The urine showing this reaction had been concentrated twenty times and

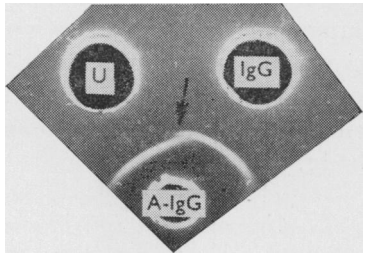

Fig. 8. Double immunodiffusion pattern developed with anti-bovine IgG (A–IgG) against urine (*U*) (× 20 concentrated) collected from a new-born pig 5 hr after being given dialysed bovine colostrum. The pattern shows a reaction of partial immunological identity between urine and serum IgG protein. The full development of the spur (↓) is thought to be inhibited by low molecular weight non-precipitable IgG fragments present in the urine.

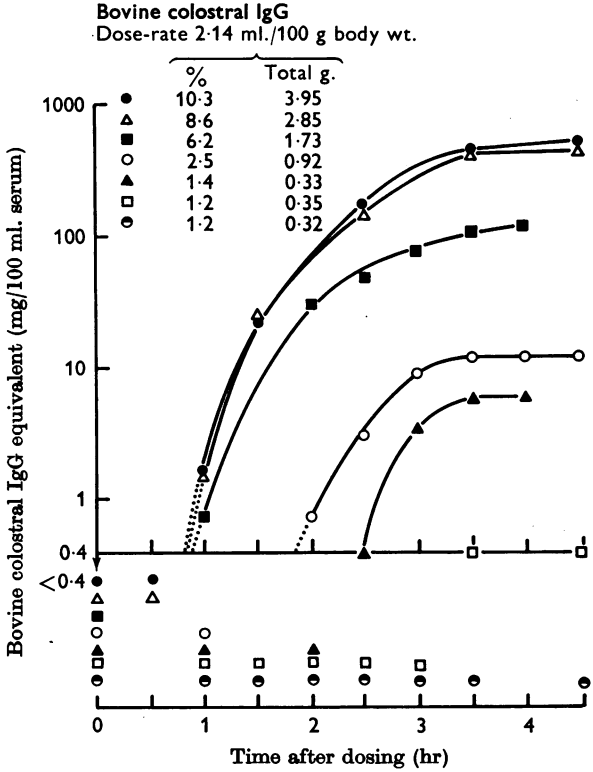

Fig. 9. QID results showing the increase with time in bovine colostral IgG equivalent in the serum of new-born pigs after feeding dialysed bovine colostrum containing different concentrations of colostral IgG. Volume administered, 2·1 ml. /100 g body weight.

had been collected from a pig 5 hr after feeding bovine colostral IgG (Fig. 8). The short stumpy spur shown in Fig. 7 was obtained repeatedly and, since the urine samples were not fractionated, it is suggested that the further development of this spur was inhibited by fragments of IgG having antigenic determinants still capable of combining with, but not precipitating, the antibody.

The absorption of bovine colostral IgG by the new-born pig

Dialysed bovine colostrum was given by stomach tube to new-born pigs, 2·1 ml./100 g body weight, as a series of different IgG concentrations,

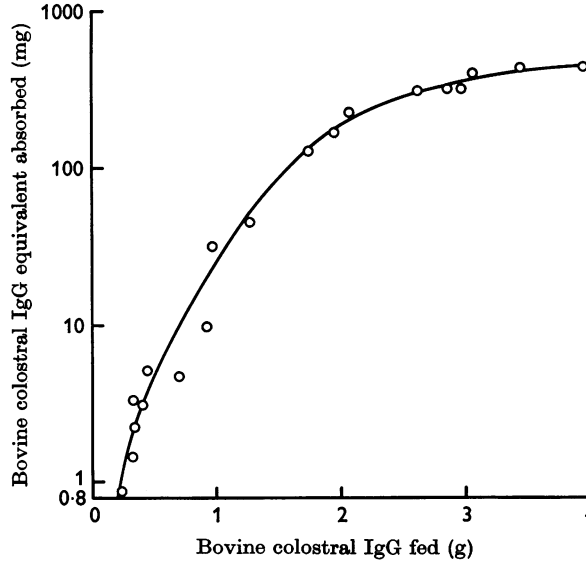

Fig. 10. QID results showing the relation between the total amount of bovine colostral IgG fed to new-born pigs (2·1 ml./100 g body weight) and the total amount absorbed as bovine colostral IgG equivalent. Calculations assumed a plasma volume of 5 % body weight.

1·2–10·3 g/100 ml. The weight of colostral IgG given varied from 0·32 to 3·95 g. Figure 9 shows the rise in IgG equivalent in pig serum taken at intervals for up to 4½ hr after feeding. The results show first that, regardless of the amount fed, the concentration of IgG in serum did not rise above the lower limit for detection (0·4 mg/100 ml. serum) during the first 30 min after feeding, and was detected 1 h after feeding only if relatively large amounts had been fed. The appearance of IgG at detectable levels took 3½ hr when 0·35 g was given and never occurred after feeding 0·32 g. When larger amounts of colostral IgG were fed, there was a rapid increase in the IgG equivalent of the serum during the second hour and fairly constant

levels of immune globulin were seen 3½–4½ hr later. The final serum concentration depended on the amount fed but the two were not directly proportional. The relation between the amount of IgG fed and the total amount absorbed is shown in Fig. 10. From this graph it can be seen that if 500 mg colostral IgG were fed, then about 5 mg (1 %) would be absorbed. Increasing the amount fed to 1000 mg increased the amount absorbed to 50 mg (5 %), while 2000 mg fed increased the absorption to 10 %. As the amount fed was further increased the percentage absorbed remained fairly constant so that of 4000 mg fed, 11 % was absorbed. Thus the efficiency of absorption was poor when the amount of IgG fed was less than 2 g and approached a maximum with the highest dose fed, about 4 g of bovine colostral IgG.

DISCUSSION

The QID test, used to estimate concentrations of specific proteins in pig serum, could not distinguish between intact globulin and immunologically precipitable degradation products. Although degraded IgG was shown to be present in significant amounts compared with undegraded globulin, the latter almost always unified the precipitin line so that comparisons were easily made with patterns produced by the standard IgG dilutions. Very occasionally, two lines of precipitate appeared which could not be read against the control. This resulted from fragments which were not unified by undegraded IgG. The presence of fragments was confirmed by both immunoelectrophoretic and double immunodiffusion analyses in agar, when they cross-reacted with the undegraded IgG. Ultracentrifuge analyses for the 7*S* component in post-feeding pig serum (i.e. for undegraded IgG) showed values up to 50 % lower than those found from the QID estimations. The isolation of fragments in the Y-W cell, followed by QID estimations for IgG equivalent, accounted for 58–77 % of this discrepancy. Absolute values for the isolated fragments remain uncertain because their sedimentation velocity was unknown and an arbitrary correction was made, assuming a sedimentation position similar to the main peak isolated in the centripetal compartment. Obviously the presence of degradation fragments is a severe drawback to the quantatitive use of this test and this might partly explain the apparent variation in the IgG serum levels obtained after feeding similar concentrations of bovine colostrum. This difficulty was overcome when the whole process of protein transfer was studied *in vitro* (Pierce & Smith, 1967).

Very low levels, between 1 and 4 mg of pig IgG/100 ml. serum were detected in pigs before suckling; this protein is aquired passively *in utero* (Myers & Segre, 1963). Similar low concentrations of pig IgG have been reported by other workers (Rutqvist, 1958; Lecce & Matrone, 1960). The

concentrations were too low to interfere with IgG estimations in pig serum taken after feeding colostral or serum IgG. Concentrations of IgG of 1·3 and 2·1 mg/ml. serum, reported in the serum of unsuckled pigs by Olsson (1959) and Payne & Marsh (1962), would have been easily detected by ultracentrifugal analysis in the present experiments. The fact that there was no peak corresponding to $s_{20w} = 7$ confirmed the QID test results and those of other workers, that only small amounts of IgG were initially present.

No bovine immune proteins were detected in pig serum during the first half hour after feeding (i.e. less than 0·6 mg/100 ml.) but by 45 min the ingested immune globulin was usually detected, the time to detection then being proportional to the concentration and/or amount fed when this was less than 1·7 g IgG. Final plateau concentrations were reached in the serum at about the same time (4 hr after feeding). HSA was absorbed when fed together with bovine colostral IgG, both reaching high concentrations 4–5 hr later (total protein concentration of ingested substances—520 mg/100 ml. serum). In contrast to these results, when bovine colostral and pig serum IgG were given together, the final total concentration of IgG equivalent was only 187 mg/100 ml. serum. The total protein transferring ability for immune globulin might have been saturated by the large amounts presented to it while the ability to transport albumin remained unsaturated.

The pig intestine transferred more pig than bovine immune globulin when presented with equally high concentrations of both in a preparation of dialysed colostrum. Competition during absorption may be the rate-limiting factor, homologous protein being preferred to the heterologous protein. If this were so, the heterologous protein would be expected to interfere with the absorption of the homologous protein so that neither would reach plasma levels normal for the individual protein. This hypothesis, presented by Morris (1957) and Brambell *et al.* (1958) for transfer of protein across rat and mouse intestine, would be more tenable for the pig, if a selective preference had been shown for the homologous protein when homologous and heterologous IgG were fed separately. Such a selectivity has been shown (Payne quoted by Wilson, 1962) but may have been masked in the present experiments by the large individual variation between pigs.

Conclusions cannot easily be drawn from results based on protein levels in the serum of the intact animal. The degree of digestion was shown to be extensive in the present work and about 90 % of the protein fed was never accounted for. Selective digestion and water absorption might also be expected to change the relative and absolute concentrations of different proteins at the absorptive surface of mucosal cells. After absorption, the rate of extravascular loss of protein during tissue equilibration is difficult

to measure but could vary with proteins of different molecular weights, for example, when final concentrations of HSA and IgG were compared. Finally, the immediate *post partum* period is one where the plasma volume is increasing rapidly (Ramirez, Miller, Ullrey & Hoefer, 1963). Any method which cannot distinguish between degradation products and intact protein will estimate fragments. These will be cleared into the urine at an unknown rate and will be equilibrating at different rates from the intact molecule.

Olsson (1959) found that the amount of protein absorbed was not proportional to the amount fed and this was shown in the present work when less than 2 g IgG was given to the pig. Only 1 % was absorbed when 0·5 g IgG was fed, but the proportion increased to 10 % when 2 g was given. Payne & Marsh (1962) reported, on the contrary, that the amount of protein absorbed was proportional to the amount fed. This was only shown in the present experiments between 2–4 g fed of which 10 % was absorbed. If protein digestion was inefficient in the new-born ungulate, and there is evidence that pepsin digestion is reduced (Hill, 1956), this might explain why relatively more is absorbed as the amount fed is increased. The delay in appearance of ingested protein in the serum when low concentrations were fed suggests some increase in protein concentration within the intestine is necessary to initiate pinocytosis. During this delay proteolysis would continue.

We are grateful to Mr N. Buttress for ultracentrifugal analyses and to Mr M. Rennie and Mr K. Burton for their technical assistance throughout this work.

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