3. The presence of equimolar concentrations of pyridoxal phosphate prevented the inhibition by isoniazid. Stable hydrazones of isoniazid did not inhibit transaminase activity. These compounds, which were stable in the presence of heavy suspensions of B.C.G. organisms, had tuberculostatic activity comparable with isoniazid.

4. Transaminase activity was found in extracts of cells which had been exposed to isoniazid under bactericidal conditions.

5. It appears improbable that the bactericidal activity of isoniazid can be explained by its action on a transaminase or other enzyme requiring pyridoxal phosphate.

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Determination of Iron in Biological Material by Spectrophotometry of Ferric Perchlorate

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The determination of iron in inorganic samples by conversion into ferric sulphate or ferric perchlorate has been described by Bastian, Weberling & Pallila (1953, 1956). These salts absorb strongly at a region in the ultraviolet where interference from other elements is negligible. It therefore seemed likely that iron in biological material could be estimated by digestion with perchloric acid followed by spectrophotometry of the diluted digest. An accurate and convenient method results; as described, samples should contain 0.2–1 mg. of iron, but much smaller quantities of iron can be estimated by modification of the volumes. For convenience, the present procedure yields 100 ml. of solution for the optical measurement. It is, however, quite practicable to finish with a final volume of 10 ml. With this modification, iron can be estimated in the range 0.02–0.1 mg.

METHOD

Reagents. AnalaR perchloric acid (60%; sp.gr. 1.54) is taken to be 10N. By tenfold dilution of the concentrated acid, N-perchloric acid is prepared. AnalaR concentrated nitric acid is used. All reagents must be iron-free. Several batches of AnalaR acids have been used; they have all given acceptably low blank values.

Samples. An appropriate amount of material containing up to 1 mg. of iron is weighed or measured into a 250 ml. conical flask. For blood, 2 ml. is taken; for tissue such as liver or spleen, 2 g. of finely chopped material is weighed out; for urine, quantities varying from 5 to 50 ml. or more are necessary.

In the special case of serum-iron estimation, this method does not present any advantage over existing procedures. This is because the concentration of iron in the serum is exceedingly low and the method chosen must therefore be very sensitive. In addition, preliminary treatment of the serum with a precipitant of acid protein is necessary to remove traces of haemoglobin; the resulting filtrate is suitable for immediate development of colour with one of the conventional reagents.

Digestion. Nitric acid (5 ml.) is added to the contents of the flask and the mixture is boiled gently on a hot-plate for at least 1 hr., or longer if necessary, until a brown or yellow mobile liquid results. Further additions of nitric acid may be necessary to prevent drying. To the cooled solution 5 ml. of 10N-perchloric acid is added and the flask is returned to the hot-plate. The temperature is raised and the contents are allowed to evaporate to dryness. The heating should be adjusted so that 1½–2 hr. elapse between the addition of the perchloric acid and complete dryness. The residue is treated with 10 ml. of 10N-perchloric acid, which is heated until boiling to achieve complete solution of the residue. The cooled solution is washed quantitatively into a 100 ml. volumetric flask and

Table 1. *Comparison of iron determinations by different methods*

Sample	TiCl ₃ titration (mg./100 ml.)	Spectro- photometry as cyanmet- haemoglobin (mg./100 ml.)	Perchloric acid digestion (mg./100 ml.)
Blood	57.6	57.4	57.8
Blood	46.8	46.7	47.5
Blood	40.4	41.5	40.6
Blood	41.5	41.5	42.1
Blood	41.6	42.1	41.4
Blood	42.4	42.4	42.4
Blood	43.4	43.7	44.5
Blood	41.4	42.1	41.5
Blood	48.7	49.1	50.7
Blood	44.1	44.5	46.4
Liver	16.0*	—	20.0*
Spleen	114.0*	—	116.0*
Urine (cardiac failure)	—	—	1.3
Urine (haemoglobinuria in remission)	—	—	0.63
Urine (active haemoglobinuria)	—	—	12.2

* mg./100 g. wet wt.

made to the mark with water. If there is a considerable amount of potassium present in the residue, a crystalline precipitate of potassium perchlorate will form (this happens commonly with urine samples). It is readily removed by centrifuging the solution, and recovery experiments have shown that no iron is retained in the precipitate.

Standard. A permanent solution is prepared by dissolving 500 mg. of pure iron (Johnson, Matthey and Co. Ltd.) in a mixture of 60 ml. of nitric acid and 100 ml. of water, then diluting the solution to 1 l. A volume (2 ml.) of this stock standard (1 mg. of iron) is pipetted into a flask, 5 ml. of 10N-perchloric acid is added and the mixture is evaporated to dryness. The residue is dissolved in 10 ml. of 10N-perchloric acid and the solution made to 100 ml. with water. A simple solution of iron metal in dilute perchloric acid is unsuitable as a standard because the resulting ferrous perchlorate does not have the required ultraviolet absorption.

Blank. The blank used is N-perchloric acid. If, however, nitric acid of satisfactory purity is not available, it will be necessary to prepare a blank solution by evaporating to dryness a quantity of nitric acid equal to that used in the digestion, followed by treatment of the residue as specified above.

Spectrophotometry. The apparatus used was a Unicam quartz spectrophotometer SP. 500 fitted with a hydrogen-discharge lamp. Test and standard solutions are measured in 1 cm. silica cuvettes at 240 m μ . The instrument is set to 100% transmission with a cuvette filled with blank solution. The optical density is strictly proportional to the iron concentration up to a density of 0.8 at least, corresponding to 1 mg. of iron/100 ml.

Bastian *et al.* (1953) have emphasized the effect of temperature on the optical density. In the present method, measurements are made at room temperature (about 18°) and all solutions are allowed to stand for about 0.5 hr. before measurement to attain the same temperature.

RESULTS

The values obtained on a series of blood samples are given in Table 1, and compared with the

Table 2. *Optical density of various cations, after digestion with perchloric acid, at mM concentration and 1 cm. path length*

	240 m μ	260 m μ
Iron	4.2	2.9
Sodium	0.002	0.002
Potassium	0.004	0.004
Calcium	0.018	0.013
Magnesium	0.000	0.001
Copper	0.098	0.025

precise but more exacting titration method of King & Wootton (1956). The table also shows the results obtained by spectrophotometry of cyanmethaemoglobin, a method which has been shown to give results in close agreement with the total blood iron. Comparative results on a sample of liver and spleen are recorded by the first two methods, although, for liver, the amount of iron in the sample was insufficient to allow an accurate titration. Some typical values of urine iron are shown in the table but no comparative values by other methods are included since there does not appear to be any satisfactory alternative procedure.

As a test of the precision of the method, a large sample of blood has been estimated on three separate occasions; each time six replicate portions of 2 ml. were digested. Measurements were made at 240 and 260 m μ . The mean value obtained for the iron content of the blood was 67.6 mg./100 ml., with the coefficient of variation of a single estimate equal to 2.4% at 240 m μ and 2.7% at 260 m μ .

Interference from the presence of other cations is, in general, negligible. Vanadium and molybdenum have an absorption comparable with that of iron; of the other elements only copper is likely to interfere, at least in animal specimens. If the sample

contains equimolar quantities of iron and copper, the iron result will be almost 2% too high, but, as shown by Bastian *et al.* (1956), this interference can be reduced by measurement at 260 m μ instead of 240 m μ , with some sacrifice of sensitivity. The effect of other common cations is given in Table 2.

DISCUSSION

Spectrophotometry of ferric perchlorate has proved to be a quick and simple method for analysis of total iron. The determination can be completed during the course of one day and the total working time is small. It is possible to shorten the method a little by measuring the optical density of the diluted perchloric acid digest (without taking to dryness), but it is essential to remove the last traces of nitric acid, and it is therefore convenient to evaporate the digest completely and redissolve the inorganic material in a fresh portion of perchloric acid. Recovery experiments have shown that no iron is lost even when the dry residue is heated for several hours.

Digestions with perchloric acid have a bad reputation for explosions. In the present method, organic matter receives a thorough treatment with boiling concentrated nitric acid before any perchloric acid is added. We have carried out many

digestions in this way without incident. In the final stages a solution of inorganic salts in perchloric acid is evaporated to dryness; in the absence of organic matter this process is acceptable as safe. However, in view of the consequences of mismanagement, all digestions are conducted behind a plastic screen and precautions are taken to avoid contact of hot liquid or vapour with inflammable materials.

SUMMARY

A rapid and precise method for determining iron in biological samples is described. Iron in the range 0.02–1 mg. is estimated by digestion with nitric acid–perchloric acid and ultraviolet spectrophotometry at 240 m μ . Precautions, and the effect of other cations, are discussed.

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Studies in Detoxication

73. THE METABOLISM OF ALKYL BENZENES: PHENYLACETYLENE AND PHENYLETHYLENE (STYRENE)*

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In the previous papers in this series (Smith, Smithies & Williams, 1954*a–c*; Robinson, Smith & Williams, 1955; Robinson & Williams, 1955; El Masri, Smith & Williams, 1956), the metabolism of alkylbenzenes containing saturated side chains was investigated. These studies have now been extended to include the unsaturated derivatives styrene and phenylacetylene. Of these compounds styrene, a compound of considerable importance in the plastics and other industries, has been previously investigated. In man, rats and rabbits, it appears to be metabolized largely by loss of one of its side-chain carbon atoms to yield benzoic acid, which is excreted as hippuric acid (Spencer, Irish,

Adams & Rowe, 1942; Carpenter, Shaffer, Weil & Smyth, 1944), and in rats and rabbits it is reported that 50–90% of the dose may be excreted as hippuric acid. Recently, however, Danishefsky & Willhite (1954) reported briefly some experiments using [β - ^{14}C]styrene and found that, when styrene was injected subcutaneously into rats (dose about 0.1 ml./100 g. rat), about 12% of the radioactivity appeared in the expired air as carbon dioxide whereas over 70% appeared in the urine. Spencer *et al.* (1942) suggested that a small amount of a mercapturic acid was formed, but, apart from hippuric acid, small amounts of unchanged styrene and respiratory CO_2 , the metabolites of styrene have not been identified. Phenylacetylene has not been investigated hitherto.

* Part 72: Mead, Smith & Williams (1958).