

THE STABILITY OF AQUEOUS SOLUTIONS OF FERROUS GLUCONATE

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A DEMAND for a liquid preparation of ferrous gluconate to be included in the British Pharmaceutical Codex and the National Formulary prompted this investigation into the conditions affecting the rate and extent of oxidation which might occur. It is generally held that ferrous iron is much more readily adsorbed than ferric and it is desirable therefore that any oxidation which may occur should be restricted to a minimum. That oxidation might occur fairly readily is evident from the high proportion of ferric iron permitted in the solid material by the specification of the United States National Formulary which allows over 17 per cent. of the total iron to be in the trivalent state. British manufacturers have been able to produce material which shows a considerable improvement on the United States specification, many commercial samples examined showing about 6 per cent. of the iron in the ferric state, whilst one manufacturer's product consistently contains as little as 2 per cent. or less. A further indication that considerable oxidation in solution is to be anticipated is afforded by the known fact that oxidation of ferrous iron depends upon the degree of ionisation of the dissolved salt, ferrous sulphate for example being about 10 times more stable than ferrous acetate.¹

EXPERIMENTAL

In the first instance a number of elixirs were prepared according to different formulæ and were stored in well-closed amber glass containers varying from 20 to 40 fl. oz. in size, the ferrous iron content being determined from time to time. These preparations at first showed a steady deterioration in ferrous iron content, but in some of the smaller containers it was observed that oxidation became more rapid as the stock of solution diminished. It thus became obvious that the volume of air in the container was of prime importance in determining the extent to which oxidation would occur, and that a strict control of container size and of the volume of air above the liquid would be necessary in a comparison of the effect of different antioxidants and conditions of storage on the rate and extent of oxidation of ferrous gluconate in solution.

Details of tests. For each sample to be examined 15 × 2 fl. oz. containers fitted with metal screw caps containing a rubber liner were employed. One of these bottles was completely filled with the sample (a total volume of nearly 60 ml.) whilst 4 other bottles contained 48, 36, 24 and 12 ml. respectively. The 10 remaining bottles each contained 12 ml. of the sample, the volumes filled in being measured as accurately as possible. At the time of filling and closing the containers, portions of the sample were assayed for total iron and ferrous iron content by the methods described below. The bottles containing 12 ml. of sample were opened successively

at intervals of 24 or 48 hours and when the rapidly falling ferrous iron content had become relatively stationary the 5 bottles containing varying quantities of solution were opened and the ferrous iron content of each determined. The reproducibility of the test was examined by charging 5 sets of containers with the same preparation; Table I gives the figures

TABLE I

(a) SHOWING THE RATE OF OXIDATION OF FERROUS GLUCONATE WHEN 12 ML. OF AN AQUEOUS SOLUTION IS STORED IN A CLOSED 2 FL. OZ. CONTAINER

Total iron content of solution at time of preparation 0.798 per cent.
 Ferrous iron content of solution at time of preparation 0.759 per cent.

Time (days)	Ferrous iron content (per cent.)				
1	0.674	0.665	0.670	0.682	0.668
2	0.616	0.609	0.600	0.612	0.614
3	0.585	0.590	0.578	0.580	0.592
4	0.576	0.575	0.568	0.580	0.570
6	0.567	0.570	0.571	0.569	0.565
7	0.564	0.564	0.560	0.568	0.564
9	0.570	0.558	0.564	0.564	0.560

(b) SHOWING THE EXTENT OF OXIDATION IN CLOSED 2 FL. OZ. CONTAINERS AFTER 10 DAYS

Volume of sample in container, ml.	Ferrous iron content (per cent.)				
Full (about 58 ml.)	0.755	0.760	0.756	0.758	0.758
48	0.732	0.740	0.728	0.736	0.734
36	0.702	0.706	0.696	0.700	0.706
24	0.664	0.648	0.656	0.662	0.650
12	0.560	0.564	0.572	0.566	0.551

obtained, which indicated that the method could be used with confidence to compare the stability of preparations. The conditions obtaining in this test were thought to be more comparable with those to be met with in practice than if the solution had been exposed to free access of air.

Methods of assay. The methods of assay used throughout the series of determinations were as follows:—

(i) *For total iron content.* A suitable portion of the solution was diluted with water to 75 ml. and 15 ml. of dilute sulphuric acid and 0.25 g. of zinc dust were added, the flask being immediately closed by means of a stopper fitted with a bunsen valve. After being set aside for 30 minutes the reaction liquid was rapidly filtered through an asbestos pad, flask and filter being washed with a dilution of 1 volume of dilute sulphuric acid with 9 volumes of water. The combined filtrate and washings, after addition of 3 drops of solution of *o*-phenanthroline-ferrous complex as indicator was titrated immediately with 0.1N ceric ammonium sulphate.

(ii) *For ferrous iron content.* A suitable portion of the solution was diluted with water to 75 ml. 15 ml. of dilute sulphuric acid and 3 drops of solution of *o*-phenanthroline-ferrous complex were added and the solution was titrated immediately with 0.1N ceric ammonium sulphate.

The application of this method of determination of iron to ferrous gluconate gives an end-point which fades back fairly rapidly from the greenish-blue indicative of complete oxidation to orange-red, but if the first sharp change is recorded as the end-point, results of a high degree of

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reproducibility are obtainable. These methods of assay were chosen because of the ease and rapidity with which they could be carried out and because blank determinations showed that they were not invalidated by any of the ingredients employed in the formulation tests carried out. The applicability of the methods to the commercial samples examined was verified by a comparison of results for total iron content, obtained by the method described above, with results obtained by digesting the sample with nitric acid, precipitating and redissolving the iron and titrating with potassium dichromate.

This method of test was applied to liquid preparations of ferrous gluconate in order to determine the effects of formulation and of storage conditions upon stability.

Formulation. A number of solutions of ferrous gluconate containing added ingredients as antioxidants or buffers were prepared and examined together with 4 proprietary preparations containing ferrous gluconate in solution.

Results are given in Table II. The effect of varying the concentration of ferrous gluconate in solution was also examined, results being recorded in Table III.

TABLE II

SHOWING THE RATE OF DETERIORATION OF LABORATORY FORMULATED AND COMMERCIAL SAMPLES WHEN 12 ML. OF THE SAMPLE IS STORED IN A CLOSED 2 FL. OZ. CONTAINER

The figures recorded represent percentage loss of ferrous iron

Time (days)	Preparations				
	1	2	3	4	5
1	16.6	9.6	11.2	17.4	15.6
2	22.1	14.2	15.4	22.8	20.4
3	25.8	18.3	20.2	25.6	23.8
4	24.6	21.4	22.6	25.1	24.8
5	25.0	23.5	24.8	25.4	25.8
7	25.6	24.6	25.7	26.1	25.3
8		24.7	25.0		25.6
9		24.9	26.1		
10		24.5	25.2		

- Preparation 1. A 7.5 per cent. w/v solution of ferrous gluconate in water.
2. As (1) with the addition of 7.5 per cent. w/v of dextrose monohydrate.
 3. As (1) with the addition of 20 per cent. w/v of dextrose monohydrate.
 4. As (1) with the addition of 2 per cent. v/v of dilute hypophosphorous acid.
 5. As (1) with citric acid and sodium citrate added to adjust to pH 4.5.

Time (days)	Commercial samples			
	Manufacturer			
	A	B	C	D
1	17.8	2.9	14.6	12.6
2	23.4	4.2	18.2	17.1
3	26.8	7.6	21.4	20.8
4	29.1	9.6	22.8	23.0
5	28.8	11.0	23.8	23.4
7	29.4	12.3	24.7	23.2
8	29.0	13.2	25.4	23.6
9		14.1	25.0	
10		14.9		

Storage Conditions.

The effect of storage in completely and partially-filled containers upon the ultimate extent of oxidation is already apparent from the figures given in Tables I and II, and

TABLE III

SHOWING THE EFFECT OF CONCENTRATION UPON PROPORTION OF FERROUS GLUCONATE OXIDISED

Original ferrous iron concentration	Final ferrous iron concentration	Percentage oxidation
1.03 per cent.	0.90 per cent.	12.6
0.52 " "	0.42 " "	19.2
0.21 " "	0.15 " "	28.6
0.10 " "	0.068 " "	32.0

Figure 1, based upon the mean results given in Table I, shows this relationship more clearly. To determine whether the ratio of the volume of air to that of the solution plays any part in determining the rate at which oxidation occurs, a number of bottles were filled at each of the volume levels with a 7.5 per cent. solution of ferrous gluconate in distilled water, the liquid in one set of 4 bottles containing 48, 36, 24 and 12 ml. respectively being titrated daily. Only in the case of the bottle containing 12 ml. was any deterioration noted on the second day, so a further series was set up, titrations being carried out at 2-hourly intervals. The results of this series are shown in Table IV.

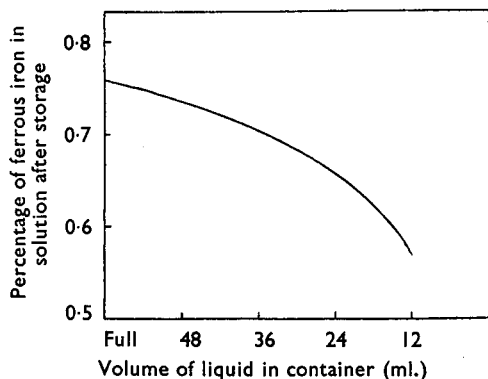


FIG. 1. Storage of ferrous gluconate solution in partially filled containers.

were filled as previously described and placed on a window ledge facing south, the results obtained being recorded in Tables V and VI. These results show that after an initial oxidation the ferric iron became reduced, the ferrous iron content increasing beyond what was originally present until it approximated to the total iron content. The reduction was accompanied in the smaller volumes by the deposition of a brownish-yellow crystalline precipitate.

The precipitate formed on exposure to light was prepared in greater yield by exposing a larger volume of considerably oxidised solution to the light, and isolated by filtration followed by washing with water, ethanol and ether. Pale brownish-yellow in colour, the separated residue was insoluble in water and organic solvents, but soluble in dilute hydrochloric acid, yielding a solution which gave reactions characteristic of ferrous iron. After removal of the iron the resulting neutralised solution yielded an immediate precipitate with calcium chloride, insoluble in acetic acid, but

TABLE IV

SHOWING THE RATE OF DETERIORATION IN CONTAINERS CHARGED WITH DIFFERENT VOLUMES OF SOLUTION FERROUS IRON CONTENT OF THE SOLUTION AT TIME OF PREPARATION 0.764 PER CENT

Ferrous iron content of the solution at time of preparation 0.764 per cent

Time (hours)	Volume of solution in the container			
	48 ml.	36 ml.	24 ml.	12 ml.
2	0.753	0.754	0.750	0.758
4	0.744	0.740	0.741	0.737
6	0.746	0.730	0.733	0.732
8	0.740	0.718	0.722	0.721
10	—	0.709	0.705	0.714
12	—	0.703	0.691	0.698
26	—	0.706	0.648	0.626
28	—	0.704	0.643	0.611
30	—	—	0.649	0.597
32	—	—	0.646	0.583

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redissolving in dilute hydrochloric acid. A portion of the calcium precipitate when heated with diphenylamine and syrupy phosphoric acid yielded a pale blue colour which was intensified by the addition of ethanol to the cooled reaction liquid. These reactions, characteristic of oxalates, gave reasonable grounds for believing that the precipitated material might be ferrous oxalate and a portion was assayed on this assumption. The iron was oxidised, precipitated as ferric hydroxide, ignited and weighed, whilst the filtrate and washings from the iron determination were just acidified with acetic acid and precipitated with calcium chloride, the residue, after washing, being titrated with potassium permanganate. The results obtained were as follows:—

Iron, 30.3 per cent. ; $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ (calculated from the permanganate titration), 97.8 per cent.

The formation of this insoluble residue was reported as long ago as 1936 by Neiger and Neuschul², who carried out an elementary analysis, but did not identify the compound. The analytical figures quoted by these authors are compatible with the finding that the residue is ferrous oxalate dihydrate.

The possibility that oxalate may have been present in the ferrous gluconate used to prepare the solutions was considered, but of two batches of material from different manufacturers, both of which yielded the precipitate from aqueous solution in the light, neither gave a positive reaction when the test for oxalic acid of the United States National Formulary specification was applied.

Among other observations, Neiger and Neuschul stated that solution of ferrous gluconate in water containing appreciable proportions of invert sugar considerably retarded the deposition of the ferrous compound, although not its formation in solution. Accordingly, tests were carried out in which the ferrous gluconate was dissolved in a 20 per cent. solution of dextrose monohydrate and exposed to light in partially-filled bottles. No precipitate was formed in 17 days, while in the absence of dextrose a precipitate had formed in 9 days. No precipitate was formed in completely filled bottles, even in the absence of dextrose, during a period of 6 weeks.

TABLE V
SHOWING THE EFFECT OF STORAGE IN DAYLIGHT

Time (days)	Ferrous iron in solution calculated as a percentage of the total iron content.
When prepared	94.2
1	88.0
2	84.8
3	82.6
4	83.8
5	86.6
7	93.0
8	94.8
9	97.5
10	99.6
11	99.3

TABLE VI
FERROUS IRON IN SOLUTION CALCULATED AS A PERCENTAGE OF THE TOTAL IRON CONTENT IN CONTAINERS CHARGED WITH DIFFERENT VOLUMES OF SOLUTION AFTER 5 WEEKS STORAGE IN DAYLIGHT

Volume of solution	Ferrous Iron
Full	99.4
48 ml.	99.7
36 ml.	88.2
24 ml.	79.8
12 ml.	72.1

} Pale brown
} crystalline
} deposit

DISCUSSION OF RESULTS

1. *Factors affecting the rate of oxidation.* It is clear from the figures quoted in Table II that the traditional antioxidants associated with ferrous iron formulation exert little or no effect in preventing the oxidation of ferrous gluconate. A slight improvement might be claimed when a moderate percentage of dextrose is added, but no substantial effect is to be observed. Dilute hypophosphorous acid, instead of retarding the oxidation appears to encourage it—a result which parallels the experience of Huyck when examining solutions of ferrous sulphate.³

A solution buffered to pH 4.5 with citric acid and sodium citrate showed just as rapid a deterioration as an unbuffered solution, contrary to the finding of Stone⁴. This author also stated that the stability of ferrous gluconate solution is considerably improved as the pH is lowered, but he does not indicate the acid used to bring about the adjustment. If a strong mineral acid were introduced the ferrous iron would naturally be stabilised to a certain extent because of the increased ionisation to be expected. The inclusion of a small proportion of citric acid in a solution containing syrup of orange did, however, improve the flavour of the product.

Of the commercial samples examined one was found to deteriorate at a markedly slower rate than any other solution examined, although the loss was still considerable; the remaining commercial samples deteriorated at least as rapidly as the solutions prepared in the laboratory. The ratio of the volume of air to that of solution apparently played no part in determining the rate of oxidation, as is shown in Table IV.

2. *Factors affecting the extent of oxidation.* The ratio of the volume of air to the volume of solution has the most pronounced effect upon the extent of oxidation which will occur in a closed container as is shown in Figure 1. It is clear that the oxidation reaction proceeds until an equilibrium is set up, probably depending both on the oxygen tension and the ratio of ferrous to ferric iron. The concentration of iron in the solution also affects the extent to which oxidation will proceed. As is to be expected, the proportion of total iron which is converted to the ferric state increases as the concentration of total iron is decreased.

It is evident from the many samples examined that, providing the solution is kept in a well-filled and sealed container, no significant deterioration of ferrous iron content may be expected within 3 months. In practice this period may well be considerably longer and indeed Talman, in a recent review⁵, mentions a period of 12 months. As soon as the container is opened and doses are removed the ferrous iron content begins to fall to an increasing extent as the volume of air trapped in the bottle becomes greater in proportion to the volume of liquid remaining.

3. *The effect of light.* The effect of light in arresting and ultimately reversing the oxidation of ferrous to ferric iron in solutions of ferrous gluconate which has been reported by Neiger and Neuschul² has been confirmed. The relatively insoluble ferrous compound which is deposited from solutions stored under such conditions has been identified as ferrous oxalate. It is a well-known fact that solutions of ferric oxalate, when stored in the light, are reduced to ferrous oxalate and this reaction has

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formed the basis of a proposed test to assess the light-protecting properties of glass for medicine bottles⁶. It therefore seems possible that ferric oxalate may be formed in solution by the oxidative breakdown of ferrous gluconate. This theory has not been adequately tested, however, and it may well be that the oxalate is formed, as the result of a photochemical reaction, from some other product of the oxidation of ferrous gluconate. The possibility that the oxalate may have been present in the original material due to the method of manufacture has been considered, but has been disregarded since no reaction for oxalate was obtainable in the samples used. Moreover the formation of the precipitate only in those cases where considerable oxidation of the ferrous gluconate might be expected, i.e., in the bottles containing smaller volumes of solution, argues that the presence of the oxalate is connected with the oxidation reaction.

The formation of this precipitate has been shown to be retarded by the presence of 20 per cent. of dextrose and a solution of ferrous gluconate in the presence of this sugar may be expected to remain stable in respect of ferrous iron concentration and freedom from deposit if stored in well-closed, completely filled containers exposed to direct daylight. Once the bottle is opened and doses are removed a suitably formulated preparation will retain its high ferrous iron content and remain free from deposit throughout the time that is required to consume 4 fl. oz. at the rate of 3 teaspoonfuls daily, provided that the container is kept well closed between withdrawals of doses and the bottle is stored in direct daylight. A comparison of the ferrous iron content of such a preparation stored in the daylight and protected from light throughout its period of use is shown in Figure 2. If it is argued that the presence of oxalate is undesirable then the question of the desirability of prescribing ferrous gluconate in solution under any conditions of storage, whether in the light or the dark, should be reviewed anew.

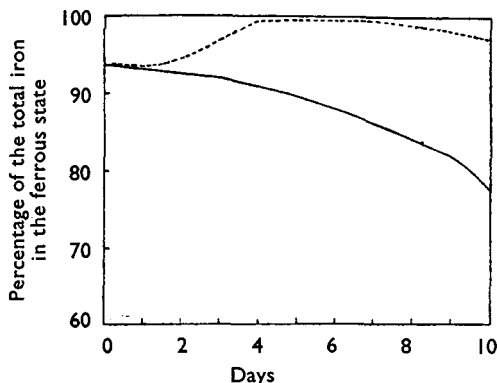


FIG. 2. Storage of ferrous gluconate elixir in 4 fl. oz. containers, withdrawing sample at the rate of 3 teaspoonfuls daily.

— storage in dark
- - - storage exposed to daylight.

CONCLUSIONS

It is concluded from the foregoing experiments that:—

(i) solutions of ferrous gluconate should be dispensed only in completely filled containers of small size; the inadvisability of dispensing from a

Winchester stock is obvious since, when the Winchester is only partially full, portions withdrawn may already be some 30 to 50 per cent. oxidised.

(ii) the maximum concentration of ferrous gluconate compatible with the formation of a stable solution should be employed—10 per cent. is suggested as a suitable figure.

(iii) a suitable flavouring agent consists of about 20 per cent. of syrup of orange together with 0.3 per cent. of citric acid.

(iv) if the solution is to be stored protected from light a small proportion of dextrose monohydrate—say 7.5 per cent., should be included. If storage in the light is considered to be safe the proportion of dextrose should be increased to 20 per cent.

Storage protected from light is accompanied by a decrease in ferrous iron content and a corresponding increase in ferric iron content which becomes greater as the volume of liquid in the bottle is reduced, but the general appearance of the preparation remains satisfactory.

Storage in the light of a suitably formulated product results in a slight increase of ferrous iron content due to the reduction of ferric iron present initially, and the maintenance of the ferrous iron throughout the storage period. It is, however, accompanied by the formation of a product which may be considered undesirable and may be attended by some deposition of solid matter in partially-filled bottles.

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DISCUSSION

The paper was presented by MR. C. A. JOHNSON.

MR. L. M. SPALTON (London) said that investigations with which he had been connected confirmed the authors' findings. It was claimed that ferrous gluconate was less liable to produce side effects in susceptible people than other iron salts, but a high ferric content would negate this advantage. In his view, the ferric content of ferrous gluconate should be not more than 2 per cent. of the total iron present. He did not regard the possibility of the formation of ferrous oxalate as too serious, because, although there was little information available about its toxicity, the dosage given in the French Codex was 1½ grains. There was, however, a remote possibility that a child could be given a slurry of ferrous oxalate which might be harmful. A general criticism of liquid iron preparations was that being solutions of low pH they might cause some damage to the teeth. As tablets of ferrous gluconate were stable, it seemed preferable to administer the drug in tablet form.

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DR. W. MITCHELL (London) said that it seemed a sugar-coated tablet was the best method of presentation. He suggested that if a solution was required, ascorbic acid might prove a suitable antioxidant.

DR. J. B. STENLAKE (Glasgow) said the problem required fundamental study. There was a close connection between the oxidation of the gluconate and the fairly well-known oxidation methods for carbohydrates using such agents as peroxides, which were catalysed by light and ferric ions. Was the oxalate produced as a result of a series of degradations, or was it a primary oxidation process?

DR. G. BROWNLEE (London) said that reduced iron salts such as ferrous sulphate and oxidised iron salts such as ferric sulphate are physiologically outside the tolerated limits in which life is possible. Ferrous salts taken orally are immediately oxidised and become tolerable. The proposal to stabilise a reduced preparation at an E_h at or below that of ascorbic acid is dangerous, particularly so in a liquid preparation when given to infants, in whom ferrous salts are sometimes rapidly absorbed. A difficulty which the authors face is in stabilising the iron preparations within the physiological range of pH in the absence of suitable E_h buffers. The tocopherols are possibilities and all three isomers are suitable.

DR. G. E. FOSTER (Dartford) said that no one had suggested that there might be some ferrous oxalate present initially. He had found calcium oxalate in a stored sample of calcium borogluconate solution. It seemed to him that, as a result of contamination of the organisms used in the preparation of commercial gluconic acid, some oxalic acid might be produced and thus the ferrous gluconate might initially contain some ferrous oxalate.

MR. C. L. J. COLES (Greenford) said that at the beginning of the paper the formulæ of a number of elixirs were mentioned. What was the pH of these products? What was the temperature at which these samples were stored? Was the sample buffered at pH 4.5 more or less stable than the elixirs referred to earlier? What was the pH of the sample referred to in paragraph (iii) of the conclusion, containing 0.3 per cent. of citric acid? Had the authors any experimental evidence concerning the effect of pH on the rate of oxidation?

MR. E. G. SPEAKMAN (Birmingham) said that the use of ascorbic acid did not solve the problem of oxidation of the preparation in half-filled bottles.

MR. G. RAINE (Manchester) said he had found that a formulation containing glycerol 50 per cent. v/v and sucrose 20 per cent. w/v with 2 per cent. dilute hypophosphorous acid considerably retarded the oxidation. When ascorbic acid was added to ferrous gluconate solutions a red colour was produced which disappeared in a day or two, and ascorbic acid was scarcely detectable after a few days; nevertheless, the resulting solution was less readily oxidised than a simple ferrous gluconate solution. A mixture of ferrous gluconate with syrup, citric acid and ascorbic acid appeared to stabilise the ascorbic acid to some extent, and he suspected

the ferrous iron was less rapidly oxidised. He had been unable to devise a method of estimating ferrous iron in the presence of ascorbic acid. The method used to protect the preparation from light was not described. Ferrous gluconate solutions were more rapidly oxidised in amber bottles than in clear glass bottles. Some experiments had shown, however, that storage in complete darkness caused less rapid oxidation than storage in amber bottles, and one sample of ferrous gluconate solution had been as stable when stored in complete darkness as when stored in daylight. The formulation with glycerol, sucrose and dilute hypophosphorous acid had been equally stable under both conditions. No proof had been given that ferrous oxalate was present in solution in any formulation. He had obtained only inconclusive evidence of the absence of oxalate in a mixture containing glycerol and glucose, stored for a month in daylight. His experiments had shown that ferrous oxalate was not appreciably soluble in the mixture. Could animal experiments be undertaken to determine the toxicity of formulations, exposed to air and light, and which had not deposited? The loss of 25 per cent. of ferrous iron content in 10 days was considerable. In his experiments, using partly-filled bottles and removing the corks for a minute or two each day so as to replace the air above the fluid, only in exceptional formulations were there losses of more than 20 per cent. in 4 weeks. The quality of ferrous gluconate available commercially seemed to vary considerably, and the stability in solution varied in consequence. He had prepared a sample of ferrous gluconate which complied with the N.F. requirements and found under all conditions that it was more stable than commercial samples. He hoped that ferrous gluconate of a much higher degree of purity would become available. The various problems of treating iron deficiencies made it desirable that an iron compound should be available in liquid form as well as in tablets. It was also possible that pure ferrous gluconate might be given by intramuscular injection with considerable benefit, instead of the saccharated iron oxide injection solution which was usually given only by the intravenous route.

MR. D. N. GORE (Dorking) said that in tablets of ferrous sulphate the presence of ascorbic acid had a considerable stabilising effect. There was evidence that ascorbic acid potentiated and helped the clinical effect of iron, but he had always assumed that this was through keeping it in the ferrous state and not a function of ascorbic acid *per se*.

MR. F. A. J. TALMAN (Liverpool) asked whether the authors had any information on the effect of sucrose concentrations on the rate of oxidation. He had found some evidence that increasing sucrose content tended in some cases significantly to decrease the rate of oxidation.

MR. C. A. JOHNSON, in reply, said they had examined a number of commercial tablets and had noticed no serious loss in ferrous iron content on storage. The use of ascorbic acid had been considered, but an American paper by Stone had shown the undesirability of this because of the colour formed. The solid ferrous gluconate used had been examined by the method of the U.S. National Formulary for oxalate, and negative results were obtained, but he could not be certain that there was no oxalate

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present. The pH of the samples made without any acid present were about 5.6 to 5.8. The pH of the preparation containing hypophosphorous acid was 4.8 and that containing citric acid was 4.5. All preparations were stored under ordinary laboratory conditions, and most of the work was done after March, 1954, with room temperatures of 50° to 70° F. Some had been stored in the refrigerator, with no effect on oxidation retardation. The effect of pH on the rate of oxidation had not been examined in detail. American work showed that at a pH of 1.9 the oxidation was apparently very small, whereas with a pH of 6.9 there was an 85 per cent. loss of ferrous iron in 48 hours in a solution which was oxygenated. The pH there may have been adjusted using mineral acids, but the presence of these was undesirable. The effect of sucrose concentrations had not been examined. All the preparations were stored in white flint glass bottles and "storage in the dark" meant storage in a closed cupboard, but opened daily for a few minutes. There was no evidence of preparations being more stable in these conditions than when stored in amber bottles. They had had a number of samples of ferrous gluconate, one of which had been very pure, containing only about 1.5 per cent. of the total iron in the ferric state. It produced about the same rate of oxidation however. There was clinical evidence that the presence of ascorbic acid, if administered with iron, allowed absorption of ferrous iron to be increased, but he felt that ascorbic acid was not the answer in a liquid preparation of ferrous gluconate.