

Adsorption of ^{59}Fe on glassware and its effect on data deduced

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SYNOPSIS The attachment of ^{59}Fe to glassware under conditions of hospital laboratory procedure forms the subject of this communication. Adsorption of this nature can be extreme and can invalidate data derived or prejudice intended dose levels. It is aggravated by sterilization by autoclave.

Data indicating the severity of the phenomenon are presented and methods of controlling it are discussed.

During the early days of using ^{59}Fe ferric chloride there was general awareness that it might be adsorbed on glassware. It was recommended that stocks should be stored in acid medium and laboratory samples were usually handled promptly (Huff, Hennessy, Austin, Garcia, Roberts, and Lawrence, 1950). In our own experience similar precautions were not, however, consistently extended, even in up-to-date laboratories, to plasma-bound or protein-bound radioiron or to other dilutions made from stocks, and as ^{59}Fe ferric citrate is now used instead of ^{59}Fe ferric chloride for injection *in vivo* the practical point of stabilization by some suitable measure is no longer stressed. The practice of stabilization has often either been allowed to lapse or has been taken for granted at many stages (Leoffler, Rappoport, and Collins, 1955; Bothwell, Hurtado, Donohue, and Finch, 1957).

In the course of general studies we found evidence that at dilutions used in hospital laboratories adsorption of preparations of ^{59}Fe on glass could be gross and at a level far beyond anything of which we had been aware or to which we could find references in the literature.

The observations upon which we base our study and our conclusions are as follows:

1 Count rates on repeat aliquots of normal saline dilutions of ^{59}Fe ferric citrate were supported by count rates on the empty soda glass universal containers. Our findings are amplified by the demonstration of similar adsorption on soda glass test tubes. Attention is also drawn to the limited reliability of recounts made on aliquots which are stored whole and counted again in the initial tubes.

2 Both ^{59}Fe salts become attached to the neutral glass of dose ampoules, and we show how sterilization exacerbates this defect and the possible serious interference with intended dose levels.

3 ^{59}Fe ferric chloride has a greater adsorptive tendency than ^{59}Fe ferric citrate, and we investigated the place of small amounts of iron carrier in limiting adsorption.

4 The value of adding sequestrene (a chelating agent) and of acidification with 0.1N hydrochloric acid was assessed, and the situation when bound to plasma.

5 We assessed the place of specially constituted citrate-saline as recommended by the Radiochemical Centre for use with ^{59}Fe ferric citrate.

6 We made short trials of plastic containers.

7 The consistency within limits of 1% sequestrene and 0.1N hydrochloric acid as protective diluents for procedures *in vitro* is shown.

MATERIALS

Pyrex volumetric flasks and volumetric pipettes were E-mil Gold Line standard; universal containers were of the customary soda glass; counting tubes were $\frac{1}{2}$ in. diameter soda glass. Stock solutions of ^{59}Fe ferric chloride and ^{59}Fe ferric citrate were supplied by the Radiochemical Centre, Amersham, at a concentration of 100 μC per ml; dose solutions were dispensed from these at a concentration of 1 μC per 1 ml by the Regional Department of Clinical Physics and Bio-engineering and screened in an ionizing chamber accurate to within 3%. Both were in neutral glass ampoules.

Samples in the hospital laboratory were counted in a well-type sodium iodide scintillation detector coupled to an IDL 1700 scaler. The equipment was checked for stability throughout the studies using a solid wax

standard incorporating ⁵⁹Fe; reproducibility was accurate to within 3%.

RESULTS

ADSORPTION ON GLASS AND EFFECT OF STERILIZATION (NORMAL SALINE DILUTIONS) Adsorption of ⁵⁹Fe on glass and loss of counts in contained fluids is so gross at the dilutions under study that this is clearly seen in the graphs, and accompanying detailed description seems unnecessary. Dilutions handled in Pyrex and soda glass contained 1 μC radioactivity per 100ml and those in neutral glass ampoules had 1 μC per 1ml (Figs. 1, 2, 3, and 4).

In Fig. 1 the irregular and excess loss in count

rates on serial aliquots must represent erratic rates of adsorption during the period of observation. Crude screening of the empty soda glass universal containers by setting them on top of the well counter in which aliquots were counted showed high count rates still present. This adsorbed material was recovered from the glass in 10% ferric chloride.

In Fig. 2 high levels of adsorption on soda glass tubes is shown. This had occurred even when small amounts of iron carrier had been added initially. It was recoverable proportionately when 10% ferric chloride was used but had been completely masked when tubes were recounted full, but it is also of special significance to note that recounts on the retained aliquots were not quite consistent

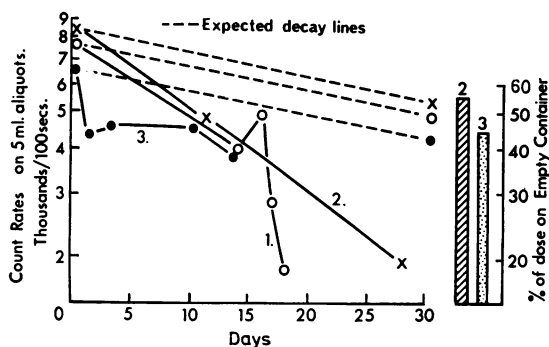


FIG. 1.

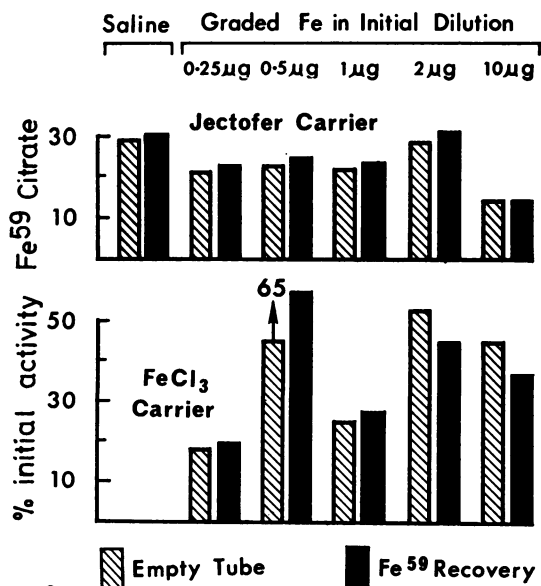


FIG. 2.

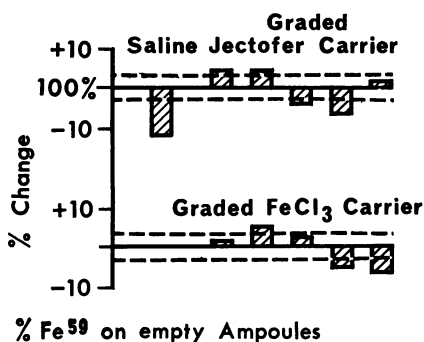


FIG. 3.

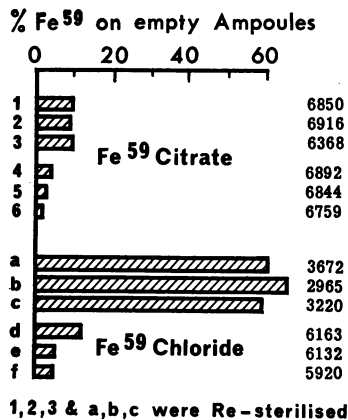


FIG. 4.

FIG. 1. Fall in count rate on serial aliquots from ⁵⁹Fe ferric citrate diluted 1 μC per 100ml in saline and stored in universal containers.

FIG. 2. Adsorption on emptied test tubes at 51 days correlated with quantitative recovery in 10% ferric chloride.

FIG. 3. Fluctuation of recounts on stored ⁵⁹Fe ferric citrate aliquots (broken lines represent limits of equipment error).

FIG. 4. Adsorption of radioactivity on neutral glass ampoules with count rates on aliquots of 1ml diluted in 100ml sequestrene listed alongside.

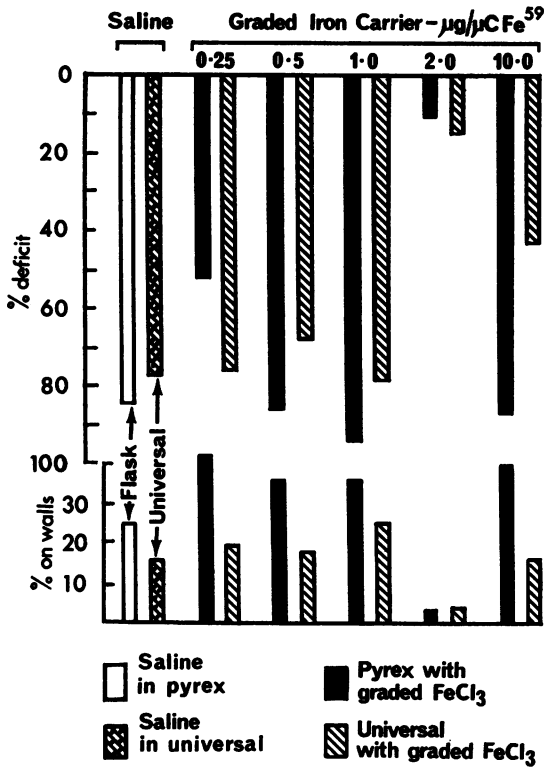


FIG. 5. Percentage deficit at 14 days in dilutions of $1\mu\text{C}$ ^{59}Fe ferric chloride in 100 ml saline and with graded doses of iron carrier added. Recovery from glass surfaces is represented on the lower ordinate.

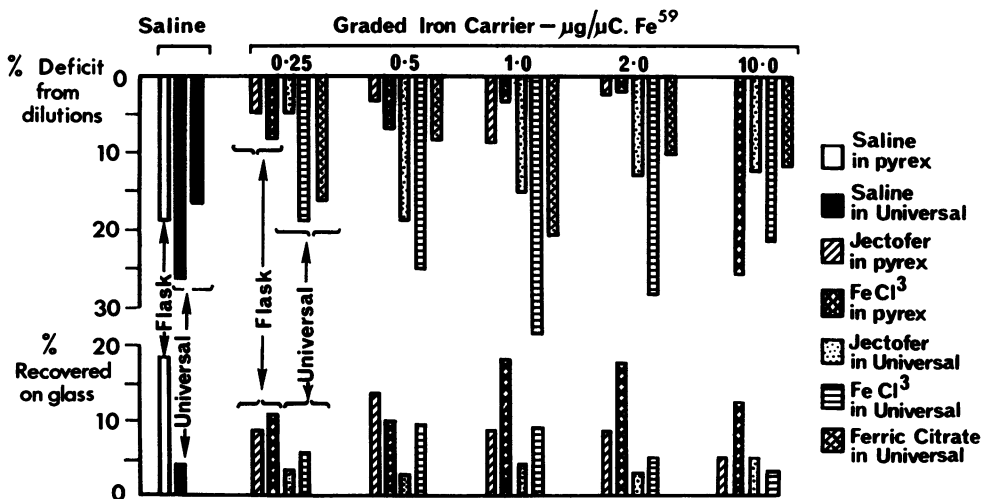


FIG. 6. Losses at 14 days using ^{59}Fe ferric citrate in saline, and with graded Fe carrier added and correlated with radioactivity recovered from the glass.

(Fig. 3). Fluctuation in one instance showed an 11% deficit on the initial value. With the volumes used surface levels protruded above the well counter and presumably irregular distribution of adsorbed material on the tube walls must now have rendered the conditions unsuitable for consistent detection.

In Fig. 4 adsorption is seen on neutral glass dispensing ampoules. With both preparations it is aggravated by sterilization and in the case of ^{59}Fe ferric chloride it amounted to around 60% in the set of ampoules shown. Figures listed alongside the histograms show the effect on subsequent count rates when 1ml fluid presumed to contain $1\mu\text{C}$ is diluted to 100ml with sequestrene. Similar data were encountered in two further batches of ampoules though the amount of iron adsorbed varied widely.

TYPE OF FE SALT Figures 5 and 6 show the deficits at 14 days in both ^{59}Fe ferric chloride and ^{59}Fe ferric citrate dilutions of $1\mu\text{C}$ per 100ml in normal saline and with small amounts of iron carrier added. Any place for iron carrier would require to be carefully and selectively assessed both from the point of view of quantity and biological behaviour should it have had a useful effect. Amounts added were therefore intentionally small but nevertheless amounted to one to 30 times the Fe content of the isotope preparations being used. Jectofer was included as a carrier because of its known safety *in vivo*. None, however, proved to have a profitable protective effect and deficits overall were again greater

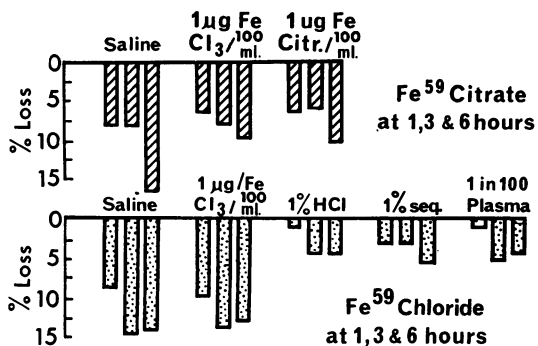


FIG. 7. Percentage deficit at one, three, and six hours in Pyrex of both isotope solutions diluted to 1 µC per 100 ml in saline and in protective diluents.

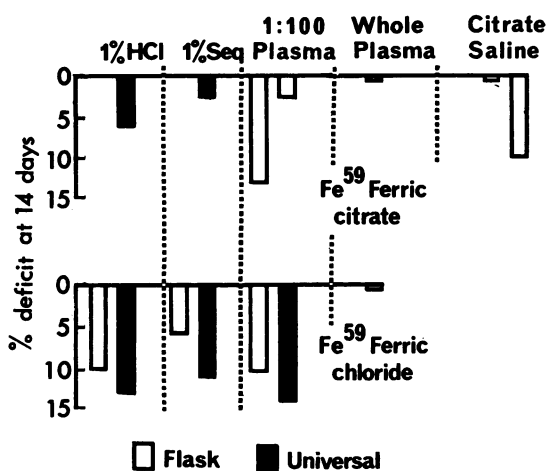


FIG. 8. Percentage losses at 14 days when protective agents were used.

with the ⁵⁹Fe ferric chloride solution that with the ⁵⁹Fe ferric citrate. Recovery tests with concentrated ferric chloride again showed large quantities of radioactivity recoverable from the glass surfaces.

'BINDING' AGENTS AND ACIDIFICATION Attention was given to 'binding' agents and acidification because attachment to plasma or purified globulin and the addition of hydrochloric acid were the practice with concentrated ⁵⁹Fe ferric chloride in the early days. These tests included assessment of count rates during the immediate hours after dilution and serial pH observations.

Representative data are given in Figures 7 and 8. It is noteworthy that with saline both isotope salts showed losses approaching 10% by one hour and around 15% by six hours when diluted at 1 µC per

100ml in Pyrex flasks. Losses with weak iron carriers, also now reviewed in these early hours, were similar. When the isotope was added to 1% sequestrene or 0.1N hydrochloric acid already in the flask losses at these intervals were reduced but were not eradicated, and with plasma diluted 1 in 100 in saline losses up to 5% were encountered within a six-hour period. At 14 days (Fig. 8) losses with protective agents and in diluted plasma could be 12 to 14%.

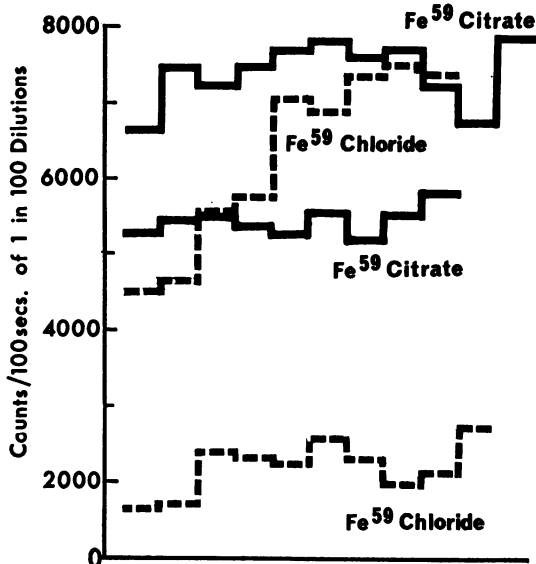


FIG. 9. Starting count rates on serial aliquots of 1ml diluted to 100 ml taken from four ampoules dispensed to contain similar concentrations of radioactivity (see text).

The situation with stored labelled whole plasma is different. Dilutions made at 14 days and counted immediately showed no loss from the expected value (Fig. 8).

Estimations of pH serially from 0 hours to 14 days in a selection of these tests showed no sequential alteration, even when large deficits from adsorption were occurring. The pH level was that of the diluent.

A summation of these adsorptive tendencies and the immediacy of their commencement emerges in Figure 9. The graphs show sets of the starting count rates on aliquots from dilutions of 1 µC per 100 ml made in Pyrex from four neutral glass ampoules each dispensed at a concentration of 1 µC per 1ml and sterilized before use. The differing range from each ampoule reflects adsorption on the neutral glass before further dilutions are made. Ampoule 4 yields less than one third of the count

rates in ampoule 1 and the other two are intermediate. Within each series variable levels reflect immediate uptake on the Pyrex flask. The lowest levels at the start of each series represent dilutions made into normal saline, those in the middle are dilutions into weak graded iron carrier, and the higher levels at the end reveal the protective effect from sequestrene, hydrochloric acid, or plasma already placed in the flask.

COMPLEXING WITH CITRATE/SALINE Enquiries at the Radiochemical Centre at Amersham elicited that for dispensing ⁵⁹Fe ferric citrate a solution of citrate/saline containing 10 mg sodium citrate and 5.9 mg sodium chloride per millilitre was regularly used. This procedure was therefore introduced for our dilutions and the results are shown in Figure 10. Control of losses and irregular dispersion was not complete although it was so greatly improved by comparison with normal saline. Some instability continued to be reflected at 14 days in changed values brought about by shaking. Screen counts on

glass surfaces did not, however, show the deposition experienced with normal saline.

PLASTIC FOR CONTAINERS The place of plastic for containers invited attention and a comprehensive screen was run (Fig. 11). In no instance among the combinations used did count rates on retained aliquots show the deviations experienced in the glass tubes that were recounted (Fig. 3). Also, the emptied plastic containers registered no adherent radioactivity. The variable starting levels seen must again have been due to adsorption by the ampoule before dilutions were made.

CONSISTENCY OF CONTROL PROCEDURES One per cent sequestrene and 0.1N hydrochloric acid had been clearly shown to have protective qualities with both isotope preparations under study. Consistency was now proven by consistent levels obtained on serial aliquots from one ampoule when 1 μC was pipetted into 100 ml of each reagent and counted immediately (Fig. 12). The greater prior adsorption

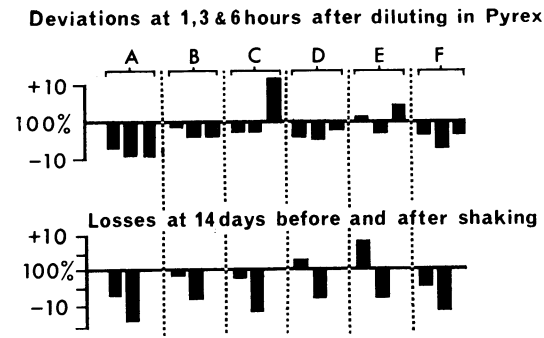


FIG. 10. Deviations in dilutions of 1 μC in 100 ml taken from six ampoules when special citrate/saline is used to prepare and dilute ⁵⁹Fe ferric citrate. Ampoules D, E, and F had been re-sterilized.

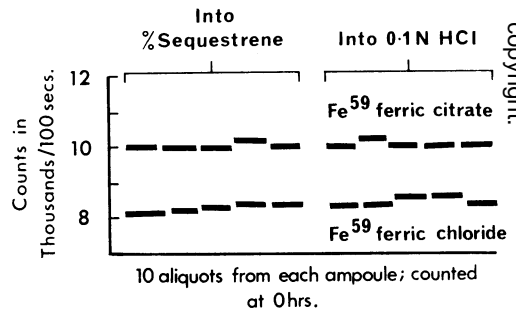
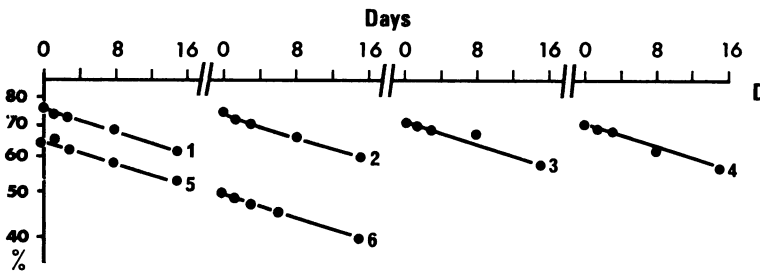


FIG. 12. Count rates at 0 hours when serial aliquots of 1 μC are diluted into 100 ml of selected diluents.



(1) ⁵⁹Fe ferric citrate in special citrate/saline. (2) Same from a re-sterilized ampoule. (3) ⁵⁹Fe ferric citrate in normal saline. (4) Same from a re-sterilized ampoule. (5) ⁵⁹Fe ferric chloride in saline. (6) Same from re-sterilized ampoule.

FIG. 11. Serial counts up to 14 days on aliquots retained in plastic tubes.

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of ^{59}Fe ferric chloride on the ampoule glass still emerges as both ampoules were dispensed to contain the same concentration of radioactivity per millilitre.

DISCUSSION

The data represented are self explanatory in terms of adsorptive features.

Attention must also be paid to the effect of adsorption on the data derived. The amount of activity used could clearly be much less than intended and could prejudice the recordability of results. In clinical studies the margin of current dosage must be high as the intended dose must often be reduced by the large fractions shown to adhere to ampoules. Review of our own organ counts *in vivo* showed some trend in correlation between low counts on dose standards and low organ counts. If predicted blood volume is used delay in counting or preparing standards could mask evidence of defective erythroid production. In published work adsorption on glass may have contributed to scatter in data in some oral ^{59}Fe tests, as to our knowledge doses in many of these were made in Pyrex and transferred to glass tumblers, although in group studies trends of a profitable nature emerged. Presumably pipettes must also adsorb from saline dilutions since uptake on glass containers has been shown to begin so immediately.

The basis for such adsorption is the availability of readily precipitated ions such as $\text{Fe} + 3$ for chemisorption when hydroxyl ions are supplied from container surfaces. Such conditions exist with ferric solutions in glass, and it is curious that the extent of exchange with radioactive ferric solutions and glass should not have made a greater impact on hospital laboratory workers before now. Complexing by citric acid, acidification, and chelation are known as preventives. The first two are in regular use for dispensing stock solutions at the Amersham Radiochemical Centre, although we have not reviewed the exact effectiveness at these concentrations. We cannot moreover find any suggestion advising users that such measures should be continued for later manipulations *in vitro* at which level of dilution the high adsorption rates just described were encountered. The greater adsorption on soda glass is

in keeping with the greater availability of hydroxyl ions from this glass than from Pyrex, and the value of plastic rests in the unavailability of hydroxyl ions from its smooth surface. The absence of *pH* changes was presumably due to the extremely low total concentration of Fe salt in the solutions and *pH* values simply represented the diluent used.

RECOMMENDATIONS

Clearly, knowledge of this adsorptive feature and the conditions of the intended use are sufficient to guide workers in the following preventive measures:

The recommended citrate/saline and not normal saline should be used to dilute ^{59}Fe ferric citrate to be given *in vivo*.

Dilutions for standards from both isotope solutions should be made into 1% sequestrene or into 0.1N HCl and counted at the time the dose is given, the ampoule having been shaken beforehand.

Subsequent estimations should be based on radioactive decay rates and not on recounts of the same or further aliquots.

Plastic containers are desirable at all stages possible, though each form of plastic should be assessed when it is introduced.

If the dose level is critical the actual strength of the contents of the ampoule should be estimated at the time of use.

Further investigations relating to methods of sterilization and reconsideration of postal despatch in plastic are required.

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