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THE ABSORPTION OF POLYVINYL PYRROLIDONE BY THE NEW-BORN PIG INTESTINE

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SUMMARY

1. The intestinal absorption of [^{131}I]polyvinyl pyrrolidone of mean mol. wt. 160,000 (K. 60) and 40,000 (K. 30) after oral administration has been measured in unsuckled conscious pigs less than 20 hr old. Absorption was assessed by the measurement of the concentration of [^{131}I]PVP in venous blood during the 6 hr after feeding and also by the distribution at the end of the experiment of [^{131}I]PVP between homogenates of the alimentary tract and homogenates of the rest of the animal.

2. The concentration of [^{131}I]PVP in the peripheral blood after feeding was dependent upon the mol. wt. of the polymer, when comparable amounts had been absorbed from the intestine. PVP K. 60 attained higher blood concentrations than PVP K. 30 and the blood concentrations of PVP K. 60 were close to the values to be expected if all the material which had left the intestine had remained in the blood. The lower blood concentrations found when PVP K. 30 was fed were associated with the disappearance of labelled solute from the gut and were thus the consequence of the relatively rapid escape of labelled solute from the plasma after absorption had taken place.

3. The ability of the intestine to absorb [^{131}I]PVP K. 60 declined progressively after birth but did not terminate abruptly unless the animal was fed colostrum. In unsuckled animals the rate and extent of absorption at 3 hr was much greater than at 20 hr after birth, but some absorption was still present at least 65 hr after birth.

4. The transfer of PVP K. 60 to the peripheral blood was dependent upon factors in sow colostrum, since significant absorption did not occur when PVP was fed in water or simple salt solutions.

5. The factors which accelerated absorption were present in colostrum from the goat, cow and ewe as well as that from the sow; they remained in the whey, but, in contrast to the factors which accelerate absorption in the calf, were largely inactivated by boiling. Similarly, neither phosphate, lactate, pyruvate, nor lower volatile fatty acid salts, which were effective in the calf, accelerated absorption in the pig.

6. The absorption of [^{131}I]PVP K. 30 was found to be much less dependent upon the composition of the solvent than the absorption of [^{131}I]PVP K. 60, although absorption was most rapid when PVP K. 30 was fed in colostrum.

INTRODUCTION

It is now accepted that the transfer of maternal immunity to the young pig results from the intestinal absorption of immune globulins from the colostrum and that during normal suckling such absorption is possible for only 24–36 hr after birth.

Information is now available concerning the changes in the serum proteins after suckling colostrum, the relative absorption of particular proteins in colostrum and the period during which absorption of intact protein can occur. Little is known, however, of the rate of absorption of macromolecular substances from the young pig intestine during the hours immediately after feeding, since, with the exception of the observations of Pierce & Smith (1967), no reports are to be found of the serum protein concentrations or antibody titres earlier than 12 hr after feeding, and the majority of investigators, concerned primarily with the changes in serum protein composition over a period of weeks or months, were content to take the first sample 24 hr after suckling.

In view of the lack of information concerning the rate of absorption of macromolecular substances in the pig, it was decided to attempt a quantitative investigation of the absorption of radioiodinated solutes by this species during the 6 hr immediately after feeding. Such an investigation had been undertaken by Balfour & Comline (1962) in the calf, when it was demonstrated that the rate of absorption of [^{131}I]γ-globulin was profoundly influenced by the constituents of the solvent in which it was fed. In the calf it is possible to cannulate either the thoracic or the intestinal lymph duct and to study the process of absorption in the anaesthetized animal, since it has been demonstrated that the protein passes almost exclusively into the intestinal lymphatic vessels (Comline, Roberts & Titchen, 1951).

Preliminary attempts to apply this technique to the young pig have met with little success, due to the small size and inaccessibility of both the thoracic and the intestinal lymph ducts and the depression of absorption in anaesthetized animals. It has therefore been necessary to develop alternative methods of measuring the absorption of ^{131}I labelled material in the conscious pig.

Cannulation of the right jugular vein and analysis of blood samples taken from it at hourly intervals provided one means of following the changes in radioiodine concentration in the blood. It was obvious,

however, that these measurements alone would afford little indication of the amount of radioiodinated material which had actually been absorbed from the intestine, since the concentration of [^{131}I] in the blood at any given time after feeding [^{131}I] labelled material would represent the algebraic sum of absorption from the intestine and the loss into extravascular compartments. For this reason it was necessary to obtain an independent estimate of the amount of the test dose which had been absorbed from the intestine. Advantage was taken of the small size of the new-born pig, and a direct measurement of absorption was obtained from the proportion of the radioiodinated solute fed found in homogenates of the alimentary tract at the end of the experiment.

The measurement of blood radioactivity and the distribution of the administered dose of [^{131}I] labelled material 6 hr after feeding have provided two means of demonstrating quantitatively the effects of such factors as the age of the pig and the nature of the solvent on the absorption of substances of high mol. wt.

It became apparent from preliminary results that [^{131}I]serum γ-globulin was an unsatisfactory substance for use in the assessment of absorption by this technique, since a large proportion of the solute fed was broken down within the intestine and subsequently escaped from the plasma after absorption (R. N. Hardy, in preparation). For this reason [^{131}I]polyvinyl pyrrolidone (PVP) of mean mol. wt. 160,000 has been used as the main experimental solute during this investigation. This substance has a mean mol. wt. comparable with that of the IgG and has been shown not to be broken down by enzyme activity within the intestine. Furthermore, it is readily absorbed by the small intestine of both the young pig and the young calf, and in the latter species its absorption has been shown to be influenced by solvent factors in a closely comparable way to the absorption of serum γ-globulin (Hardy, 1969).

The results of the experiments to be reported have demonstrated that the absorption of [^{131}I]PVP is influenced by factors in the solvent, in that negligible quantities of the polymer are absorbed when it is administered in water or in simple solutions of sodium chloride, whereas large quantities pass from the intestine into the peripheral blood when it is administered in colostrum. However, analysis has shown that the factors responsible for the acceleration of absorption in the pig differ in certain respects both from the factors in colostrum and from those exogenous substances shown to potentiate the absorption of serum γ-globulin and PVP from the intestine of the new-born calf (Hardy, 1969). A preliminary account of certain of these results has been published previously (Hardy, 1965).

METHODS

Pigs. The pigs used in these experiments were obtained from local farms. They were removed from the sow immediately after birth, without being allowed to suckle, and were transported in heated boxes to the laboratory where they were kept in a specially constructed incubator maintained at $27 \pm 2°$ C and relative humidity 70–80 % saturated.

Feeding technique. Colostrum was defatted by centrifugation before use. Whenever possible fresh colostrum was used, but prolonged storage at $-20°$ C did not reduce its ability to potentiate the absorption of PVP. Colostrum whey and boiled colostrum whey were prepared as described by Balfour & Comline (1962). [^{131}I]Polyvinyl pyrrolidone (PVP) of mean mol. wt. 40,000 (K. 30) was obtained from the Radiochemical Centre and similar unlabelled carrier material was employed (Polyvidone, May & Baker). [^{131}I]PVP of mean molecular weight 160,000 (K. 60) was supplied by the Radiochemical Centre and the unlabelled material used was polyvinyl pyrrolidone K. 60 (Fluka A. G.). The elution volume of [^{131}I]PVP K. 60 on Sephadex G.100 columns corresponded with the void volume of the column. No changes in elution characteristics were observed in [^{131}I]PVP recovered from the plasma or from the contents of the stomach and small intestine. Test solutions comprised unlabelled PVP in 2 % (w/v) solution in the solvent (e.g. cow colostrum) and 1–2 μc [^{131}I]PVP. Each animal was fed 45 ml. of the test solution by stomach tube in three 15 ml. doses at hourly intervals.

Blood samples. Immediately after the first feed alternate animals were lightly anaesthetized with halothane (Fluothane: I.C.I.). The right jugular vein was exposed in the neck through a small lateral incision at the level of the larynx. A fine polyethylene cannula (Portex, 49A, external diameter 1·27 mm) was passed into the vein until the tip had entered the thorax.

The cannulae were about 20 cm long and were reflected dorsally and strapped between the shoulder blades with surgical tape. The same tape served to close the incision which had previously been lavaged with a long-acting local anaesthetic (Duncaine: Duncan Flockhart & Co. Ltd.). The anaesthesia was transient and the animals were active within minutes of the vein being cannulated. The cannulae were filled with heparin–saline and terminated in a ground down No. II serum needle sealed with an adaptor.

To obtain a blood sample, the animal was placed in a restraining stand. Blood could then easily be obtained from conscious animals without undue disturbance and samples were taken at hourly intervals during the 6 hr after feeding.

Blood samples for scintillation counting were taken in precision bore 1 ml. syringes (Everett). The volumetric error involved fell within the limits of accuracy of the scintillation counter. In the unanaesthetized control animals, a single intracardiac blood sample was taken at the end of the experiment. There were no consistent differences between the absorption in control animals and that in animals in which the jugular vein had been cannulated.

The haematocrit was determined in a number of pigs from samples of heparinized blood taken before feeding and at the end of the experiment. The haematocrits observed ranged between 32 and 38 % but the variation in any individual animal before and after feeding did not exceed 3 %.

Recovery of isotope fed. The bladder and contents were removed and the volume of urine and ^{131}I concentration measured; in most animals the ^{131}I PVP K. 60 recovered from the urine was less than 1 % of the amount fed and values exceeding 3 % were not observed. The alimentary tract between the lower oesophagus and anus was removed and the stomach, small intestine and large intestine were separately

homogenized, each in 200 ml. water for 3 min at a nominal 12,000 rev/min in a commercial blender (Ato-mix: M.S.E.). Each homogenate was poured into a 500 ml. measuring cylinder and the volume adjusted to 400 ml. with the washings from the blending chamber. The diluted homogenate was a uniform suspension of tissue fragments and had the consistency of thick soup. After vigorous mixing, three aliquots were taken from each homogenate, for scintillation counting. Samples were taken with a 5 ml. automatic syringe (Dansk Laboratoriendstyr. Copenhagen) to which a length of flexible nylon tubing (Portex No. 6 External diameter 3·24 mm) had been attached. The syringe was set to deliver 3·00 ml. and samples of the homogenate were drawn into the nylon tubing when the syringe plunger was released. Since the nylon tubing had a capacity of approximately 5 ml., the radioactive solution did not enter and contaminate the barrel of the syringe. After obtaining the sample, the tip of the nylon tubing was wiped before discharging the contents into the counting vial. The same syringe and tube were used to obtain samples of the solution fed necessary for the estimation of recovery. The carcass, after removal of bladder and alimentary tract, was homogenized, after a preliminary passage through a 1/3 h.p. commercial electric mincer (Enterprise Manufacturing Co. Ltd.). Samples of the final homogenate were taken as described above.

Calculation of the rate of absorption as determined by the appearance of [^{131}I]PVP in the blood

The measurement of the radio-iodine content of blood samples taken at hourly intervals during the final 4 hr of the experimental period has enabled an estimate to be made of the rate of absorption into the blood of orally administered radio-iodinated material. Each animal was fed a standard volume of test solution, and the radio-iodine concentration in the blood has been expressed as a percentage of the concentration of radio-iodine in the solution fed (blood percentage radioactivity).

Calculation of the distribution of [^{131}I]PVP at the end of the experiment

The amount of isotope fed to each animal was calculated from the volume of solution fed and its [^{131}I] concentration. Similarly, the total recovery of isotope could be calculated from the sum of the products of the volume and [^{131}I] concentration for each homogenate and for the urine. The total recovery of labelled solute usually exceeded 90 % of that fed; animals in which less than 85 % was recovered were discarded. In all cases the percentage recovery of labelled solute in a particular homogenate should be taken to mean the recovery expressed as a percentage of the total recovery of solute, rather than as a percentage of the amount fed.

Calculation of the theoretical blood percentage radioactivity assuming all the labelled solute absorbed was retained in the circulation

If, for example, a 1200 g pig is fed 1·5 μc [^{131}I] labelled solute and 50 % is absorbed from the gut, then:

Relative number of counts fed. 45 ml. colostrum containing 25,000 counts/min.ml. = 1,125,000.

Plasma volume. 66 ml. (plasma volume = 55 ml./kg (McCance & Widdowson, 1959).

Plasma radioactivity (assuming uniform distribution). (562,500/66) = 8522 counts/min.ml.

Haematocrit for unsuckled pigs = 38 % (McCance & Widdowson, 1959).

$\therefore$ Blood radioactivity = $8{,}522 \times (62/100) = 5283$.

Blood percentage radioactivity = $(5283/25{,}000) \times 100 = 21{\cdot}1$ %.

Measurement of radioactivity. Samples were analysed in a well-type scintillation counter coupled to a Panax Type 100 c scaler.

RESULTS

The assessment of absorption

PVP K. 60

In all the experiments to be reported, the amount of [^{131}I]PVP K. 60 which was absorbed from the alimentary tract was measured both by its concentration in venous blood and, more directly, by the distribution of labelled solute between the alimentary tract and the rest of the carcass at the end of the experiment.

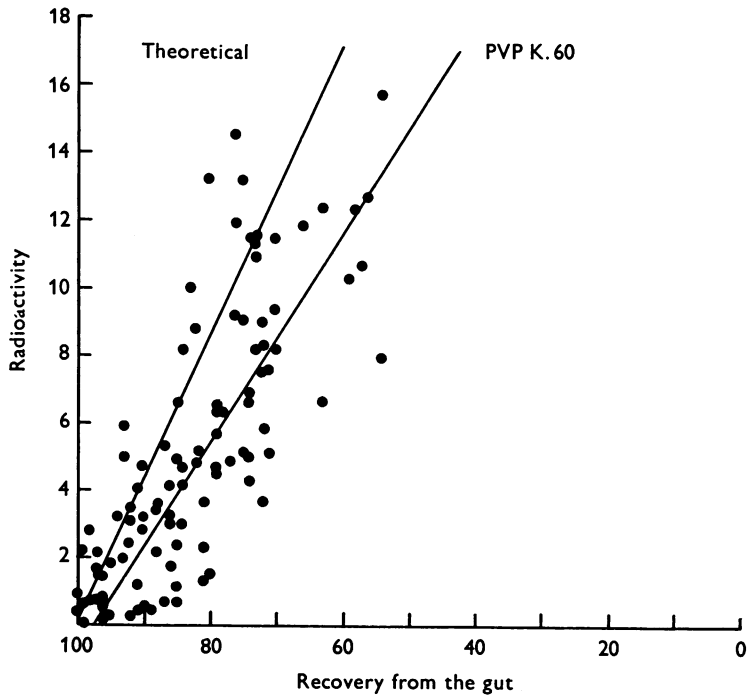

Fig. 1. Pigs fed [^{131}I]PVP K. 60. Relation between blood percentage radioactivity 6 hr after feeding (ordinate) and the percentage of the [^{131}I]PVP recovered, found in the alimentary tract (abscissa). Regression shown was highly significant ($P < 0{\cdot}01$). Theoretical regression was calculated for a 1200 g pig assuming all the [^{131}I]PVP absorbed was retained within the plasma.

There was a highly significant regression between these two parameters of absorption and it can be seen from Fig. 1 that this regression approximated to the theoretical relation for a 1200 g pig, calculated as shown on p. 637. For reasons of clarity the blood percentage radioactivity will be used subsequently as the index of absorption when comparing results between individual animals.

PVP K. 30

It is apparent from the regression between blood radioactivity and recovery from the gut in pigs fed PVP K. 30 that, after absorption, this solute escaped from the plasma in greater quantities than did PVP K. 60 (Fig. 2).

All animals used in these experiments were fed a standard volume of 45 ml. solution, irrespective of body weight, for which reason it would be

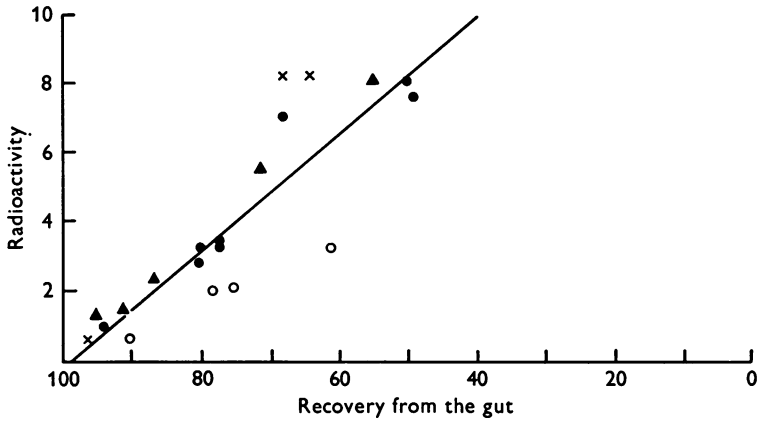

Fig. 2. Pigs fed [^{131}I]PVP K. 30. Relation between blood percentage radioactivity 6 hr after feeding (ordinate) and the percentage of the [^{131}I]PVP recovered found in the alimentary tract (abscissa). Regression shown was highly significant ($P < 0{\cdot}01$). Weight of pigs: × = < 1000 g; ▲ = 1000–1200 g; ● = 1200–1400 g; ○ = > 1400 g.

expected that differences in body weight and hence plasma and extracellular fluid volume would produce variations in the relation between the blood percentage radioactivity and the amount absorbed from the gut. Figure 2 demonstrates that the variation from the calculated regression was related to the weight of the individual animals in the expected manner.

Factors influencing the absorption of [^{131}I]*PVP K.* 60

Experiments were undertaken to determine the effect of the age of the animal and the nature of the experimental solvent on the absorption of [^{131}I]PVP K. 60. This solute was chosen for detailed examination, first, because it had a nominal mean mol. wt. approximating to that of the major component of serum γ-globulin, secondly, because it was not broken down by enzymes within the gut and thirdly, because it was largely retained in the plasma during the period immediately after its absorption from the intestine.

Age and solvent factors

There was a marked decline during the first 20 hr after birth in the ability of young pigs to absorb [^{131}I]PVP K. 60, for which reason the animals used in these experiments have been divided into three groups on the basis of age. The results of 109 experiments in which pigs were fed [^{131}I]PVP K. 60 in nine different solvents are summarized in Table 1, in which the blood percentage radioactivity and the standard error of the mean and the number of animals in each experimental group are recorded.

TABLE 1. The effect of age on the blood percentage radioactivity in pigs fed [^{131}I]PVP K. 60 in various solvents

	Solvent	Age when fed (hr) 0–5	6–14	15–20
A	NaCl 56·7 mM KCl 44·8 mM	0·3 ± 0·2 (4)	0·2 (2)	0·2 (3)
B	Sow colostrum	12·3 ± 1·0 (5)	6·1 ± 0·7 (4)	3·3 ± 0·5 (4)
C	Cow colostrum	9·2 ± 0·6 (10)	6·2 ± 0·9 (7)	4·4 ± 0·4 (10)
D	Goat colostrum	10·4 ± 0·8 (8)	—	6·2 ± 1·8 (8)
E	Ewe colostrum	—	11·6 (2)	8·8 (2)
F	Cow colostrum whey	9·2 ± 0·8 (4)	5·2 ± 1·4 (4)	2·6 ± 1·4 (4)
G	Boiled cow colostrum whey	2·3 ± 0·4 (12)	0·6 ± 0·3 (6)	3·2 ± 0·9 (6)
H	Goat colostrum whey	9·7 (2)	—	—
I	Boiled goat colostrum whey	3·2 (2)	—	—

In this table the figures indicate mean ± S.E. of mean; figures in parentheses show number of animals in each group.

From the results in Table 1 (A) it is clear that very little absorption of [^{131}I]PVP K. 60 occurred when it was fed in a simple chloride solution containing similar concentrations of sodium and potassium to cow colostrum whey (see Balfour & Comline, 1962). The blood radioactivity had not exceeded 1 %, 6 hr after feeding and at this time 90–100 % of the [^{131}I]PVP recovered was found in the gut homogenates.

The absorption of [^{131}I]PVP from sow colostrum (Table 1B) was much greater than that from chloride solution. In pigs less than 6 hr old the mean blood percentage radioactivity was 12·3 ± 1·0, declining to 6·1 ± 0·7 between 6 and 14 hr of age and to 3·3 ± 0·5 between 15 and 20 hr. The difference in blood percentage radioactivity between pigs 0–5 hr old and

those 6–14 hr old was highly significant ($P < 0{\cdot}01$). The individual results obtained with some animals fed sow colostrum are illustrated in Fig. 3.

The ready availability of cow colostrum made possible a more extensive analysis of the effect of age upon absorption (Table 1C). It was found that there was a rapid decline in the ability to absorb [^{131}I]PVP with age. This

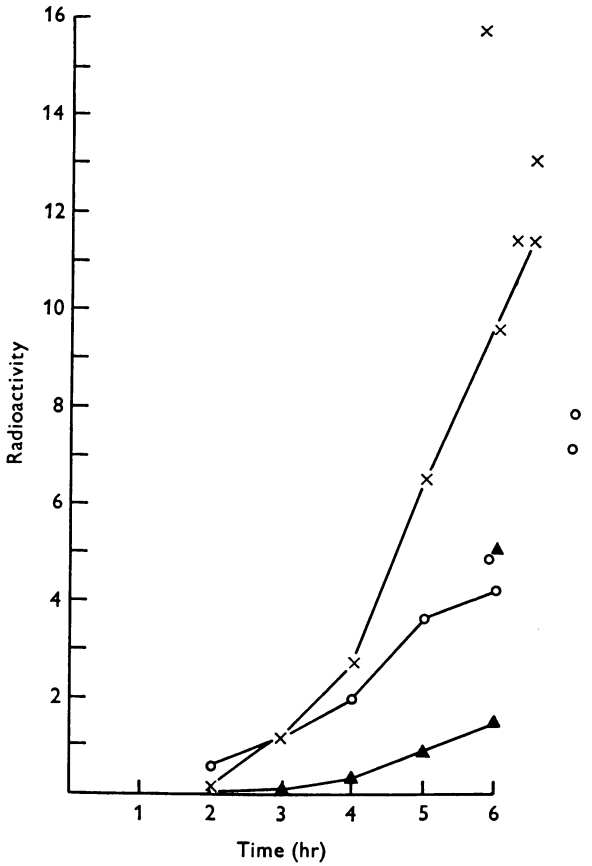

Fig. 3. Effect of age on the absorption of [^{131}I]PVP K. 60 from sow colostrum. Age of pigs when fed: 0–5 hr, × — × ; 6–14 hr, ○ — ○ ; 15–20 hr, ▲ — ▲. Ordinate, blood percentage radioactivity. Abscissa, time after feeding.

is illustrated in Fig. 4, which also indicates how much of this variation can be related to differences in the weight of the pigs. The mean blood percentage radioactivity in the 0–5 hr group was significantly greater than that of the 6–14 hr group ($P < 0{\cdot}02$), but the mean value from the latter group did not differ significantly from that of pigs 15–20 hr old.

The absorption of [^{131}I]PVP K. 60 from goat colostrum is shown in

Table 1(D), and although no experiments were performed on animals 6–14 hr of age, there was significantly less absorption in animals 15–20 hr of age than in very young pigs ($P < 0{\cdot}05$).

Since it was found necessary to use fresh ewe colostrum, few experiments could be performed using this material (Table 1E). Samples of colostrum cannot be kept at low temperatures unless the fat is removed and, since

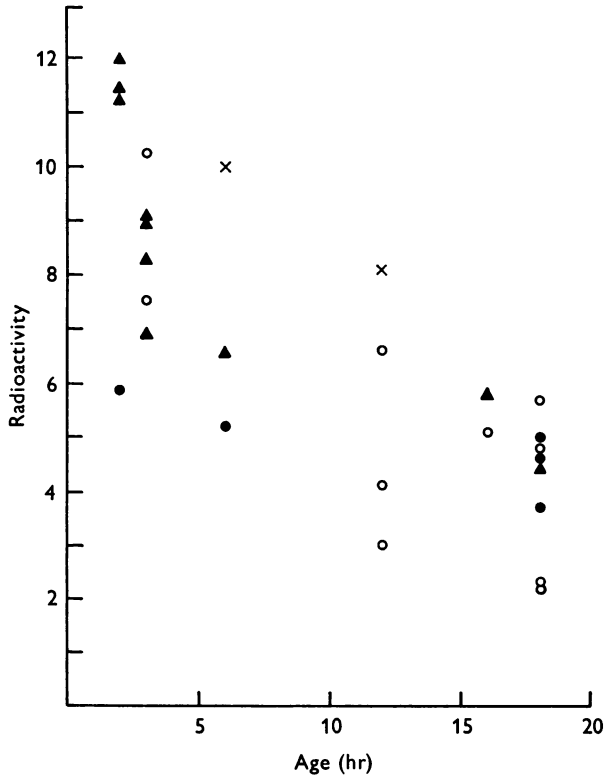

Fig. 4. Effect of age on the absorption of [^{131}I]PVP K. 60 from cow colostrum. Ordinate shows blood percentage radioactivity 6 hr after feeding. Abscissa, age of pigs when first fed (hr after birth). Weight of individual animals: × = < 1000 g; ▲ = 1000–1200 g; ● = 1200–1400 g; ○ = > 1400 g.

ewe colostrum has a high fat content and is extremely viscous, it was not possible to remove the fat by decantation or centrifugation.

The results from the four pigs fed ewe colostrum showed considerable variation, but both pigs in the 15–20 hr group absorbed better than pigs fed other solvents at this age. It would therefore seem from these limited results that ewe colostrum may be exceptionally effective in promoting absorption in the pig.

The removal of casein from both cow and goat colostrum by incubation with rennet had little effect on the ability of these solvents to promote absorption. There was no significant difference in any age group between the absorption of [^{131}I]PVP K. 60 from cow colostrum and that from cow colostrum whey. Similarly, although a statistical comparison was not possible, the absorption of [^{131}I]PVP K. 60 from goat colostrum was closely comparable with that from goat colostrum whey.

In contrast, the removal of the heat coagulable proteins from colostrum whey by boiling at pH 5·4 produced a marked reduction in its ability to promote absorption: the absorption from boiled cow colostrum whey was reduced significantly, relative to that from cow colostrum, in pigs 0–5 hr old ($P < 0{\cdot}01$) and also in pigs 6–14 hr old ($P < 0{\cdot}01$). In pigs 15–20 hr old the difference was not significant. A similar loss in the ability to accelerate absorption was seen when goat colostrum whey was boiled.

It will be noted, however, that, in pigs less than 5 hr old, the absorption of [^{131}I]PVP K. 60 from boiled cow colostrum whey remained significantly higher than that from a simple chloride solution ($P < 0{\cdot}01$).

The possible influence of colostrum fat upon the absorption of [^{131}I]PVP K. 60 was investigated in one experiment, the results of which are shown in Fig. 5. Two pigs were fed fresh cow colostrum containing the normal proportion of fat. Two pigs were fed the same colostrum after removal of the fat by centrifugation and a third pair of pigs were fed colostrum to which had been added 20 ml./100 ml. of the fat fraction removed by centrifugation from the residue of the colostrum collected.

A series of experiments was undertaken to determine the effect on the absorption of PVP K. 60 by the pig of those simple compounds known to accelerate the absorption of PVP K. 60 and serum γ-globulin by the young calf. The results are shown in Table 2, from which it can be seen that none of these simple compounds improved absorption above the basal levels seen when PVP K. 60 was fed in chloride solution (cf. Table 1A).

Absorption of [^{131}*I*] *PVP K. 60 by suckled pigs and by pigs more than 20 hr old when fed*

It has been found that pigs which were allowed to suckle naturally for a short while after birth rapidly lost the ability to absorb [^{131}I]PVP K. 60.

In one such experiment four pigs from a litter of eight were isolated from the sow without being allowed to suckle, while their siblings were permitted to suckle without disturbance for 2 hr after birth. Five hours after birth each pig was fed 45 ml. sow colostrum containing [^{131}I]PVP K. 60: the results of this experiment are summarized in Table 3.

A small number of experiments have been performed to determine to what age the unsuckled pig retains the ability to absorb [^{131}I]PVP K. 60,

since it has been possible to maintain unsuckled animals in an apparently healthy condition in the incubator for 65–70 hr after birth. The results of these experiments, which are shown in Table 4, indicate that the unsuckled animal can still absorb [^{131}I]PVP K. 60, 65 hr after birth.

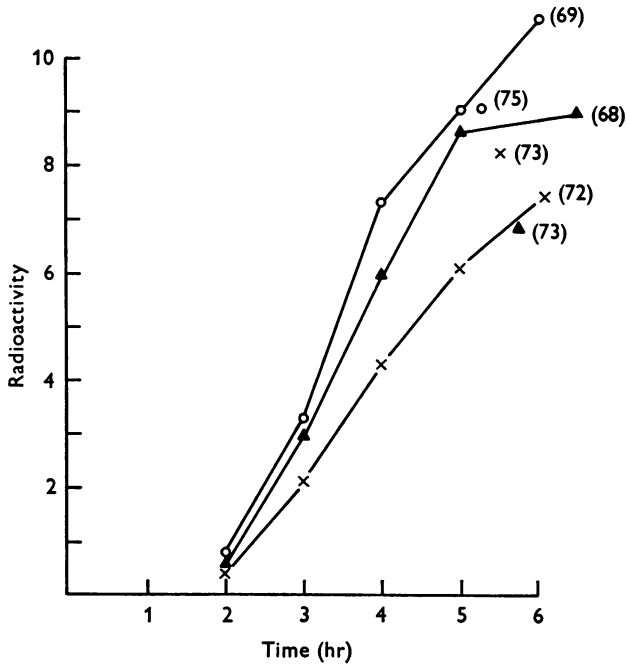

Fig. 5. Effect of colostrum fat on the absorption of [^{131}I]PVP K. 60 fed in cow colostrum. Pigs fed: defatted colostrum, ▲—▲; colostrum with natural proportion of fat, × — × ; colostrum with additional 20 ml. colostrum fat/100 ml. ○—○. Age of pigs 3 hr. Percentage of isotope recovered found in the alimentary tract for each pig is shown in parentheses. Ordinate and abscissa as Fig. 3.

The absorption of [^{131}*I*]*PVP K.* 30

It has been established that the absorption of [^{131}I]PVP K. 60 (mean mol. wt. 160,000) is extremely dependent upon factors supplied in the solvent fed. The importance of solvent factors to the absorption of [^{131}I]-PVP K. 30 (mean mol. wt. 40,000) has also been assessed using four of the solvents previously evaluated during measurement of the absorption of [^{131}I]PVP K. 60 (see Table 1). For comparison, the results of the experiments concerning [^{131}I]PVP K. 30 are summarized in Table 5. The number of pigs used in this work was small and thus a statistical analysis was not possible in all experimental groups.

The results of these experiments have shown that the absorption of

[^{131}I]PVP K. 30 is less dependent upon solvent factors and also that it is less related to the age of the animal than is the absorption of [^{131}I]PVP K. 60.

In pigs fed PVP K. 30 in chloride solution, there were no significant differences between the blood percentage radioactivities in animals in the three age groups. In comparison with animals of a similar age fed PVP

TABLE 2. Blood percentage radioactivity in unsuckled pigs 6 hr after feeding [^{131}I]PVP K. 60

Solvent	Age when fed (hr)	Blood % radio-activity
$NaH_2PO_4.2H_2O$, Na_2HPO_4, KCl 44·8 mM (56·7 mM)	6	0·2
		0·2
Na_2HPO_4 + 5 g glucose/100 ml.	6	0·4
		0·7
Sodium pyruvate 56·7 mM	12	0·7
		0·6
Sodium lactate 56·7 mM	12	0·5
		1·7
Sodium acetate 56·7 mM	3	0·2
		0·3
Sodium propionate 56·7 mM	3	0·1
		0·2
Potassium isobutyrate 56·7 mM	3	0·2, 0·2
		0·3, 0·4
	10	0·3
		0·4

TABLE 3. Absorption of [^{131}I]PVP K. 60 from sow colostrum in 5 hr old animals

	Blood % radioactivity	Recovery from the gut (%)
Pigs allowed to suckle for 2 hr	0·2	95
	< 0·1	96
	0·3	96
	0·2	97
Unsuckled pigs	10·2	81
	8·9	70
	11·8	69
	12·6	72

K. 60, however, animals in the 0–5 hr age group had a significantly higher blood radioactivity ($P < 0·01$) even though the PVP K. 30 absorbed into the circulation was lost more rapidly than PVP K. 60. In pigs fed PVP K. 30 in cow colostrum, the blood percentage radioactivity of the 0–5 hr age group was significantly higher than that of animals fed PVP K. 30

in chloride solutions ($P < 0{\cdot}01$), and was not significantly different from animals fed PVP K. 30 in sow colostrum at this age. The mean blood percentage radioactivity of pigs fed PVP K. 30 in cow colostrum in the 0–5 hr age group was not significantly different from that of pigs fed 6–14 hr after birth, although a significant decline in the blood percentage radioactivity was seen by 15–20 hr after birth ($P < 0{\cdot}01$). In the two pigs fed PVP K. 30 in goat colostrum in the 0–5 hr age group, the blood percentage radioactivity was of the same order as that in animals fed cow colostrum or sow colostrum.

TABLE 4. The absorption of [^{131}I]PVP K. 60 by unsuckled pigs more than 20 hr old

Age when fed (hr)	Solvent	Blood % radioactivity	Recovery from the gut (%)
41	Sow colostrum	4·2	86
41	Sow colostrum	3·1	89
41	Cow colostrum	3·7	90
41	Cow colostrum	3·3	88
65	Cow colostrum	2·9	92
65	Cow colostrum	3·0	84

TABLE 5. The effect of age on blood percentage radioactivity in pigs fed [^{131}I]PVP K. 30 in various solvents

	Age when fed (hr)		
Solvent	0–5	6–14	15–20
NaCl 56·7 mM KCl 44·8 mM	2·7 ± 0·6 (5)	2·1 ± 1·0 (5)	3·4 ± 0·4 (5)
Cow colostrum	6·8 ± 1·0 (5)	7·6 ± 0·7 (4)	2·4 ± 0·9 (4)
Sow colostrum	5·9 ± 1·4 (6)	—	—
Goat colostrum	7·8 (2)	—	—

In this table the figures indicate mean ± S.E. of mean; figures in parentheses show the number of animals in each group.

DISCUSSION

The choice of [^{131}I]PVP for the analysis of the factors influencing the normal permeability of the intestine of the young pig is open to criticism since the reaction of the epithelial cells to a synthetic polymer may not reflect their reaction when presented with a normal macromolecular constituent of colostrum. It has been reported previously, however, that the

young pig will readily absorb PVP K. 30 (Lecce, Matrone & Morgan, 1961*a*, *b*) and it has been shown that there is a close quantitative similarity in the young calf between the absorption of [^{131}I]PVP K. 60 and [^{131}I]serum γ-globulin with respect to the action of different solvents (Hardy, 1969). Furthermore, experience with the young calf and preliminary experiments on the young pig had already shown that [^{131}I]PVP K. 60 was not absorbed into the blood once closure had occurred and therefore that its absorption must have employed those specific pathways for macromolecular absorption available only in the very young animal.

The potential disadvantage of the use of PVP K. 60 was the variation in mol. wt. on either side of the mean value, unavoidable in polymer fractions of this type. Gel filtration of samples of [^{131}I]PVP K. 60 on Sephadex G-100 has shown, however, that the ^{131}I labelled material was eluted as a single homogeneous peak with mol. wt. exceeding 150,000. Furthermore, there was no difference in the elution characteristics of samples of PVP taken from the solution fed, the blood of experimental pigs after absorption, or samples from the intestinal tract. A detailed investigation of the factors which influenced the absorption of [^{131}I]PVP K. 60 has therefore been reported.

There was a decline with age in the ability of the young pig to absorb [^{131}I]PVP K. 60 which, since [^{131}I]PVP was not broken down within the gut, must have represented a change in the actual mechanisms responsible for the absorption of material of high molecular weight.

Previous reports of the decline in the ability of the young pig to absorb specific antibodies with increasing age (Miller, Harmon, Ullrey, Schmidt, Lueke & Hoefer, 1962; Kim, Bradley & Watson, 1966) have not demonstrated a decline in the permeability of the gut to intact protein molecules as such, because they did not exclude the possible role of increased proteolysis in the decreased antibody absorption in older pigs. In fact, there is a significant increase in the ability to digest ^{131}I bovine and porcine γ-globulin within the first 24 hr after birth (R. N. Hardy, in preparation), and Brown, Smith & Witty (1968) report that the break-down of albumin during transfer across everted sacs of small intestine first appears at about 24 hr of age. The decline in antibody absorption with age reported by other investigators can therefore be attributed to the decrease in the amount of unhydrolysed antibody available for absorption, as well as to changes in intestinal permeability.

It has been found that while unsuckled animals retained the ability to absorb [^{131}I]PVP K. 60 for at least 65 hr after birth, animals which had suckled for 2 hr immediately after birth absorbed virtually no PVP when tested at 5 hr of age. This contrast between the suckled and unsuckled animal was first noticed by Lecce & Morgan (1962) and it has since been

established that closure may also be brought about by the ingestion of suitable quantities of a variety of materials including boiled cow colostrum whey (Lecce, Morgan & Matrone, 1964) and glucose solutions (Lecce, 1966*a*). One notable difference between the results of these investigators and those reported in this paper concerns the time interval between the ingestion of the material which will cause closure and the onset of closure itself. Thus, while suckling virtually abolished the absorption of [^{131}I]-PVP K. 60 in all animals within 5 hr (Table 3), it did not curtail the absorption of PVP K. 30 until at least 48 hr of age in many animals (Lecce & Morgan, 1962), and Lecce (1966*a*) emphasizes that glucose ingestion would not terminate the absorption of intact chicken egg protein in less than 12 hr.

The reasons for these differences concerning the onset of closure are not yet clear, but it seems probable that the characteristics of the solutes used to demonstrate closure may themselves contribute towards an explanation, since there is evidence to suggest that the molecular weight of a macromolecular solute may dictate its mode of absorption by the intestine. Thus, in both the calf and the pig the absorption of [^{131}I]PVP K. 60 is largely dependent upon solvent factors, and in the calf, when absorbed, the PVP passes almost exclusively into the lymphatics. In contrast, the absorption of [^{131}I]PVP K. 30 and [^{131}I]serum albumin in both the pig and the calf is less dependent upon solvent factors and in the calf, at least, the labelled solutes pass in part directly into the portal vessels (Hardy, 1969). Furthermore the transfer of bovine plasma albumin across the epithelium *in vitro* does not require solvent factors (Brown *et al.* 1968).

It may be that the absorption pathway used by macromolecules of relatively high molecular weight, such as [^{131}I]serum γ-globulin and [^{131}I]PVP K. 60, requires solvent factors, and in the pig can be abruptly interrupted by those dietary factors which cause closure. Solutes of lower molecular weight, such as PVP K. 30 or serum albumin may be absorbed in addition by pathways not accessible to solutes of higher molecular weight, since they are less rapidly affected by those factors causing closure: indeed Brown *et al.* (1968) have reported the transfer of albumin across everted sacs of jejunum in nine day-old suckled pigs.

The age and dietary histories of the animals were not the only factors which limited the absorption of PVP K. 60, since the absorption of this material by the young pig was largely dependent on the solvent in which it was fed. Absorption of [^{131}I]PVP K. 60 from a basal solvent, containing only Na^+, K^+ and Cl^- in the approximate concentrations found in cow colostrum whey, was negligible, but the absorption was accelerated when cow colostrum or cow colostrum whey was fed. Samples of sow colostrum and colostrum from goats and ewes were also comparably effective in

promoting the absorption of [^{131}I]PVP K. 60. Colostrum fat was not essential to the process.

In contrast, the absorption of [^{131}I]PVP K. 60 from boiled cow colostrum whey was extremely poor in the new-born pig, although this solvent was as effective as whole cow colostrum in promoting the absorption of [^{131}I]PVP K. 60 and [^{131}I]serum γ-globulin in the calf (Balfour & Comline, 1962; Hardy, 1969).

The solvent factors in colostrum which accelerate the absorption of [^{131}I]PVP K. 60 appear therefore to differ in the two species; in the calf they are heat-stable and their action can be imitated by, but not attributed to, phosphate, lactate, pyruvate and certain of the lower volatile fatty acids, whereas in the pig they are destroyed by heat and cannot be replaced by those simple salts which are effective in the calf.

It has been suggested, from the other known actions of the salts which accelerate absorption in the calf, that they may act by supplying metabolic energy necessary to the absorption process (Hardy, 1969). If so, since such salts are ineffective in the pig, it seems possible that either this species is not capable of utilizing energy in this form during absorption or that a supply of exogenous metabolic energy is not the limiting factor in absorption.

Another possible mode of action of solvent factors has recently been proposed by Smith, Witty & Brown (1968), who demonstrated a facilitation by poly-L-arginine of IgG transfer across everted sacs of new-born pig intestine and suggested that this may be related to the action of the polyelectrolyte on lipid membrane components. Whatever may be the precise identity and mode of action of the factors responsible in the calf and pig, it seems that each group of absorption-promoting substances is present in both colostrum and colostrum whey.

There are indications that the pinocytotic uptake of macromolecular material by the intestinal epithelial cells is not dependent upon the presence of the specific solvent factors described in the present experiments. Payne & Marsh (1962) showed that both homologous and heterologous γ-globulins labelled with fluorescein were taken up within 6 hr by the epithelial cells when administered in buffered saline solution, and Kaeberle & Segre (1964) demonstrated a similar uptake within 2½ hr of feeding fluorescein isothiocyanate (FITC) or [^{35}S] homologous γ-globulin in buffered saline solution. Furthermore, Lecce (1966*b*), who measured the uptake of fluorescent γ-globulin by everted slices of the new-born pig jejunum, has demonstrated that under *in vitro* conditions the γ-globulin was taken into the epithelial cells when incubated in a balanced salt solution. Additional evidence for the passage of material of high molecular weight into the epithelial cells of the small intestine in the absence of

solvent factors has been obtained by measurement of the uptake of [^{125}I]PVP K. 60 when administered in water to young pigs. Under these conditions it is found that a very large proportion of the labelled PVP is taken into the epithelial cells, although little appears in the blood (R. M. Clark & R. N. Hardy, in preparation).

All these results support the contention that the solvent factors necessary for the absorption of macromolecular material into the blood do not manifest their effects during the uptake of this material by the epithelial cells, and probably therefore act by accelerating the discharge of the protein from the lacteal side of the epithelial cell.

Further evidence for this suggestion is the estimate of the time relations of the absorption of FITC labelled γ-globulin in buffered saline, deduced histologically by Payne & Marsh (1962). These authors report that the cells appear to absorb 'all the globulin they can contain' before protein is seen in the lymphatics. FITC labelled protein was in fact seen leaving the cell between 5 and 18 hr, by which time the contents of the cell were completely discharged. The experiments reported in this paper and the results of Pierce & Smith (1967) show that macromolecular material when fed in colostrum can leave the cell within 2 hr of feeding, so it must be concluded that the extremely slow absorption of FITC labelled protein reported by Payne & Marsh (1962) was a consequence of the fact that the buffered saline solvent was not able to facilitate the transfer of intracellular protein into the lymphatics at a rate comparable with that when colostrum is fed.

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