THE ASSIMILATION AND ELIMINATION OF IRON ADMINISTERED ORALLY TO THE DOG AS FERROUS ISOASCORBATE AND FERROUS AMMONIUM SULPHATE

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Radioisotopically labelled iron as ferrous ammonium sulphate or ferrous isoascorbate has been orally administered to dogs. The movement and excretion of the iron has been followed by examination of plasma, faeces and urine samples. There is a maximum in the plasma iron at approximately 1 hour after dosing. Between 2 and 15 per cent of the iron is not recovered in the faeces and no trace is found in the urine. The maximum plasma iron level attained appears, from the limited data obtained, to be linearly related to the amount administered. There is no significant difference between the two forms used. A feature of the work is the use of a simple method to assay the faecal activity, which is described.

The absorption, distribution and fate of iron after oral administration have been frequently studied by orthodox chemical methods. When compounds are compared in this way, a complete distribution study is always tedious and recoveries are poor. In particular this applies to the recovery of iron from faeces. These difficulties seem to have been minimised by the use of tracer methods.

Experimental

Materials

Ferrous isoascorbate. A preparation of the material was kindly supplied by Mr. E. H. Searle of Product Research Division, Beecham Research Laboratories, together with purified isoascorbic acid, which also was used by the Radio Chemical Centre at Amersham to prepare the labelled material. The final material was freeze dried and sealed in ampoules conveniently containing the equivalent of 20 to 25 mg. of ferrous iron of total activity approximately 15 μc.

Ferrous ammonium sulphate. This material used was analytical reagent grade satisfying the specification in the British Pharmacopoeia 1958. The labelled material was supplied to specification in 10 ml. rubber capped multi-dose bottles by the Radio Chemical Centre at Amersham, 2 ml. containing the equivalent of 20–25 mg. of ferrous iron of total activity approximately 15 μc.

Animals. Two mature and healthy bitches designated M and N were used.

M was a greyhound of 8 years and weight 18 kg. N was a mongrel of 3 years and weight 17·5 kg. Both had been trained to take a stomach tube, and were accustomed to the blood sampling technique. Pens and paws of the animals were monitored regularly for contamination.

Dose and administration of dose. Doses varying from approximately 20 mg. to 150 mg. of ferrous iron were given. The required quantity of material was dispersed in distilled water containing in 100 ml. of water 0·6 ml. of N HCl. The latter was used so that the pH of the dose approximated to that of gastric juice. The dose was given by a stomach tube to the fasting animal. The following precautions were taken to eliminate loss of dose on the walls of the tube and glassware, and to provide a control for assay purposes.

Twice the quantity of labelled iron compound required was dispersed in 200 ml. of the acidified distilled water. The solution was carefully divided into two equal parts. One part was retained as a control. The stomach tube was prewashed by pouring 100 ml. of unlabelled iron compound of concentration similar to that to be administered followed by two 50 ml. portions of distilled water. After allowing approximately 30 seconds for drainage, the tube was inserted in the animal. The solution of labelled compound was then poured down the prewashed stomach tube into the animal, and the vessel washed twice with 50 ml. distilled water, the washings being poured down as well. After allowing approximately 30 seconds for drainage, the tube was withdrawn.

Samples

Faeces and urine. Samples of faeces were collected in wide neck 16 oz. screw topped bottles as soon as they were passed and the time noted. This generally occurred once in every 24 hours. The samples were stored in a refrigerator at 0–5°. Urine samples were taken by catheter in the early part of the work, but these showed so little activity that urine examination was discontinued.

Blood. Samples of 25 ml. to 30 ml. in volume were withdrawn with an hypodermic syringe from leg veins. The blood was centrifuged before clotting to obtain the plasma which was then stored in well stoppered bottles in a refrigerator until assayed.

Activity Assay

The plasma and faeces differed much in activity. This, and their different compositions, made assay by different methods desirable. The usual corrections for background and counter paralysis time were made.

Faeces. A method of assay using the 16 oz. storage bottle was devised to eliminate transfer from the bottles.

The control specimen of labelled iron compound was transferred to a 16 oz. bottle and made up to a constant volume with distilled water. Bottles of faeces were similarly made up and shaken well to disperse the material. The bottles were centrally placed in a counting chamber containing four G10 γ-type counters connected in parallel to a ratemeter. A counting rate reading was taken and the bottle rotated through about 120° when a further reading was made. This process was repeated, three readings in all being taken. These were averaged. The control was assayed before and after the examination of a series of samples and its average activity was used for calculation. This procedure eliminated the

effect of bottle irregularities and gave statistically consistent data on the same sample, when examined in several bottles. Calculations showed that the β-activity of the ^{59}Fe was effectively stopped by the glass.

The counters, if exposed to light, gave spurious results. This effect lasted for over a minute after exposure. The counting chamber was,

TABLE I

SCHEME OF EXPERIMENTAL WORK

Dog	Expt.	Preparation	Dose size as mg. Fe^{++}	Date
M	1	Ferrous ammonium sulphate	22·5	19.8.58
M	2	Ferrous ammonium sulphate	22·5	26.8.58
M	3	Ferrous isoascorbate	22·5	2.9.58
N	4	Ferrous isoascorbate	155·0	15.10.58
N	5	Ferrous isoascorbate	128·0	5.11.58
N	6	Ferrous ammonium sulphate	22·5	10.12.58

therefore, made light-tight and time was allowed for light effects to disappear between the introduction of the sample and the measurement of its activity.

Plasma. The low activity of the samples made it necessary to use a scintillation counter. 10 ml. of plasma was accurately measured into a planchette, which was submitted to the counter. A suitable aliquot of the control sample was diluted so that 10 ml. gave approximately similar activity. The plasma activity was calculated using the dilution factor.

Equipment. A ratemeter was used for the examination of faeces. The scintillation counting equipment consisted of a photomultiplier, an

TABLE II

AMOUNT OF IRON (AS A PERCENTAGE OF THE TOTAL DOSE) NOT RECOVERED IN THE FAECES

Dog	Expt.	Not recovered per cent
M	1	2·56
M	2	2·84
M	3	2·12
N	4	13·60
N	5	10·90
N	6	16·50

E.H.T. unit of high stability, a preamplifier, a linear amplifier with provision for variable discriminator bias voltage and a scalar. Both sets of equipment were standard lines of good quality.

RESULTS

The quantity of radioactive material available for the preliminary experiments was sufficient for six experiments. The scheme of work is shown in Table I.

Excretion of ^{59}Fe in the Faeces

After administration of the dose, faeces were collected regularly; collection and assay was terminated when activity was no longer detected.

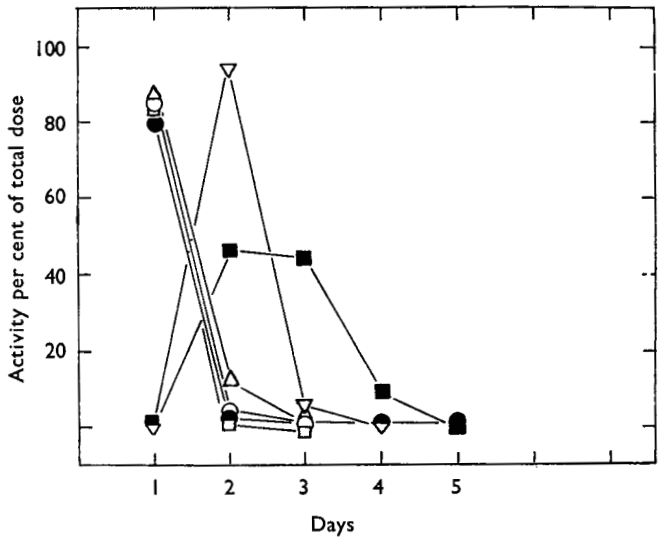

FIG. 1. Daily excretion of iron (as per cent of the total dose) in faeces.
△ Expt. 1. 22·5 mg. Fe^{++} as ferrous ammonium sulphate.
▽ Expt. 2. 22·5 mg. Fe^{++} as ferrous ammonium sulphate.
■ Expt. 3. 22·5 mg. Fe^{++} as ferrous isoascorbate.
● Expt. 4. 155 mg. Fe^{++} as ferrous isoascorbate.
○ Expt. 5. 128 mg. Fe^{++} as ferrous isoascorbate.
□ Expt. 6. 22·5 mg. Fe^{++} as ferrous ammonium sulphate.

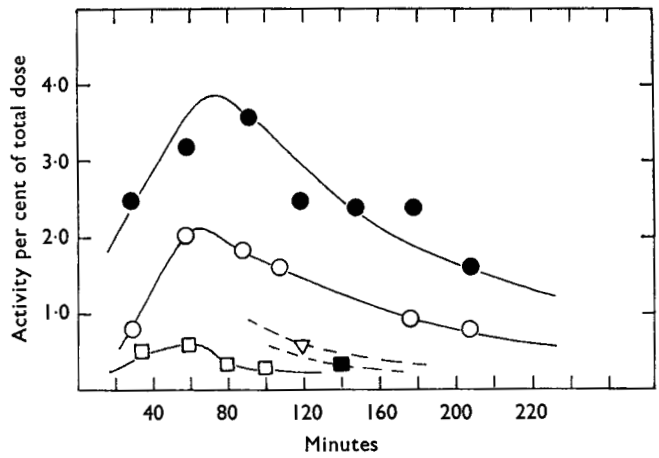

FIG. 2. Variation in plasma iron level (as per cent of the total dose) with time.
▽ Expt. 2. 22·5 mg. Fe^{++} as ferrous ammonium sulphate.
■ Expt. 3. 22·5 mg. Fe^{++} as ferrous isoascorbate.
● Expt. 4. 155 mg. Fe^{++} as ferrous isoascorbate.
○ Expt. 5. 128 mg. Fe^{++} as ferrous isoascorbate.
□ Expt. 6. 22·5 mg. Fe^{++} as ferrous ammonium sulphate.

The samples have been grouped into 24 hour periods to facilitate comparison.

It was originally intended to use one dog only for the work. Dog M, unfortunately, succumbed to an infection and died; the second animal was then introduced. Nevertheless, some of the data obtained on each animal can be compared. In particular experiments 1, 2 and 6 involve similar quantities of iron.

That such comparisons are limited can be seen from Table II, in which the percentage unrecovered is quoted.

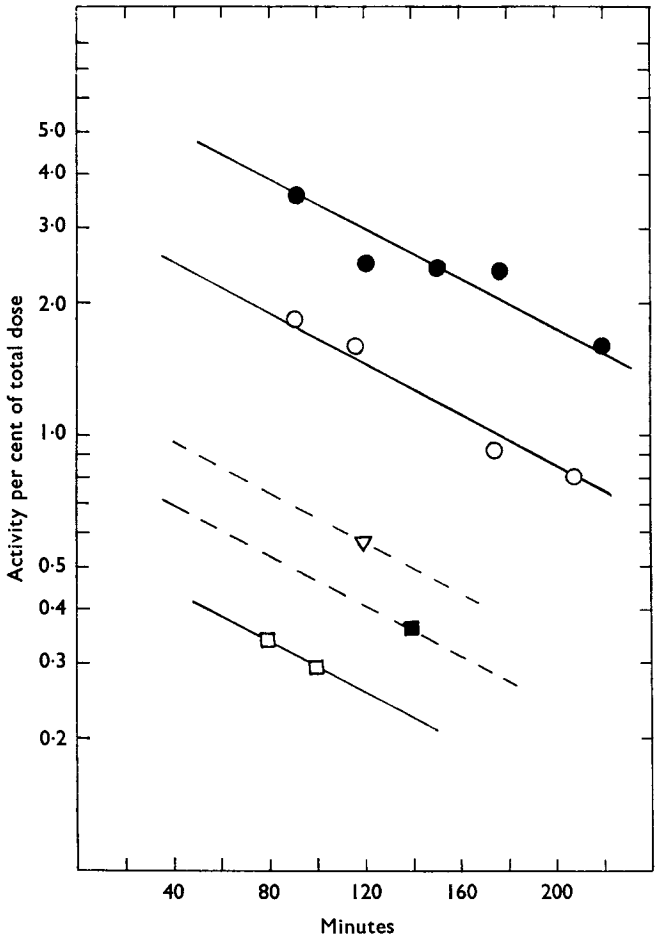

FIG. 3. Decrease in plasma iron level (as a per cent of the total dose) with time from one hour after dosing.

▽ Expt. 2. 22·5 mg. Fe^{++} as ferrous ammonium sulphate.
■ Expt. 3. 22·5 mg. Fe^{++} as ferrous isoascorbate.
● Expt. 4. 155 mg. Fe^{++} as ferrous isoascorbate.
○ Expt. 5. 128 mg. Fe^{++} as ferrous isoascorbate.
□ Expt. 6. 22·5 mg. Fe^{++} as ferrous ammonium sulphate.

Dog N clearly excretes less of the iron administered than dog M. Moreover, from Figure 1 its excretion pattern appears more regular. Here the relative amount of iron excreted is shown as a function of time.

Plasma ^{59}Fe Levels

Initially measurements of plasma activity were made only on samples obtained 2 and 24 hours after administration of the dose. Later as the experimental procedure was developed samples were taken at approximately 30 minute intervals for 4 hours. The results of these measurements are shown in Figure 2. The levels are given as a percentage of the total

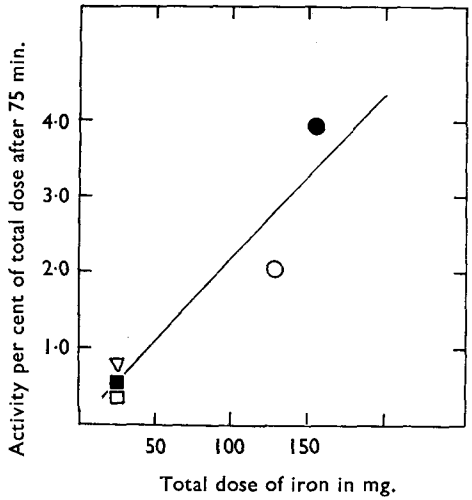

FIG. 4. Variation with dose size of the plasma iron level (as a per cent of the total dose) estimated to exist 75 minutes after dosing.

▽ Expt. 2. 22·5 mg. Fe^{++} as ferrous ammonium sulphate.
■ Expt. 3. 22·5 mg. Fe^{++} as ferrous isoascorbate.
● Expt. 4. 155 mg. Fe^{++} as ferrous isoascorbate.
○ Expt. 5. 128 mg. Fe^{++} as ferrous isoascorbate.
□ Expt. 6. 22·5 mg. Fe^{++} as ferrous ammonium sulphate.

dose administered. This is calculated from the measured activity of the 10 ml. plasma sample by assuming that each animal has a total plasma volume of 800 ml.

Despite the variation in faecal iron recovery the data in Figure 2 appears consistent in that the plasma levels are approximately proportional to the quantity of iron administered. The curves are generally uniform in shape and the few levels obtained on dog M fit in with those from dog N.

The maximum level is reached approximately 75 minutes after administration.

The consistency in plasma level patterns is also supported by the results obtained when the logarithm of the iron level is plotted against time. Figure 3 shows that the relation is linear, and with dog N, where comparison is possible, the lines have similar slopes. Only the plasma levels of samples taken after 75 minutes are given in Figure 4. If the lines are extrapolated to 75 minutes, the plasma level obtained approximates to the

maximum value and this can be used to compare the ease with which a particular preparation is assimilated. The data, so obtained, can be augmented by assuming a similar relationship for the plasma levels obtained earlier in dog M. This assumption is questionable but not unreasonable, particularly when the results of experiments 1, 2 and 6 are compared. Here, identical doses were given and the resulting levels are similar when account is taken of the time of sampling.

The plasma levels at 75 minutes, obtained by this extrapolation procedure, are plotted against the size of the dose in Figure 4.

Qualitatively, bearing in mind the limited amount of data available, there appears to be a linear relationship between the quantity of iron administered and the maximum plasma iron obtained. This relationship does not appear to be influenced by the form in which the iron is presented.

Discussion

The results of some investigations suggest that only a limited section of the intestinal tract in dog[1] facilitates the uptake of iron. Granick[2] states that the most active region is just below the pyloric sphincter. Interpretation of the maximum shown to occur in many plasma level-time curves can of course lead to a similar conclusion. In this work the maximum occurs approximately 1 hour after dosing. Thus, the upper twentieth of the tract appears most active which is consistent with the findings of others.

The animals were fasted for some hours before and after dosing. Consequently the only iron passing through the active region is that from the dose together with a small amount excreted in the bile. However, since the latter is about 1 mg. in 24 hours,[3] for man, it may reasonably be neglected for doses of 22 mg. and over in this present work on dogs.

Thus, after allowing for scatter, the increase in plasma iron appears directly proportional to the dose in the range studied. Whilst it is unwise[5] to accept without question the assumption that the increase in plasma iron is directly related to the iron converted into haemoglobin, many[3,4] consider the increase to be a good measure of this. If this is accepted, then the present results are probably consistent with the contention[3,4,6] that iron uptake is not primarily regulated at any stage in the passage from the lumen to the plasma. The relative amount of iron absorbed does not appear to decrease with increase in the quantity administered. This is interesting, since in the range 1 to 10 mg. iron/kg. weight, a decrease would be anticipated. Here it should be noted that no attempt had been made to simulate iron deficiency in the dogs, though some iron is lost by taking blood samples.

The exact form of the plasma iron level-time curve in the first hour is a matter for conjecture, owing to the limited number of activity measurements in this period. It is clear, however, that continuous monitoring of the blood would permit a more detailed study, based on the form of the curve in the initial period, of the transfer of iron from the lumen to the plasma. This in turn would facilitate a comparison of various iron preparations.

Continuous monitoring of doses as low as 10 μc presents difficulties. Consequently some form of sampling would have to be retained for the measurement of long-term changes in plasma activity, since the latter may fall inside the wider fiducial limits associated with continuous monitoring.

The method employed for faecal activity measurement is, to the best of the authors' knowledge, new. It is considerably less laborious than that previously reported[5,7].

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After Dr. Rapson presented the paper there was a DISCUSSION.