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Determination of gastric-emptying profiles in the rat: influence of cil structure and volume

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Summary

A simple non-invasive technique was developed for the determination of the gastric-emptying rate of oils in rats, employing a gamma camera and ^{99m}Tc-sulphur colloid as the oil phase marker. Using this method the gastric emptying of 3 oils, arachis oil, Miglyol 812 and liquid paraffin, was investigated. It was shown that both the oil volume and chemical structure altered the rate of gastric emptying.

Introduction

The enhanced oral absorption of several drugs in the presence of oils has been attributed to the inhibition of gastrointestinal motility (Jamali and Axelson, 1977; Chakrabarti and Belpaire, 1978). As oils of varying chain-length and chemical saturation have different effects on gastric emptying and intestinal transit (Hunt and Knox, 1968; Cooperman and Cook, 1976) the chemical structure of an oily vehicle may be critical in determining the extent of drug absorption. The oral absorption of DDT has been shown to be altered in the presence of different oils (Palin et al., 1980) and this investigation was undertaken to determine whether such differences were due in part to a variation in the gastric-emptying rate of the formulations.

It has been suggested that the oil volume used in many animal studies on drug absorption is too large for comparison with experiments conducted in man (Yamahira

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et al., 1978). Reduction of the oil volume in which DDT was administered to rats from 1 ml to 30 μl, was shown to alter the differences in DDT absorption from the different oils (Palin, 1981). 30 μl was selected as being equivalent on a body weight basis to a 10 ml dose in a 70 kg man. The effect of this reduction in oil volume on the gastric-emptying profile of each oil was investigated to determine whether this factor had contributed to the changes in DDT absorption.

The methods described in the literature for the determination of gastric-emptying rates in rats involve dosing animals with a non-absorbable marker, killing each animal after a given time period and then determining the distribution of the marker throughout the gastrointestinal tract (Aberdeen et al., 1960; Brodie, 1966; Varga, 1976; Hinder et al., 1976). This procedure does not allow continuous assessment of gastric emptying and accurate determination of the distribution of the marker is often difficult. Imaging of technetium-99m (^{99m}Tc) labelled markers using a gamma camera and associated computer, is used for gastric-emptying studies in man. The same basic technique was adapted and applied to the rat to determine the gastric-emptying rate of the 3 oils (arachis oil, Miglyol 812 and liquid paraffin) used in the DDT study. Prior to commencing these studies, radiological measurements were conducted in rats dosed with barium sulphate suspension to determine the external view giving the optimum resolution of the stomach and intestines within the rat.

Materials and methods

Materials

The oils investigated were arachis oil BP (Evans, Liverpool), Miglyol 812 (Dynamit Nobel, Slough, Berks.) and liquid paraffin BP (Shell, London). Oleic acid was obtained from B.D.H. Chemicals, Poole. [^{123}I]Oleic acid and [^{123}I]arachis oil were prepared according to the method of Lubran and Pearson (1958) in the Radiopharmacy Department, General Hospital, Nottingham. [^{99m}Tc]Sulphur colloid was prepared using a ^{99}Mo–^{99m}Tc generator (Amersham International). The prepared colloid had a particle size distribution of 72% equal to 400–600 nm, 18% less than 100 nm and 7% greater than 600 nm.

X-Ray investigation

Three male Wistar rats, weight range 190–210 g, were dosed orally with 1 ml barium sulphate suspension. Dorsal, ventral and lateral radiographs of the rats in perspex restraining cages were taken at 1, 2 and 3 h after dosing.

Radionuclide imaging study

Each rat was fasted overnight, dosed orally with oil plus radionuclide marker and immediately placed in a perspex restraining cage at the camera face. It was possible to image 3 rats simultaneously by use of a small rack such that the rats were positioned one above the other. For the large oil volume study 90 × one-minute scintigrams were taken, whereas for the small oil volume study 72 × 75-second scintigrams were taken. The data from the studies were stored on magnetic tape for analysis.

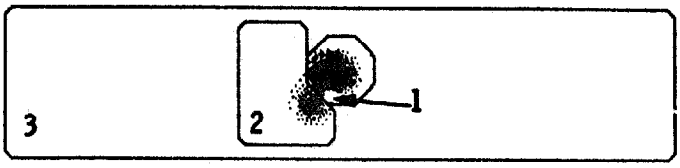

Fig. 1. Regions of interest constructed on scintigrams of rats. 1 = stomach region; 2 = intestinal region; 3 = whole body region.

A preliminary investigation was performed to determine the most suitable oil phase marker available. [^{123}I]Arachis oil, [^{123}I]oleic acid and [^{99m}Tc]sulphur colloid were investigated. [^{99m}Tc]Sulphur colloid was found to be the most suitable marker (see Discussion) and was used in all further investigations.

Formulation for large oil volume study was 0.5 ml (500 μCi) [^{99m}Tc]sulphur colloid + 1.0 ml oil (arachis oil, Miglyol 812 or liquid paraffin) or 1 ml water.

Formulation for the small oil volume study was 15 μl (150 μCi) [^{99m}Tc]sulphur colloid – 30 μl oil (arachis oil, Miglyol 812 or liquid paraffin) or 30 μl water.

Analysis of radionuclide imaging study

Using the data stored in the computer regions of interest (ROI) of a standard size were constructed around: (1) the stomach; (2) the intestine; and (3) the whole body (see Fig. 1) and the counts within the regions computed. The counts for the stomach region and the intestinal region were standardized by reference to the whole body counts to allow for variation in the radioactivity of the dose administered. Assuming that the total dose is in the stomach on the first image, the ratio stomach: whole body counts at t = 0 will be 1 and the ratio intestinal: whole body counts will be zero. Graphs were constructed of the decrease in the stomach: whole body counts ratio with time and the increase in the intestinal: whole body counts ratio with time.

Results

Radiographs of rats orally dosed with barium sulphate suspension showed that the right lateral view gave the best single plane discrimination of the areas of interest; the stomach and intestinal regions. This view was therefore used in all subsequent scintigraphic studies.

Fig. 2 shows an overlay produced from a lateral radiograph and a lateral scintigram taken immediately after dosing a rat with [^{99m}Tc]sulphur colloid. It can be seen that initially the activity was in the stomach and the mouth regions. After a period of time the activity moved out of the stomach region into the area corresponding to the intestines. By computer analysis it was possible to follow the decrease in stomach counts and the concomitant increase in intestinal counts and thus monitor gastric emptying.

The high activity present in the stomach region prevented the accurate determination of the movement of activity into the intestinal region. However, the rise in

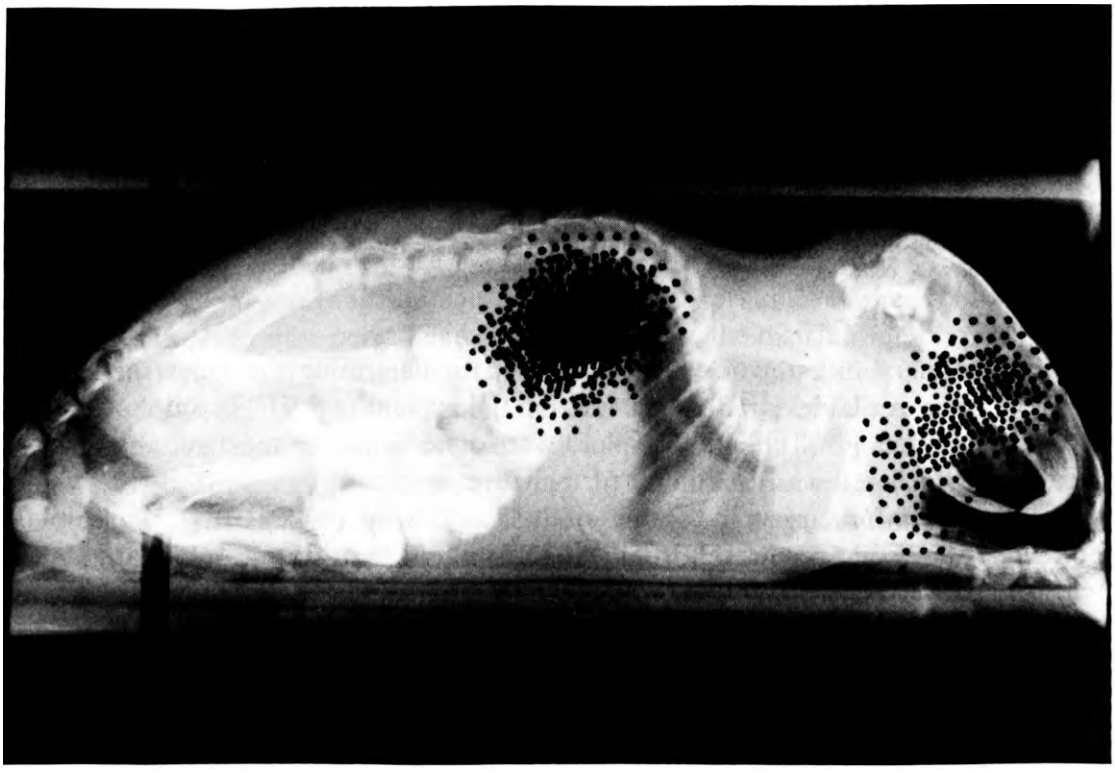

Fig. 2. Composite radiograph and scintigram of a rat following oral administration of 1 ml barium meal and 1 ml [^{99m}Tc]sulphur colloid, respectively.

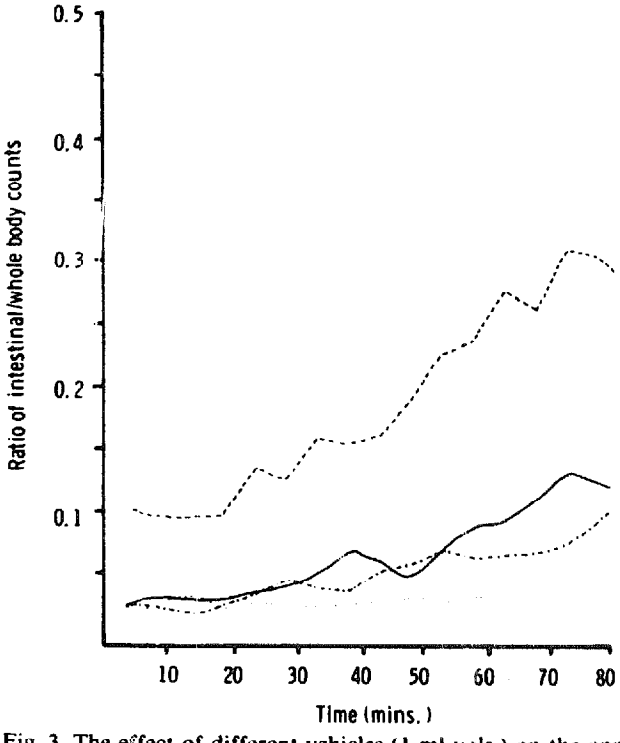

Fig. 3. The effect of different vehicles (1 ml vols) on the appearance of [^{99m}Tc]sulphur colloid in the rat intestine. Each value is the mean for 6 animals. ·····, arachis oil; -·-·-, Miglyol 812; ———, liquid paraffin; — — —, water.

TABLE I

EMPTYING INDEX FOR THE DIFFERENT FORMULATIONS (MEAN ± S.D.)

Oil	Marker	Oil volume	No. per group	Emptying index
Arachis oil	[^{123}I]arachis oil	1.2 ml	3	0.051 ± 0.001
	[^{123}I]oleic acid	1.2 ml	3	0.035 ± 0.007
	[^{99m}Tc]S-colloid	1.0 ml	6	0.032 ± 0.019
		30 μl	6	0.063 ± 0.028
Miglyol 812	[^{123}I]oleic acid	1.2 ml	3	0.021 ± 0.007
	[^{99m}Tc]S-colloid	1.0 ml	6	0.081 ± 0.067
		30 μl	6	0.223 ± 0.128
Liquid paraffin	[^{122}I]oleic acid	1.2 ml	3	0.124 ± 0.059
	[^{99m}Tc]S-colloid	1.0 ml	6	0.119 ± 0.099
		30 μl	6	0.176 ± 0.065
Oleic acid	[^{123}I]oleic acid	1.2 ml	3	0.061 ± 0.034
Water	[^{99m}Tc]S-colloid	1.0 ml	6	0.214 ± 0.108
		30 μl	6	0.207 ± 0.095

Statistical comparisons between treatment groups were made using Mann-Whitney U-test; emptying index for 30 μl arachis oil significantly lower than for 30 μl Miglyol 812, liquid paraffin or water ($P < 0.05$), for 1 ml water significantly higher than for 1 ml oils ($P < 0.05$), for Miglyol 812 (1 ml) significantly lower in the presence of [^{123}I]oleic acid than [^{99m}Tc]sulphur colloid ([^{99m}Tc]S-colloid, $P < 0.05$).

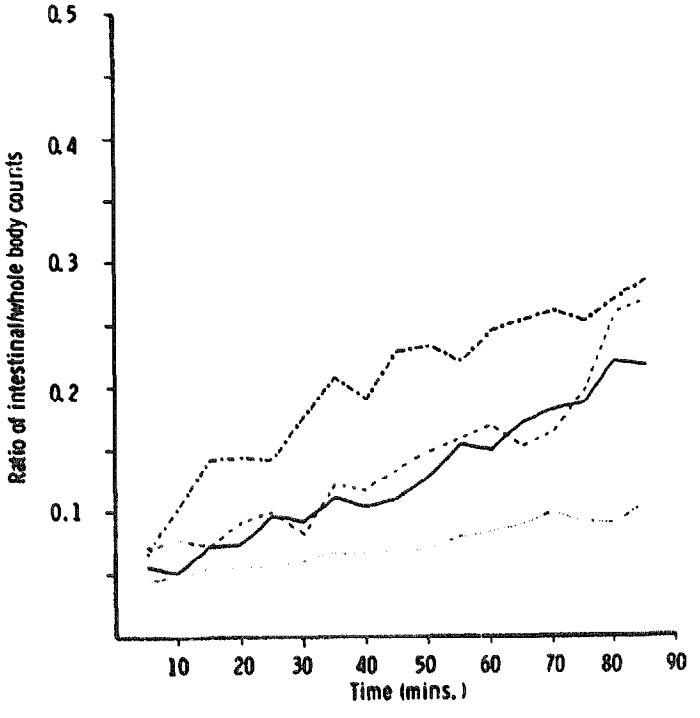

Fig. 4. The effect of different vehicles (30 μl vols.) on the appearance of [^{99m}Tc]sulphur colloid in the rat intestine. Each value is the mean for 6 animals. ·····, arachis oil; ·–·–·, Miglyol 812; ———, liquid paraffin; — — —, water.

intestinal activity as gastric emptying occurred was clearly illustrated by plotting the intestinal:whole body counts ratio against time (see Fig. 3). Gastric emptying was therefore determined in terms of the activity entering the intestinal region.

The gastric-emptying rate of each formulation was calculated as the difference in the intestinal:whole body counts ratio at t = 5 min and at t = 80 min and referred to as the emptying index (see Table 1). Statistical comparison of the different formulations was made using the Mann-Whitney U-test. The emptying index for the 1 ml volumes of liquid showed there was no difference between the emptying of the 3 oils although each emptied more slowly than water ($P < 0.05$, see Fig. 3). The mean values for the emptying index for the oils had the rank order: liquid paraffin > Miglyol 812 > arachis oil. With the smaller volumes of oil (30 μl) the emptying index was lower ($P < 0.05$) following administration in arachis oil compared to water, liquid paraffin and Miglyol 812 (see Fig. 4), but there was no difference between water, liquid paraffin and Miglyol 812.

Discussion

Preliminary studies showed that gastric emptying in the rat could be followed by using the gamma camera after dosing with a suitable γ-emitting radionuclide. An ideal radionuclide marker for the oil phase is one which remains associated with the oil phase, exerts no independent physiological effect on gastric emptying and is not absorbed.

The first two criteria may be satisfied by the attachment of a radionuclide, such as ^{123}I, to the oil molecule. However, iodination could only be achieved with arachis oil as the other two oils are saturated. [^{123}I]Arachis oil was administered to rats and the results used for comparison with the other formulations.

The potential of spiking the oils with a small quantity of a second miscible oil was investigated using [^{123}I]oleic acid (0.2 ml). However, the gastric emptying of Miglyol 812 was determined to be slower in the presence of [^{123}I]oleic acid than [^{99m}Tc]sulphur colloid (see Table 1). Oleic acid is a long-chain unsaturated fatty acid and therefore may be expected to inhibit gastric emptying (Hunt and Knox, 1968) whereas [^{99m}Tc]sulphur colloid is physiologically inert. It would seem possible that the presence of a marker lipid will influence the gastric emptying of the oil under investigation and is therefore unsuitable for the present study.

Investigations using [^{99m}Tc]sulphur colloid suggested that this would be the most suitable marker, as it is unlikely to exert any independent effect on gastrointestinal transit and is not absorbed. However, it is immiscible with oil being a solid of very small particle size and it was possible that the gastric emptying of the colloid and not that of the oil was being monitored. Comparison of the gastric-emptying profiles of [^{99m}Tc]sulphur colloid administered in the different oils suggests that the emptying of the colloid is altered by the presence of the oils. Moreover, similar results for the rank order effect on gastric emptying of the 3 oils were obtained using the miscible oil, [^{123}I]oleic acid, as a marker. It was concluded that the effect of each oil on gastric emptying could be determined from the changes in the gastric emptying of

[^{99m}Tc]sulphur colloid in the presence of the different oils.

No statistically significant differences were found between the emptying index for 1 ml volumes of the 3 oils, although the mean values had a rank order of: liquid paraffin > Miglyol 812 > arachis oil. It was noticed that in most of the rats dosed with 1 ml volumes of liquid paraffin the [^{99m}Tc]sulphur colloid emptied from the stomach faster than in the presence of 1 ml volumes of arachis oil. However, due to large intra-group variation and analytical errors (see below) a statistically significant ($P < 0.05$) effect was not observed. Liquid paraffin is not digestible and therefore will not stimulate the neurohumoral inhibitory responses produced by digestible oils such as Miglyol 812 and arachis oil. However, the gastric-emptying rate of liquids is reduced with increasing viscosity (Yamahira et al., 1978; Levy and Jusko, 1965), and it is possible that this factor reduced the gastric emptying of liquid paraffin compared with water. More rapid gastric emptying of liquid paraffin compared to Miglyol 812 and arachis oil may account for the earlier peak plasma concentration and lower total absorption of DDT from this oil (Palin et al., 1980).

At low oil volumes the gastric emptying of [^{99m}Tc]sulphur colloid in the presence of water, liquid paraffin and Miglyol 812 was shown not to differ, but was slower in the presence of arachis oil. Arachis oil contains long-chain saturated and unsaturated fatty acids which have a greater inhibitory effect on gastric emptying than the medium-chain saturated fatty acids of Miglyol 812 (Hunt and Knox, 1968; Cooperman and Cook, 1976). This may account for the lower emptying index for 30 μl arachis oil compared to Miglyol 812. At the greater oil volume this difference between the two oils may not have been seen due to the inhibitory effects of increased viscosity. The viscosity of the chyme is unlikely to be increased by the administration of 30 μl volumes of oil, and this may explain the similarity in the emptying index for liquid paraffin and water.

Comparison of the gastric-emptying profiles for 1 ml and 30 μl of each liquid suggests that with the larger oil volumes there is a delay in gastric emptying and that the rate of emptying is reduced. This is consistent with the literature reports on the effect of increasing the administered volume and stomach distention on gastric emptying (Aberdeen et al., 1960). The volume of oil administered may therefore be critical in determining gastrointestinal drug absorption, influencing both the time lag prior to gastric emptying and the rate of movement from the stomach into the intestine. In addition, for liquid paraffin and Miglyol 812 it can be seen that for the large oil volume gastric emptying was slower than for water whereas for the small oil volume there was no difference. This suggests that the volume administered may determine whether an oil will alter drug absorption compared to an aqueous formulation.

Several problems were encountered with the computer analysis of the radionuclide imaging study which gave rise to errors in the counts recorded and may have attributed to the large intra-group variation noted through the study. The analysis of the data did not account for activity moving through the intestinal region lying in the same horizontal plane as the stomach. In addition animal movement during imaging could not be compensated for and inaccurate activity within the regions may have been recorded. Errors also resulted from the incomplete resolution by the

camera of the anatomical structure being examined.

Despite these difficulties this method provides a relatively simple, non-invasive technique for the determination of the complete gastric-emptying profile in the rat. The results have shown that both the oil volume and the nature of the oil are important in determining the gastric-emptying rate. The choice of oil and the volume administered is therefore critical when determining gastrointestinal absorption of a drug from an oily vehicle.

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