Experimental studies on the value of the reference substances polyethyleneglycol, bromsulphthalein, and ^{51}Cr as indicators of the fluid content in the intestinal lumen¹

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EDITORIAL SYNOPSIS These studies have shown that calculation of total intestinal content based on the concentration of non-absorbable markers in the aspirate may give values which are up to 50% too high.

Experimental and clinical studies on the function of the liver, the bile ducts, and the pancreas, together with studies on the motility and absorptive function of the small intestine, are generally based on intestinal intubation combined with aspiration at various levels of the intestinal content using different techniques (Blankenhorn, Hirsch, and Ahrens, 1955). In the interpretation of the results obtained in a number of these studies the aspiration of the intestinal content has been assumed to be of a quantitative nature (Lagerlöf, 1942; Dreiling and Janowitz, 1962), but apparently such an assumed requirement is not always fulfilled and recent investigations of the motility and absorptive capacity of the small intestine have been based on the application of non-absorbable (reference) substances. Infusion into the intestinal canal of these markers, uniformly mixed with the intestinal content, is assumed to provide a standard of the total intestinal content, or rather an expression of the quantity of fluid which, within the experimental period, passes through a certain section of the intestine. In most of these investigations polyethyleneglycol has served as marker (Shaffer and Critchfield, 1947; Sperber and Ekman, 1953; Borgström, Dahlqvist, Lundh, and Sjövall, 1957; Lundh, 1958; Aberdeen, Shepherd, and Simmonds, 1960; Dahlqvist and Borgström, 1961; Fordtran, Levitan, Bikerman, Burrows, and Ingelfinger, 1961; Fordtran, Soergel, and Ingelfinger, 1962; Schedl and Clifton, 1961

and 1963; Wiggins and Dawson, 1961; Bennett and Simmonds, 1962; Dahlqvist and Thomson, 1963; Clifton and Schedl, 1963; Clodi, Fordtran, and Ingelfinger, 1963; and Jacobson, Bondy, Broitman, and Fordtran, 1963). Some investigators have used polyvinylpyrrolidone (Mehnert and Förster, 1961 and 1963), in some cases labelled by ^{131}I (Clifton and Schedl, 1963), bromsulphthalein (Fordtran *et al.*, 1961; and Jacobson *et al.*, 1963), phenol red (Schedl and Clifton, 1961; Clifton and Schedl, 1963), and various chromium compounds (Nakayama, Nakamura, Yamamoto, and Tamiya, 1960); even indole-cyanide-green has been found adequate (Fordtran *et al.*, 1961).

Systematic investigations of the value of this technique are available only to a limited extent. Jacobson *et al.* (1963) found the method useful for a determination of the total intestinal content in rats and dogs whereas the method was assumed to be of minor value in determinations of volumes in closed intestinal loops. Fordtran *et al.* (1961) were of the opinion that the method was expedient but these authors fail to give any direct comparison of calculated and actual intestinal contents. Wiggins and Dawson (1961) found, however, that estimates of the fat absorption were misleading if obtained by polyethyleneglycol as reference substance, the explanation most probably being ascribable to the heterogenous distribution of polyethyleneglycol in the intestinal content.

These factors are of major interest, theoretically as well as practically, and the object of the present

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experimental investigations has been to clarify (1) whether aspiration through intestinal tubes represents quantitative recovery of intestinal contents or, if this is not the case, whether the aspirated volume is a usable expression of the total intestinal content at the point of aspiration; (2) whether the volume of intestinal contents passing a given point in the intestine can be determined with sufficient accuracy by means of reference substances; and (3) whether various reference substances are equally well suited for the purpose.

We have preferred to use polyethyleneglycol 4000 which so often has been used in previous investigations of this type, together with bromsulphthalein and ^{51}Cr in the form of $\text{Na}_2^{51}\text{CrO}_4$, the two latter being chosen because of the readiness with which they are analysed.

METHODS

The investigations have been carried out by a uniform technique, rabbits and one dog serving as experimental animals. The technique is shown in Figure 1. Through an incision into the stomach immediately orally to the pylorus, two polyvinyl¹² tubes are inserted under nembutal anaesthesia. These tubes are carried through the pylorus and about 2 cm. downwards into the duodenum; subsequently a ligature is arranged around the pylorus

¹²Pharmaseal EHR; external and internal diameters: 2.7/1.9mm.

FIG. 1. Diagram of the intestinal tubes used in the present investigation.

so as to preclude regurgitation of intestinal content into the stomach.

Distally to the pylorus, 30 to 40 cm., three tubes of the same type are inserted through an incision into the jejunum. The tubes are carried retrograde into the intestine until the tips are at sites 10 to 15 cm. (tube A), 15 to 20 cm. (tube B), and 20 to 25 cm. (tube C) distally to the tubes inserted through the stomach. The jejunal incision is closed by a purse string suture, care being taken to avoid tightening which may compromise the passage of intestinal content to the distal intestinal loops. Finally the abdominal cavity is sutured.

Infusion is carried out from calibrated tubes at a constant rate, through one of the proximal tubes by physiological saline containing polyethyleneglycol, bromsulphthalein, and ^{51}Cr , and through the other by isotonic glucose, the latter being representative of the digestive secretion in which the reference substances are to be distributed. No attempt is made to infuse the two solutions at the same rate. The period of infusion covers 50 or 60 minutes.

Within the same interval the intestinal content is aspirated through tubes A, B, and C, either by mechanical suction (intermittent negative pressure of about 100 mm. Hg) or by siphonage fractionated into intervals of five or 10 minutes. The aspirates obtained from the individual tubes and within the individual periods are measured and concentrations of the markers in each portion are determined.

Occasionally, after infusion and aspiration is complete, a ligature has been arranged around the intestine, directly distally to the site of insertion into the jejunum. The intestinal section between the pylorus and this ligature is excised *in toto*; the intestinal content is evacuated by a slight, manual pressure; a few of the intestinal specimens have been rinsed a couple of times in physiological saline; finally the intestinal wall is homogenized and the ^{51}Cr concentration determined in one or more aliquots of intestinal content, rinsing water, and intestinal wall.

Polyethyleneglycol is determined according to the method of Hyden (1956); bromsulphthalein is photometrically determined at 580 nm., and ^{51}Cr is counted in an Echo well counter.

The total intestinal content, equalling the quantity of fluid passing through a definite section of the intestine in a definite space of time, is calculated as the quantity of reference substance infused within the same period, divided by the average concentration of reference substance in the intestinal content aspirated within this period. On the assumption that the markers are applicable, this calculated volume should be of the same order of magnitude as the total quantity of fluid infused within the same period and, indeed, whether calculations are based on one or the other of the reference substances should have no influence on the values obtained. Always provided that there is no essential net transport of water into or out of the intestinal segment under examination, it might not be unreasonable to expect that results obtained by a calculation from samples discharged through tubes A, B, and C, were of the same order of magnitude in the individual experiments.

Finally, in order to illustrate how the markers are truly mixed with the intestinal content, a few experiments were carried out using rabbits as experimental animals. By the usual technique two tubes were inserted into the duodenum and ⁵¹Cr, dissolved in physiological saline, (cf. experiment 1) and isotonic glucose (cf. experiment 2), were infused through these tubes. After infusion for an adequate period, usually for about 10 minutes, the duodenum was frozen instantaneously and excised in quick-frozen condition. Autoradiography at -15°C. was carried out on duodenal specimens of perfectly identical thickness.

RESULTS

RELATIONSHIP BETWEEN VOLUMES ASPIRATED AND INTESTINAL CONTENT Table I records the volumes infused and aspirated. The infused volumes have varied greatly apparently, in total as well as in terms of kilogram per body weight, these variations being unrelated to the proportions of infused volume to be aspirated. Likewise it is seen from the table that siphonage and mechanical aspiration provide percentage aspiration of the same order of magnitude.

Table II shows the percentage of infused volume which in the individual experiments has been drawn through tubes A, B, and C, respectively. Variations from experiment to experiment are considerable and the range of distribution is even wider than shown in Table II since the rate of discharge may vary

from one 10-minute period to the next within the individual experiments. On an average the discharge is most excessive through tubes A and B. In these experiments there is no net transport of water through the wall of the intestinal segment examined (cf. later) and hence the intestinal content at tube B will be inferior to that at tube A. But, the average discharge through tubes A and B being equal, the explanation must be that volumes aspirated and total intestinal content are unrelated.

The reason why discharge through tube C is less excessive than discharge through tubes A and B may be that nothing but minor quantities of the infused material reach this tube. The yield obtained in total by three tubes ranges between 87 and 100%. If results were based on the yield from one tube, which is the general procedure in intubation experiments, the aspirated volume would be subject to very great variations (6 to 63%).

TOTAL RECOVERY OF MARKERS Table III shows the total recovery of the markers applied as a percentage of the total quantity of infused material. This recovery is subject only to quite small variations and, on an average, the recovery of the three reference substances is uniform. The recovery of the infused fluid, however, ranges at a significantly higher level than recovery of each one of the three reference substances.

TABLE II

ASPIRATED VOLUMES FROM THE DIFFERENT TUBES AS PERCENTAGES OF THE INFUSED VOLUMES

No.	Percentage of Volume Infused			
	Tube A	Tube B	Tube C	Total
1	63	31	6	100
2	26	28	39	92
3	22	35	36	93
4	13	44	29	86
5	36	2	63	100
6	6	70	10	87
8	37	52	8	97
9	56	32	1	90
Mean	32.4	36.8	24.0	93.1

TABLE III

PERCENTAGE RECOVERY IN THE ASPIRATES OF INFUSED VOLUMES AND INFUSED REFERENCE SUBSTANCES

No.	Volume	⁵¹ Cr	Bromsulphthalein	Polyethylene-glycol
1	99.6	84.0	92.3	85.5
2	92.1	78.4	81.8	84.5
3	93.2	73.3	50.5	69.3
4	85.7	72.6	81.3	80.4
5	100.3	87.8	89.7	85.7
6	86.7	78.2	81.1	82.2
8	97.1	90.5	92.6	92.7
9	89.7	75.6	78.8	82.8
Mean ± S.D.	93.1 ± 5.6	80.1 ± 6.7	81.0 ± 13.5	82.9 ± 6.6

TABLE I

INFUSED AND ASPIRATED VOLUMES

No.	Animal	Body Weight (kg.)	Infused		Aspirated		Mechanical Suction
			ml.	ml./kg.	ml.	% of Infused	
1	Rabbit	6.0	116	19.3	115	100	—
2	Rabbit	6.0	197	32.8	181	92	—
3	Rabbit	4.0	163	40.8	152	93	+
4	Rabbit	5.5	369	67.1	316	86	+
5	Rabbit	5.3	193	36.4	194	100	—
6	Rabbit	4.9	268	54.7	232	87	+
8	Dog	10.0	366	36.6	355	97	—
9	Rabbit	3.5	269	76.9	241	90	—

The concentration of markers in all of the aspirates is proportionally of the same order of magnitude as the concentration of these substances in the infused solution. This, combined with the uniform recovery, makes it justifiable to conclude that the three substances are identically treated in the intestinal canal and hence they must be equally adequate for the purpose.

In all of the experiments an average of about 20% of the infused quantity of reference substance was seen to remain in the experimental animal.

Table IV shows the percentage recovery of infused ^{51}Cr in the proximal part of the intestine after completion of infusion and aspiration of samples. These values vary considerably from one experiment to another, but in all experiments, these values even included, a loss of about 10% of infused marker is seen, equalling the loss of infused volumes of fluid. The concentration of the various markers in the aspirates, in percentage of the concentration in the infused solution, is without systematic variation in aspirates from tubes A, B, and C. This may be ascribed to the absence of a net transport of water to and from the intestinal content between tubes A and C. Thus the comparable deficiencies as regards recovery of fluid and reference substance may be due to the phenomenon that about 10% of the infused volume passes through all three aspiration tubes and remains in the distal part of the intestine, which feature is indicated also in pilot experiments.

TABLE IV

PERCENTAGE RECOVERY OF INFUSED ^{51}Cr IN THE PROXIMAL PART OF THE INTESTINE

No.	Intestinal Content	Rinsing Water	Intestinal Wall
4	—	—	9
5	—	—	3
7	6	—	7
8	1	0.3	1
9	3	0.2	6

TOTAL INTESTINAL VOLUME Calculations of the total intestinal volume based on amounts of infused reference substance and its concentration in the aspirates show considerable variations. In most of the experiments calculations of volumes in periods immediately following onset of infusion and aspiration are just as satisfactory as calculations throughout the experiment. In some experiments calculations of volumes based on the initial aspirates are completely misleading, probably because of insufficient mixing of the markers and the intestinal content. For this reason Table V includes such calculations of volumes only as are based on aspirates obtained within the latter half of the

TABLE V
VOLUMES CALCULATED ON THE BASIS OF THE CONCENTRATIONS OF THE DIFFERENT MARKERS IN ASPIRATES FROM THE DIFFERENT TUBES AS PERCENTAGES OF THE INFUSED VOLUME

No.	Tube	^{51}Cr	Bromsulphthalein	Polyethylene-glycol
1	A	105	99	97
	B	120	113	120
	C	—	—	—
2	A	157	150	148
	B	143	130	130
	C	105	102	94
3	A	131	185	148
	B	131	189	138
	C	136	200	140
4	A	118	108	109
	B	119	105	106
	C	124	106	107
5	A	107	108	123
	B	—	—	—
	C	108	102	104
6	A	113	109	106
	B	105	103	101
	C	161	145	142
8	A	138	130	135
	B	116	105	94
	C	—	—	—
9	A	109	104	100
	B	115	107	97
	C	—	—	—
M $\pm$ S.D.	A	122.3 $\pm$ 18.3	124.1 $\pm$ 29.8	120.8 $\pm$ 20.9
M $\pm$ S.D.	B	121.3 $\pm$ 12.3	121.7 $\pm$ 31.1	112.3 $\pm$ 17.2
M $\pm$ S.D.	C	126.8 $\pm$ 22.3	131.0 $\pm$ 42.6	117.4 $\pm$ 22.1
M $\pm$ S.D.	all	123.1 $\pm$ 16.9	125.0 $\pm$ 32.1	117.0 $\pm$ 19.2

experimental period, viz., after a period of intestinal equilibration covering 25 to 30 minutes. In a few experiments the aspirates obtained through one or the other tube may have been very sparse and hence analysis of the markers was found to involve a considerable inaccuracy. Such calculations are highly misleading and the results have been rejected. Results from calculations of volumes are recorded in Table V. The calculated volumes are given as percentages of the volume simultaneously infused. Qualities of results seem to vary greatly, both from one experiment to the other and within the individual experiments; whether one or the other of the markers was used seems to be immaterial to the results; it is equally unimportant whether aspirates from one tube or the other form the basis of calculations. The standard deviation is considerable in all of the three substances applied here although it is of the most considerable magnitude in the case of bromsulphthalein.

AUTORADIOGRAPHIC DISTRIBUTION OF MARKERS Findings by autoradiographic examination are depicted in Fig. 2, illustrating the distribution of ^{51}Cr in representative duodenal cross sections from the proximal 20 cm. of the intestines of two rabbits. The distribution of the markers in the various slides is seen to vary greatly, being uniform only in a few.

FIG. 2. Distribution of infused ^{51}Cr in representative duodenal sections from two rabbits.

DISCUSSION

The results show beyond doubt that aspirates obtained through a single tube are anything but complete even in these experiments where the ratio of tube calibre to duodenal calibre is by far more favourable than is the case otherwise in experiments in man. The introduction of mechanical suction in tubes of the sizes applied here fails to augment the aspirate, a feature found also in studies on pancreatic function in man (Thaysen, Müllertz, Worning, and Bang, 1964). In addition the results show that the volume aspirated through a single tube is not applicable as an expression of the total intestinal content at the site of aspiration (Table II).

With a view to calculations of intestinal volumes, it must be stated that even in such model experiments as those presented here conditions in the small intestine may be difficult to evaluate because it is impossible to provide a standard of the transport of fluid through the intestinal wall. The striking similarity of concentrations of the three markers in aspirates obtained through the three tubes in the individual experiments suggests strongly that the net transport of fluid has been of minor importance in the intestinal sample under examination, and hence sites of location of the aspiration tube within an area of 5 to 10 cm. seem to have no influence on the results.

An absorption of the markers applied might represent one source of error although it can almost be considered as non-existent, since polyethyleneglycol with the molecular weight applied here (about 4000) in several experiments has been found not to be absorbable (Shaffer and Critchfield, 1947; Sperber, and Eckman, 1953; Aberdeen *et al.*, 1960; and Jacobson *et al.*, 1963). The same applies to chromium compounds (Ebaugh, Clemens, Rodnan, and Peterson, 1958; and Waldmann, 1961). In some experiments even bromsulphthalein has been found

unabsorbable (Owen, 1951) compared with the experience of other investigators who found a faint absorption (Lorber and Shay, 1952; Lorber, Oppenheimer, Shay, Lynch, and Siplet, 1953; and Ritter, 1960). Such absorption could hardly be of any importance in our experiments on account of the short intestinal segments and brief intervals used. This is supported by the fact that the recovery of bromsulphthalein and the calculation of volumes on the basis of bromsulphthalein are of the same order as the corresponding values calculated on the basis of ^{51}Cr and polyethyleneglycol (Table III).

Few studies are available which are of the same type as the one discussed here. Jacobson *et al.* (1963) perfused the entire intestinal canal in rats and dogs and found fair accordance between total volumes aspirated and volumes calculated on the basis of the markers polyethyleneglycol and bromsulphthalein. In these experiments, however, very large volumes were perfused, even up to 300 ml./kg./hour. Studies on closed loops in which the infused volumes were much smaller showed that calculations of volumes based on concentrations of markers would give values which were up to 50% too high. The experimental arrangements in the studies of Jacobson *et al.* (1963) and our own are not fully identical, the latter authors concerning themselves with the total volume infused, the former with the volume which may be evacuated from the intestine. The fluid content in the investigated intestinal segments cannot be precisely estimated in any of these experiments. The difference between this volume and the quantity infused in total seems to be of minor importance in our experiments where, as already mentioned, no measurable net transport of fluid through the intestinal wall is demonstrable. Complete elimination of a fluid transport, however, is not possible. Taking the nature of the infused solutions into account, it is most likely that such transport is

directed out of the intestine (absorption); if so, the error in the estimated volumes recorded in Table V is even more marked.

It is impossible to decide whether or not an essential augmentation of the infusion volume might improve these results. Our figures fail to show whether changes within the area 20 to 70 ml./kg./hour have any influence on the accuracy with which the intestinal volume may be calculated, but it can hardly be precluded that an essential increase, *e.g.*, up to 300 ml./kg./hour, might provide different results. Infusions of this order, however, must not be considered physiological since they surpass by far the normal transport of fluid in the intestinal canal and consequently such results should be considered with a certain reserve.

In an isolated and unpublished experiment we have used a very small infusion volume but here the calculation of volume was completely misleading.

In human experiments Fordtran *et al.* (1961) found that the ratio of concentrations of the three markers applied (polyethyleneglycol, bromsulphthalein, and indo-cyanide-green) in aspirates from the intestines equalled the ratio of concentrations in the infusion solution; moreover during constant infusion the concentration of polyethyleneglycol was found to fluctuate only by $\pm 10\%$ in samples of intestinal content aspirated at brief intervals, for which reason the method seems to be adequate for a valuation of the water kinetics in the intestine. By analogy with this we have found the relative concentration of markers to be highly constant which shows merely that the reaction of the intestinal canal to the markers applied is uniform. The demonstrated fluctuations in the concentration of polyethyleneglycol concentration, *viz.*, of $\pm 10\%$, involve *per se* an error in calculations of volumes, amounting to 25%, which is in fair accordance with the average error found by us (Table V).

Figures in Table V indicate that the three markers are equally suitable, or not suitable, for determinations of the intestinal content. Because of the higher standard deviation in determinations based on bromsulphthalein, it is not unreasonable to consider this substance the one least suitable. The most satisfactory results involving errors below 10% must be considered fully applicable in the clinical routine, but in the individual experiments it can hardly be decided whether errors of 10 or 50% are involved in the calculations. To this should be added that the method is more readily applicable under the conditions discussed here than is otherwise the case in a normally functioning intestinal canal, since possibilities of a uniform mixture of markers and intestinal content, quite naturally, are most

perfect if infusion continues uninterruptedly rather than interruptedly, as is the case in the normal excretion of digestive secretion.

Our results show that a homogenous mixture of markers and intestinal content is hardly obtainable by the method discussed here (Fig. 2). No doubt this, in connexion with the varying adhesion of markers to intestinal mucosa (Table IV), represents the explanation of the poor parallelism of actual and calculated volumes. Proportions of the three markers being equal, despite their considerable chemical heterogeneity, it is hardly reasonable to believe that more satisfactory results might be obtained by the introduction of other reference substances.

According to these results estimation of the individual total secretion of organ-specific substances, for instance bile acids, pancreatic enzymes, and so on, is not possible by the applied technique. The use of meals containing markers for estimating gastrointestinal motility (Aberdeen *et al.*, 1960; Mehnert and Förster, 1963), physiological dilution of the meals in the intestine (Borgström *et al.*, 1957), and net transport of water and various water-soluble substances in the meal (Borgström *et al.*, 1957; Dahlqvist and Borgström, 1961; and Mehnert and Förster, 1963) is in fact based on the assumption that admixture of the intestinal content at any moment is ideal, the reference substance then being distributed homogeneously in the intestinal content. As shown here this is not necessarily the case, and the results obtained must be taken with some caution.

Investigation of the net transport of water and water-soluble substances in different parts of the intestine by infusing solutions containing both marker and test substances in homogeneous solution (Clodi *et al.*, 1963; Fordtran *et al.*, 1961, and Fordtran, Soergel, and Ingelfinger, 1962; Schedl and Clifton, 1961; 1963) is only to a minor degree affected by the results published here. In these cases the admixture problems are not vital, but the adhesion of the markers to the intestinal wall may to an uncertain degree affect the results, depending on whether the marker is concentrated at the peripheral part of the intestine parallel with the net absorption of water or whether the adhesion of the substance to the intestinal wall is a more specific reaction. This problem is not solved by the results published here, but the finding that the recovery of markers is significantly lower than the recovery of water (Table III) points against a specific adhesion. This is, to a minor degree, supported by the observation that only limited amounts of the marker adhering to the mucosa are found in the rinsing water (Table IV).

SUMMARY

The possibilities are examined of determining the total content of fluid in the intestinal lumen by means of aspiration of the intestinal content through tubes inserted at random combined with infusion of non-absorbable (reference) substances.

Two tubes were inserted into the duodenum in rabbits and in one dog; through one tube the reference substances polyethyleneglycol 4000, bromsulphthalein, and $\text{Na}_2^{51}\text{CrO}_4$ (^{51}Cr), dissolved in physiological saline, were infused and through the other a solution of isotonic glucose. The intestinal content was aspirated through three tubes, inserted retrograde, 10 to 25 cm. distally to the site of infusion.

Aspiration through a single intestinal tube is seen to vary greatly and to be anything but complete. There is no interrelation between the volumes aspirated and total content in the intestine at the site of aspiration.

Recovery of the reference substances applied is identical although at a significantly lower level than recovery of the infused volume. Calculation of the total intestinal content, based on concentrations of the markers in the aspirates, gives values which are up to 50% too high.

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