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PASSAGE OF I^{131}-ALBUMIN AND I^{125}-GAMMA GLOBULIN INTO THE SMALL INTESTINE OF CALVES*)

By

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The important discovery by *Gordon* (1959) and by *Schwartz & Jarnum* (1959) that in certain gastrointestinal disorders — so-called protein-losing gastroenteropathies — plasma proteins are being lost into the lumen of the gastrointestinal tract, has greatly focussed the attention upon the role of the digestive system in the normal catabolism of serum proteins. The presence of serum albumin and gamma globulin in the intestinal secretions of normal humans and experimental animals has since been confirmed by many authors working with different techniques (*Holman et al.* 1959; *Birke et al.* 1960; *Armstrong et al.* 1960; *Ullberg et al.* 1960; *Wetterfors et al.* 1960; *Campbell et al.* 1961; *Tarver et al.* 1961; *Barandun et al.* 1962; *Glenert et al.* 1962; *Birke et al.* 1963; *Andersen et al.* 1963; *Dich & Nielsen* 1964). However, there is conflicting evidence concerning the quantitative significance of the protein transfer across the intestinal wall. Thus, *Glenert et al.* (1962) found that 40—60 per cent of total albumin catabolism occurred in the gut. *Franks et al.* (1963 ab) on the other hand, claim that the small intestine is unimportant in albumin catabolism. *Dich & Nielsen* (1964) found that appr. 10—30 per cent of total albumin and gamma globulin catabolism

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in pigs could be accounted for by intestinal degradation. *Andersen et al.* (1963) found that 20—75 per cent of gamma globulin catabolism occurred in the gut, while *Birke et al.* (1963) concluded that the amounts of gamma globulin passing the gut wall in healthy persons was probably negligible.

The present communication reports experiments with calves fitted with permanent, isolated intestinal loops into which the passage of I^{131}-albumin and I^{125}-gamma globulin were studied simultaneously. The purpose of the study was to examine the quantitative aspects of the transfer, to study the effect of instillation of salt solutions upon the transfer, and to compare the intestinal passage of two proteins with different molecular weights.

METHODS

Surgical procedure: 5 crossbred calves were used. At the age of 2—3 months they were fitted with isolated re-entrant loops at various sites of the small intestine. The loops were prepared essentially according to the technique described by *Hogan & Phillipson* (1960) and by *Ash* (1962)*). After the operation the calves were kept in small pens where they had access to hay of

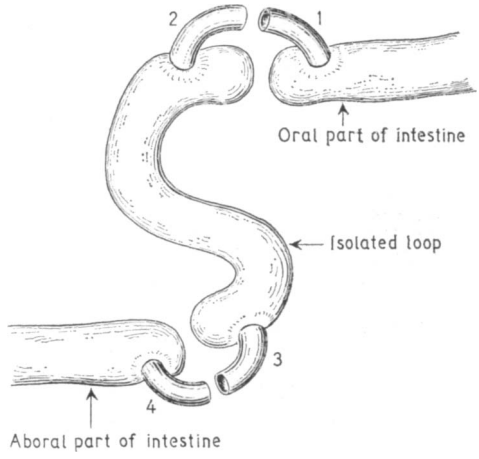

Fig. 1.

*) The cannulae used in the operations were kindly supplied by Mr. R. W. Ash, Rowett Research Institute, Aberdeen, whose help is gratefully acknowledged.

Table I. Experimental and surgical data from 5 calves with isolated, re-entrant loops.

Calf	Weight kg	Surgical preparation	Interval between operation and start of experiment (days)	Experimental life, days
II	71	Ileal loop, 60 cm above coecum. Length of loop: 50 cm. Length of small intestine: 16.6 meters.	14	22
III	70	Jejunal loop, 3.8 meters from pylorus. Length of loop: 72 cm. Length of small intestine: 16 meters.	16	33
IV	46	Ileal loop, 55 cm above coecum. Length of loop: 55 cm. Length of small intestine: 18.3 meters.	14	30
VI	52	Jejunal loop, 7 meters from pylorus. Length of loop: 65 cm. Length of small intestine: 15.7 meters.	18	37
VII	45	Ileal loop, close to coecum. Length of loop: 55 cm. Length of small intestine: 18.2 meters.	14	34

good quality. Twice daily they were given milk (4—6 litre pro die). Usually, the postoperative recovery was uneventful.

No experiment was started earlier than 2 weeks after the operation. By this time the influence of the operation upon the metabolism of serum proteins has subsidied. At the beginning of the experiment all calves were in excellent clinical condition. Table I and Fig. 1 show details of surgical and experimental procedure.

Preparation of I^{131}-albumin and I^{125}-gamma globulin. Commercial bovine albumin and gamma globulin were used.**) Immunoelectrophoresis proved that the preparations were pure albumin and gamma globulin. The proteins were labelled with carrier-free I^{131} and I^{125} according to the method described by *McFarlane* (1958). The mean ratio of iodine bound to protein was ½—1 atom/molecule. More than 99 per cent of the radioactivity was precipitable with trichloroacetic acid (TCA). Immunoelectrophoresis of some of the preparations proved migrat-

**) Armour Pharmaceutical Co., Ltd, and Pentex Ltd.

ion as unlabelled albumin and gamma globulin, and autoradiography — after mixing with bovine serum and electrophoresis — proved that radioactivity was confined to the albumin and gamma globulin fractions. The labelled proteins were diluted with bovine albumin and injected into the animals within 24 hours.

Counting procedure. All samples were counted in a NaI-scintillation counter with discriminator. Thus, I^{131}-activity and I^{125}-activity could be measured independently. However, I^{125}-activity was measured 2—3 months after the experiments. Any radioactivity due to I^{131} was, therefore, negligible.

General experimental procedure. 2—3 days before the start of the experiment the calves were given appr. 5 ml Lugol's iodine solution orally. This was continued for 3—4 days to prevent thyroid uptake of the label. Doses were 200—300 micro-Curies I^{131}-albumin and 100—150 micro-Curies I^{125}-gamma globulin. Injections were given through a polythene catheter inserted into the jugular vein. Plasma volume was calculated from the radioactivity in a plasma sample taken 15 minutes after the injections. Before the daily collection of loop secretions the loops were washed thoroughly with saline to remove digesta. The loop was bypassed by leading the digesta directly from cannula 1 to cannula 4 (cf. Fig. 1). Thus, the fluid flowing into the measuring cylinder was clear succus entericus. The presence of albumin and gamma globulin in the loop secretions was confirmed by immunoelectrophoresis. In most experiments samples were collected daily for 4 hrs. To study the influence of flow through the loop upon the intestinal transfer of proteins, hyper- and isotonic solutions of magnesium sulphate were instilled through the loops. With 1 hour-intervals the loop fluid was sampled directly into a cylinder containing a 20 per cent TCA-solution. After centrifugation activity not bound to proteins was measured in the clear supernatant fluid. Plasma samples were taken daily throughout the experiment.

After the experiments the calves were sacrificed and post mortem examination carried out. In all calves firm adhesions had developed around the cannulae. In calf II a fistula had developed between the loop and the oral ileum, close to cannula 1. Besides this, no gross pathological lesions were found at necropsy. Histology revealed some dilatation of the lymphatic vessels of villi intestinales. The cause of this has not been ascertained; obviously, it was not due to inhibited drainage of lymph from the

exposed bowel. No inflammatory processes were found in any loop*).

Calculation of data. The intestinal clearance proposed by *Glenert et al.* (1962) has been employed. This parameter indicates the volume of plasma (ml) that is cleared per minute by intestinal degradation of albumin and gamma globulin. It is derived from the equation $\frac{Q_I}{Q_p \times t}$, where Q_I is protein-bound radioactivity in succus entericus, Q_p is the radioactivity in 1 ml plasma and t is the length of the collection period (minutes). For reasons of comparison intestinal clearances were referred to 10 per cent of the total length of the small intestine. Also, in some calves, the "metabolic clearance" was calculated: This parameter is derived from the degradation constant ($K_{1\,2}$ = percentage of vascular albumin or gamma globulin pool broken down *per day*) and the plasma volume (PV): $\frac{K_{1\,2} \times PV}{1440}$ = ml plasma containing the mass of albumin or gamma globulin broken down per minute. The ratio of intestinal to metabolic clearance will, then, show the percentage of total albumin and gamma globulin catabolism that must be assumed to occur in the intestine.

Turnover rates of albumin and gamma globulin were calculated according to *Matthews* (1957).

RESULTS

Metabolic clearances were calculated in calves III, IV, VI and VII. For I^{131}-albumin it ranged from 0.15 to 0.22 ml plasma/min. and for I^{125}-gamma globulin from 0.14 to 0.20 ml plasma/min.

Data from experiments where succus entericus was flowing spontaneously from the loop are listed in Table II. It appears that there is little difference between the average intestinal clearances in the 5 calves, the range being 0.016—0.041 ml/min./10 % intestine for I^{131}-albumin and 0.005—0.036 ml/min./10 % intestine for I^{125}-gamma globulin. However, in individual experiments with the same loop variation was considerable. Fluctuations of more than 100 per cent within the same collection period have been common. In calf II the fistula mentioned above

*) Histological examination was kindly made by Sigurd Andersen, D. V. M.

Table II. Experimental data obtained with spontaneous flow of succus entericus. Intestinal clearances were determined 0—136 hours after the injections of isotopes.

Calf	Protein	Intestinal clearance, ml plasma/min./10% intestine		Protein-bound radioactivity, % of total excretion		Ratio gamma glubulin clearance / albumin clearance
		Range	No. of determinations	Average	Range	
II	I^{131}-albumin	0.006—0.095	12	53	11—86	Only albumin injected
III	I^{131}-albumin	0.024—0.068	5	96	94—100	0.65 (range 0.46—0.96)
	I^{125}-γglobulin	0.012—0.041	5	79	53—93	
IV	I^{131}-albumin	0.025—0.091	5	95	89—100	0.56 (range 0.44—0.62)
	I^{125}-γglobulin	0.011—0.055	6	79	61—90	
VI	I^{131}-albumin	0.017—0.072	3	84	79—91	0.86 (range 0.71—1.04)
	I^{125}-γglobulin	0.012—0.075	3	78	72—85	
VII	I^{131}-albumin	0.004—0.024	3	42	19—55	0.32 (range 0—0.67)
	I^{125}-γglobulin	0 —0.016	3	48	—	

caused occasional admixture of digesta to the loop secretions. In such collections the protein-bound radioactivity was low (11—59 per cent, mean 33 per cent). In secretions where such admixture did not occur protein-bound radioactivity ranged from 53—86 per cent (mean 67 per cent). This gives evidence of a rapid digestion of the excreted proteins when digesta are passing through the intestine.

Except for calf VII, where gamma globulin clearances were low, albumin and gamma globulin clearances were of the same order of magnitude. The ratio between the intestinal clearances of gamma globulin and albumin ranged from 0.65 to 0.97 (average figures). Protein-bound I^{131}-activity ranged from 42—96 per cent of total I^{131}-excretion in the loop. Protein-bound I^{125}-activity was lower: 48—79 per cent of total excretion (in two determinations (calf VII) no I^{125}-activity appeared to be protein-bound).

The radioactivity in shed mucosal cells was negligible.

There was no distinct difference between the intestinal clearances of jejunal and ileal loops (Table II).

In calf VI the protein transfer was studied during a 24-hour collection period, 5 days after the injection of the labelled proteins. The procedure in this experiment was a preliminary wash-

ing with saline to remove digesta from the loop. At the end of the collection period the loop was washed again to obtain any protein that might have accumulated during the long collection period. The results obtained in this experiment are shown in Table III. It appears that intestinal clearances are subject to very considerable variations. During the final washing of the loop (the last 10 minutes of the experiment) much protein has been washed out. Average figures from this experiment were 0.016 ml/min./10 % intestine for I^{131}-albumin and 0.015 ml/min./10 % intestine for I^{125}-gamma globulin. In calf VII a similar experiment was carried out over a collection period of appr. 8 hours. In this experiment the intestinal clearances were 0.017 ml/min./10 % intestine (I^{131}-albumin) and 0.003 ml/min./10 % intestine (I^{125}-gamma globulin).

Table III. Results of a 24 hour-collection in calf VI. The collection was started 5 days after the injections of I^{131}-albumin and I^{125}-gamma globulin.

Collection period	Intestinal clearance, ml/min./10 % intestine		Protein-bound radioactivity per cent of total excretion		Volume of collected loop-fluid ml
	I^{131}-albumin	I^{125}-gamma globulin	I^{131}	I^{125}	
1^{50} pm — 9^{00} pm	0.010	0.009	87	85	40
9^{00} pm — 8^{45} am	0.020	0.018	90	88	87
8^{45} am — 1^{40} pm	0.008	0.008	88	86	17
1^{40} pm — 1^{50} pm	0.233	0.211	77	73	45

From Table III it appears that the bulk of radioactivity was protein-bound, even in the final washings, a fact that gives evidence of very little breakdown within the isolated loop.

Effect of instillation of iso- and hypertonic solutions through the loop. Isotonic (3.7 %) and 10 % magnesium sulphate solutions were used. Usually, 250 ml (37°C.) were instilled through cannula 2. An instillation rate of appr. 80 drops per minute was found appropriate. With higher instillation rates the calves would sometimes show slight symptoms of colic, probably due to distension of the loop. The results of these experiments are listed in table IV.

Instillation of a 3.7 % magnesium sulphate solution caused an increased intestinal clearance in calves IV and VI. The in-

Table IV. Effect of instillation of isotonic and 10 % $MgSO_4$ and digesta through the loop.

Calf	Solution instilled		Intestinal clearance, ml/min./10 % intestine		
			Pre-instillation level	During instillation	After instillation
III	3.7 % $MgSO_4$	albumin	0.040	0.034	0.010
		gamma globulin	0.026	0.029	0.008
	10 % $MgSO_4$	albumin	0.040	0.122	0.035
		gamma globulin	0.026	0.055	0.012
IV	3.7 % $MgSO_4$	albumin	0.040	0.075	0.041
		gamma globulin	0.027	0.068	0.028
	10 % $MgSO_4$	albumin	0.040	0.128	0.012
		gamma globulin	0.027	0.071	0.007
VI	3.7 % $MgSO_4$	albumin	0.037	0.054	0.009
		gamma globulin	0.036	0.051	0.011
	10 % $MgSO_4$	albumin	0.037	0.057	0.026
		gamma globulin	0.036	0.049	0.022
VII	3.7 % $MgSO_4$	albumin	0.016	0.017	—
		gamma globulin	0.005	0	—
	10 % $MgSO_4$	albumin	0.016	0.087	0.031
		gamma globulin	0.005	0.063	0.023
	Sterile digesta	albumin	0.016	0.054	
		gamma globulin	0.005	0.024	
	Non-sterile digesta	albumin	0.016	0.080	
		gamma globulin	0.005	0.030	

crease was of the order 1.5—2.5 times the pre-instillation levels. In calves III and VII intestinal clearances remained unchanged or decreased during instillation with 3.7 % magnesium sulphate solution.

In all calves instillation of a 10 % solution of magnesium sulphate was followed by an immediate increase of the intestinal clearances of both proteins. For I^{131}-albumin the increase was 1.5—5 times pre-instillation levels, while I^{125}-gamma globulin was increased by 1.3—13 times.

In calf VII it was attempted to estimate the influence of passage of *digesta* through the loop (Table IV). Sterilized and non-sterilized jejunal digesta (obtained at necropsy from a cow) were instilled (200 ml over 15 minutes). I^{131}-albumin clearances

increased by 3.4 and 5 times and I^{125}-gamma globulin clearances by 4.8 and 6 times pre-instillation levels. The percentage of protein-bound activity was of the same order of magnitude after instillation of sterile and non-sterile digesta; however, digesta taken from cannula 1 — coming from the oral end of the gut — contained practically no protein-bound radioactivity, indicating a complete breakdown of all excreted proteins in the bowel.

DISCUSSION

The data submitted in this paper lend support to the general opinion that the digestive tract is involved in the catabolism of plasma proteins. However, individual experiments have shown that the intestinal transfer of proteins is subject to enormous variation (cf. Table II). This variability highly reduces the reliability of calculations based upon intestinal and metabolic clearances. Furthermore, the passage of digesta is obviously important in the transfer of proteins (Table IV, calf VII), a fact that further impedes quantitative approach. Consequently, our results have led us to the conclusion that quantitation of the intestinal transfer as related to total catabolism is difficult or even impossible with the present technique.

Although the clearances of albumin and gamma globulin were of the same order of magnitude, gamma globulin clearances were, in most experiments, slightly lower than albumin clearances. The reason for this may, partly, originate in the fact that protein-bound I^{125}-activity was consistently lower than protein-bound I^{131}-activity. This may indicate that bovine gamma globulin is more susceptible to breakdown within the isolated loop than albumin. In spite of the small quantitative difference between the transfer of the two proteins, the results reported above justify the conclusion that there is no *qualitative* difference between the intestinal excretion of albumin and gamma globulin in the bovine.

No consistent difference was found between the clearances of jejunal and ileal loops. This is in keeping with results obtained by us in pigs (*Dich & Nielsen* 1964).

Passage of large volumes of fluid through the loop tends to bring about an increase of intestinal clearance. This is more pronounced when hypertonic solutions are used, but may also be evident after instillation of isotonic solutions. Furthermore it has been shown that the passage of digesta through the loop

causes an increase of the transfer rate. This increase is very considerable and it may be even greater than found in our experiments since much of the protein excreted into the loop during the instillation had been broken down before collection could be made. When digesta were sampled directly from cannula 1 practically no radioactivity appeared to be protein-bound. It must be assumed that during the normal passage of digesta through the gut excreted proteins are broken down completely, thus constituting an amino acid "reservoir" available for reabsorption and — perhaps — re-utilization in the protein syntheses of the body such as proposed by *Nasset et al.* (1955).

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SUMMARY

Intestinal transfer of I^{131}-albumin and I^{125}-gamma globulin was studied in 5 calves fitted with re-entrant intestinal loops. The passage of the two proteins across the intestinal wall proved highly variable (cf. Table II). However, the transfer of I^{131}-albumin and I^{125}-gamma globulin was of the same order of magnitude, which is taken as evidence of similar transfer mechanisms of albumin and gamma globulin, in spite of different molecular weights. Instillations of hypertonic magnesium sulphate solution and of intestinal digesta were followed by increased transfer rates. It appears, therefore, that the passage of digesta will influence the transfer of proteins along the gut. It is emphasized that quantitation of the transfer as related to total catabolism of plasma proteins is subject to considerable inaccuracy.

ZUSAMMENFASSUNG

Der intestinale Durchtritt von J^{131}-Albumin und J^{125}-Gammaglobulin bei Kälbern.

Der intestinale Durchtritt von J^{131}-Albumin und J^{125}-Gammaglobulin bei 5 Kälbern mit isolierten Darmsegmenten wurde untersucht. Ausserordentliche Variation wurde festgestellt (Tabelle II), wogegen

eine Vergleichung zwischen dem Durchtritt von Albumin und Gammaglobulin erwies, dass dieser von gleicher Grösse war. Dies wird als Schein daran gehalten, dass der Durchtritt von beiden Eiweissstoffen dem selben metabolischen Muster folgt, trotz verschiedenes Molekülgewicht. Instillationen von hypertonischen Magnesiumsulfatlösungen und von Ingesta rufen eine Steigerung im Durchtritt hervor. Es ist deshalb sehr wahrscheinlich, dass die Darmpassage für den Durchtritt von Plasmaproteinen dem Darm entlang eine Rolle spielt. Es wird betönt, dass die quantitative Berechnung des Durchtrittes in Relation zum totalen Eiweiss-Katabolismus unsicher ist.

SAMMENDRAG

Udskillelse af J^{131}-albumin og J^{125}-gammaglobulin til tyndtarmen hos kalve.

Den intestinale udskillelse af J^{131}-albumin og J^{125}-gammaglobulin er undersøgt hos 5 kalve forsynede med isolerede tarmslynger. Der fandtes overordentlig stor variation i udskillelsen (tabel II), hvorimod en sammenligning mellem udskillelsen af albumin og gammaglobulin viste, at denne var af samme størrelsesorden. Dette er taget som udtryk for, at udskillelsen af de to proteinstoffer følger samme metaboliske mønster trods den forskellige molekylvægt. Instillation af hypertoniske magnesiumsulfatopløsninger samt af tyndtarmsindhold efterfulgtes af en forøget udskillelse af begge plasmaproteiner. Det må derfor anses for sandsynligt, at tarmpassagen spiller en rolle for udskillelsen af serumproteiner ned gennem tarmen. Det understreges, at beregninger af udskillelsens betydning i forhold til proteinnedbrydningen som helhed, er underkastet betydelig usikkerhed.

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