LXXXIII. THE IONISABLE IRON IN FOODS.

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(*Received February 11th, 1936.*)

SINCE it is now generally agreed that "haematin" iron in foods is largely unavailable for haemoglobin formation, and therefore is probably not absorbed [Elvehjem *et al.*, 1933; Lintzel, 1931], it is clear that a knowledge of the total iron in a foodstuff may be of little use from a dietetic standpoint. A knowledge of the ionisable iron is probably of much more value, although it cannot be assumed that the whole of this iron will be available, or that the whole of the "haematin" iron will be unavailable. Widdowson and McCance [1936] have made use of the present figures to calculate the ionisable iron intakes of 63 normal men and the same number of women.

Ionised iron in foodstuffs and biological material may be determined by several methods [Tompsett, 1934, 1, 2; Hanzal, 1933; McFarlane, 1934; Burmester, 1934], but the use of $\alpha\alpha'$-dipyridyl, introduced by Hill [1930], is probably the most convenient, and results obtained by means of this reagent have been found to agree in general with the biological assays of available iron in the same materials. Discrepancies have been encountered with egg yolk, but these have been satisfactorily explained [Elvehjem *et al.*, 1933; Sherman *et al.*, 1934, 1, 2].

Determinations of the ionisable iron in a number of English foodstuffs have accordingly been made, using $\alpha\alpha'$-dipyridyl. This reagent was prepared according to the method of Hill and sodium hydrosulphite used as the reducing agent. The latter as purchased, was heavily contaminated with iron and was purified according to the directions of Hill.

Method for the determination of ionisable iron.

(*a*) *In flesh foods.* The raw or cooked material was cut into small pieces with a stainless steel knife and thoroughly pulped in a mortar. Five aliquots (1–5 g.) were weighed into five tubes *A*, *B*, *C*, *D* and *E* of 40 ml. capacity, graduated at 20 ml. and to all 10 ml. of sodium acetate-acetic acid buffer p_H 5·5 were added. Previously cooked foods were not heated, but raw foods were heated at this stage for 10 min. at 100°. After testing the p_H of the fluids in the tubes, 0·5–1·0 g. of hydrosulphite was added to all the tubes. 0·05 mg. of iron was added to tubes *C* and *D* and a few crystals of $\alpha\alpha'$-dipyridyl to *A*, *B*, *C* and *D*. *E* was retained as a blank. The contents of all tubes were well mixed with a glass rod and allowed to stand overnight. 5 ml. of absolute ethyl alcohol were then added, the contents again well mixed and allowed to stand for at least 8 hours, but generally overnight. The solutions were then made up to 20 ml. with distilled water and filtered through Whatman filter-papers No. 541.

The amount of iron present in each solution was then determined by matching the colour against a series of standards in a Cole and Onslow comparator, the blank being placed in the appropriate position. A few of the extracts could have been matched in a colorimeter, but usually the natural pigments were not completely decolorised by the hydrosulphite, so that a comparator method was employed.

(*b*) *In fruits and vegetables.* The material was prepared for analysis as described under (*a*) and our portions were weighed out into the graduated tubes. 10 or 15 ml. of buffer p_H 5·5 were added and the tubes heated in a water-bath for 10 min. at 100°. After cooling, the p_H was checked, 0·05 mg. of iron added to two tubes and hydrosulphite to all. The volumes were then made up to

the graduation mark (20 ml.) with distilled water and the contents of the tubes well mixed and allowed to stand overnight. In the morning the contents were well stirred and filtered after an hour. Each filtrate was then divided into two portions. To one, a few crystals of αα′-dipyridyl were added and the colour developed; the other served as a blank.

Standards. The iron standards were prepared as follows. In twelve tubes of uniform bore aliquots of a standard iron solution were placed corresponding to 0·0025, 0·005, 0·01, 0·02, ... 0·10 mg. of iron respectively. 10 ml. of buffer p_H 5·5 and 0·5–1·0 g. of hydrosulphite were added, followed 12 hours later by 5 ml. of absolute ethyl alcohol. Each solution was then made up to 20 ml. and the tube sealed. When the method was modified to avoid the adsorption of the coloured complex on solid vegetable material, a fresh set of standards was prepared which contained no alcohol. The absence of the latter did not influence the intensity of the colour.

Discussion of the method.

(*a*) *Experiments with haemin.* The use of αα′-dipyridyl as a reagent for ionisable iron is entirely dependent upon the fact that no coloured complex is formed in the presence of haematin iron. In a personal communication, Hill informed us that free iron is liberated by the action of dissolved oxygen when hydrosulphite is added to a solution of haemin. This important point has been investigated under the experimental conditions used for the determination of ionisable iron.

4 mg. of haemin[1] were dissolved in about 3·5 ml. of *N*/10 NaOH and made up to 10 ml. 10 ml. of buffer p_H 5·5 were measured into five tubes, *A*, *B*, *C*, *D* and *E*, graduated at 20 ml. To *A* and *B* 1 ml. of the haemin solution was added and to *C* and *D* about 0·5–1 g. of hydrosulphite. After standing 15 min. (to allow all the dissolved oxygen in *C* and *D* to react with the hydrosulphite), hydrosulphite was added to *A* and *B* and 1 ml. of the haemin solution added to *C* and *D*. *E* was reserved as a reagent blank. The contents of all tubes were then thoroughly mixed, made up to the 20 ml. mark and left overnight. The solutions were then filtered, divided into two parts, one of which served as a blank; a few crystals of αα′-dipyridyl were added to the other.

No difference could be detected between *A*, *B* and *C*, *D*, and therefore no evidence was obtained that a decomposition of haemin took place when hydrosulphite was added to a colloidal suspension of haemin at p_H 5·5 by the interaction of hydrosulphite and dissolved oxygen. There was however a very small difference between the solutions containing haemin and the reagent blank, certainly less than the 0·0025 mg. standard. It was thought that this slight difference might have been due to the presence of free iron in the original haemin solution. This was tested in the following way: 3 ml. of the haemin solution (0·4 mg. per ml.), 10 ml. of buffer p_H 5·5, and 7 ml. of distilled water were placed in a tube and thoroughly mixed. About 0·25 g. of A.R. sodium chloride was then added, the whole again well mixed and allowed to stand overnight. The solution was then filtered, which removed the haemin, and a few crystals of αα′-dipyridyl were added. No difference could be detected between this solution and a blank prepared in a similar manner from which the haemin was omitted. Hydrosulphite, therefore, may interact with haemin and set free traces of ionised iron, but the extent to which this occurs is so very slight that it may be discounted entirely as an interfering factor in the present work.

Further, ionisable iron determinations were carried out on representative samples of the various types of foodstuffs, to which 1 ml. of the stock haemin solution was added. The results are shown in Table I. In 6 instances there was a very small and negligible increase, in 2 there was no change and in 1 a decrease.

[1] Kindly presented by R. Hill.

Table I. *Determination of ionisable iron in presence of haemin.*

Sample	Aliquot g.	Haemin added (as a solution in NaOH) mg.	Fe standard used mg.	Difference mg.
Watercress, fresh	2	None	0·03	
		0·4	0·0325	+0·0025
Bread, wholemeal	2	None	0·0325	
		0·4	0·0350	+0·0025
Apples, raw	5	None	0·0065	
		0·4	0·0075	+0·0010
Barcelona nuts	2	None	0·0625	
		0·4	0·0638	+0·0013
Turnip, raw	5	None	0·013	
		0·4	0·013	None
Liver, ox, raw	2	None	0·06	
		0·4	0·06	None
Oatmeal	1	None	0·04	
		0·4	0·0425	+0·0025
Raisins	2	None	0·04	
		0·4	0·0385	−0·0015
Cabbage, raw	5	None	0·0275	
		0·4	0·0285	+0·0010

(*b*) *Recoveries.* With two exceptions (see under (*h*)) satisfactory recoveries of added iron were obtained from all the foods given in Tables A and B.

(*c*) *The accuracy obtainable with the comparator.* With most samples the final solution was perfectly clear, and it was possible to estimate the iron content of the solution to the nearest 0·0025 mg. It was always possible, even when the unknown was matched against the strong standards, to read to the nearest 0·005 mg. Solutions which gave a cloudy solution, even after centrifuging and filtering, could be matched against the standards to the nearest 0·005 mg. The percentage error was of course dependent on the above factors and on the iron content of the sample.

(*d*) *Incomplete extraction of the iron.* This was considered to be a possible cause of inaccuracy, and in consequence all samples were thoroughly ground in a mortar to facilitate the extraction. Experiment repeatedly showed that under the working conditions described, complete equilibrium between the tissue and the surrounding liquid had been reached when the extraction had proceeded overnight, and at least this time was always allowed.

(*e*) *Adsorption of the coloured complex.* Hill stated that a 30 % concentration of ethyl alcohol in an acid medium prevented the adsorption of the coloured complex, but recovery estimations have always been carried out as an additional safeguard. In this way it was discovered that alcohol will not prevent adsorption of the coloured complex taking place with some vegetables and fruits. In the modification already described, the $\alpha\alpha'$-dipyridyl was not added until the liquid had been filtered from the insoluble vegetable tissue. In this way the coloured iron complex was formed in the absence of solid material. The success of the modification can be seen from Table II, in which results obtained by the two methods are compared. It should be noted that a low recovery by the original method was associated with a low value for the ionisable iron.

It is important to add the hydrosulphite at the commencement of the extraction, since Tompsett [1934, 2] has shown that ferric iron forms complexes with

Table A. *Total and ionisable iron in foods of vegetable origin.*

Foodstuff	Systematic name	Total iron (mg./100 g.)	Ionisable iron (as % of total iron)	
Vegetables:				
Artichoke, globe, cooked	*Cynara scolymus*	0·55	100	
Beans, broad, raw	*Vicia faba*	1·08	93	
butter, cooked	Var. of *Phaseolus vulgaris*	1·82	71	
French, raw	*Phaseolus vulgaris*	0·57	87	
haricot, raw	Var. of *Phaseolus vulgaris*	6·65	83	
haricot, cooked	Var. of *Phaseolus vulgaris*	2·72	84	
scarlet runner, cooked	*Phaseolus multiflorus*	0·74	74	
tinned, baked	Var. of *Phaseolus vulgaris*	2·05	98	
Beetroot, cooked	*Beta vulgaris*	0·50	94	
Brussel sprout, raw	*Brassica oleracea, bullata gemmifera*	0·67	75	
Cabbage, raw	*Brassica oleracea, capitata*	0·98	62	82
cooked	*Brassica oleracea capitata*	0·76	70	92
Carrageen moss	*Chondrus crispus*	8·88	97	
Carrot, raw	*Daucus carota*	0·56	100	
cooked	*Daucus carota*	0·41	98	
Cauliflower, raw	*Brassica oleracea, botrytis cauliflora*	0·91	99	
cooked	*Brassica oleracea, botrytis cauliflora*	0·52	100	
Celeriac, cooked	*Apium graveolens rapaceum*	0·87	98	
Celery, raw	*Apium graveolens*	0·14	100	
Chicory, raw	*Chichorium intybus*	0·57	66	59
Cucumber, raw	*Cucumis sativus*	0·25	100	
Egg plant, raw	*Solanum melongena*, var. *ovigerum*	0·32	53	50
Endive, raw	*Chichorium endivia*	2·77	72	
Fennel, dry, raw	*Foeniculum vulgare*	9·06	45	
fresh, raw	*Foeniculum vulgare*	4·20	50	62
Horseradish, raw	*Cochlearia armoracia*	1·60	100	
Leek, raw	*Allium porrum*	0·77	91	
Lentil, raw	*Lens esculenta. Ervum Lens*	7·63	60	72
Lettuce, raw	*Lactuca sativa*	0·80	63	
Marrow, raw	*Cucurbita pepo*	0·44	85	
Mushroom, raw	*Psalliota campestris*	0·65	100	98
Mustard, raw	*Brassica*	1·80	50	
Mustard and cress, raw	*Brassica* and *Lepidium* species	5·70	45	39
Onion, raw	*Allium capa*	0·40	100	
Parsley, raw	*Petroselinum sativum*	10·00	50	
Parsnip, raw	*Pastinaca sativa*	0·78	89	100
cooked	*Pastinaca sativa*	0·48	100	
Pea, blue, cooked	Var. of *Pisum sativum*	1·55	77	
fresh, raw	*Pisum sativum*	1·77	74	
split, raw	Var. of *Pisum sativum*	5·41	81	
split, cooked	Var. of *Pisum sativum*	1·84	71	
tinned	Var. of *Pisum sativum*	1·87	100	
tinned (Chivers)	Var. of *Pisum sativum*	2·10	100	
Potato, raw	*Solanum tuberosum*	0·60	95	100
cooked	*Solanum tuberosum*	0·62	93	100
Radish, raw	*Raphanus caudatus* or *R. sativus*	1·68	68	55
Rhubarb, raw	*Rheum rhaponticum*	0·38	100	
Salsify, raw	*Tragophogon porrifolius*	1·33	98	
Seakale, cooked	*Crambe maritima*	0·66	91	
Spinach, raw	*Spinacia oleracea*	2·96	68	
cooked	*Spinacia oleracea*	4·15	57	
Swede, raw	*Brassica* species	0·41	100	91
Turnips, raw	*Brassica rapa*	0·33	67	66
Watercress, raw	*Nasturtium officinale*	2·08	66	
Fruits:				
Apple, eating, raw	*Pyrus malus*	0·23	81	100
cooking, raw	*Pyrus malus*	0·24	100	
Apricot, dry, raw	*Prunus armeniaca*	4·08	98	
fresh, raw	*Prunus armeniaca*	0·37	95	
Avocado pear, raw	*Persea americana*	0·53	100	
Banana, raw	*Musa paradisiaca*, subspecies *sapientum*	0·47	100	

Table A (*cont.*).

Foodstuff	Systematic name	Total iron (mg./100 g.)	Ionisable iron (as % of total iron)		
Fruits (*cont.*):					
Blackberry, raw	*Rubus fruticosus*	0·95		41	39
Cherry, raw	*Prunus cerasus* or *P. avium*	0·48		100	
Cranberry, raw	*Vaccinium oxycoccos*	0·70		61	79
Currant, dry, raw	*Vinus vinifera*	1·07		89	
black, raw	*Ribes nigrum*	0·95		100	
red, raw	*Ribes rubrum*	0·66		85	
Custard apple, raw	*Annona squamosa*	0·52		100	
Damson, raw	*Prunus* species	0·63		70	
Date, dry	*Phoenix dactylifera*	1·71		82	
Fig, dry, raw	*Ficus aurea*	4·17		96	
fresh, raw	*Ficus aurea*	0·42		95	
Gooseberry, raw	*Ribes grossularia*	0·58		40	
Grape, black raw	*Vinus vinifera* species	0·27		85	
white raw	*Vinus vinifera* species	0·21		86	
dried (raisins)	*Vinus vinifera* species	3·80		97	94
sultana	*Vinus vinifera* species	3·60		65	
Grapefruit, raw	*Citrus paradisi*	0·11		92	
Greengage, raw	*Prunus* species	0·46		84	
Lemon juice	*Citrus medica*, var. *Limonum*	0·07		80	
Loganberry, raw	*Rubus loganobaccus*	1·00		76	
Medlar, raw	*Pyrus germanica*	0·77		65	
Mulberry, raw	*Morus nigra*	1·57		50	
Nectarine, raw	*Prunus* species	0·46		87	
Olive, bottled	*Olea europaea*, var. *amora*	1·03		97	
Orange juice	*Citrus aurantium*	0·20		85	
Passion fruit, raw	*Passiflora*	1·12		100	
Peach, dry, raw	*Prunus persica*	7·60		92	
fresh, raw	*Prunus persica*	0·39		100	
Pear, cooking, raw	*Pyrus communis*	0·16		94	
dessert, raw	Var. of *Pyrus communis*	0·21	100	100	100
Pineapple, raw	*Ananas sativus*	0·22		91	
Plum, cooking, raw	*Prunus communis*	0·35		60	50
dried (prunes), raw	*Prunus communis*	3·20		72	
Victoria, raw	*Prunus communis*	0·30		47	60
Pomegranate juice, raw	*Punica granatum*	0·20		33	33
Quince, raw	*Cydonia vulgaris*	0·19		54	
Raspberry, raw	*Rubus adoeus*	1·11		82	69
Strawberry, raw	*Fragaria vesca*	0·71		52	
Tangerine juice, raw	Var. of *Citrus aurantium*	0·18		95	
Tomato, raw	*Solanum lycopersicum*	0·37	73	53	66
Nuts:					
Almond	*Prunus amygdalus*	4·54		99	
Barcelona	*Corylus maxima, barcelonensis*	3·44		91	
Brazil	*Bertholletia excelsa*	2·70		61	62
Chestnut, baked	*Castanea vulgaris*	0·87		60	42
Cob	*Corylus maxima* or *C. avellana*	1·44		94	100
Cocoanut	*Cocos nucifera*	1·98		86	
Cocoanut milk	*Cocos nucifera*	0·10		75	
Peanut	*Arachis hypogoea*	1·19		100	
Walnut	*Juglans regia*	1·83		41	
Cereal and Cereal foods:					
Biscuit, Digestive	—	1·57		91	
Biscuit, Ryvita	—	3·20		100	
Bread, brown, Daren	—	2·96		100	
Goldmedal	—	2·30		100	
Hovis	—	2·48		95	
Lyons (wholemeal)	—	2·12		72	76
wholemeal	—	3·43		83	
Bread, white, Lyons	—	1·07		87	90
Flour, white	*Triticum sativum*	1·00		93	
Oatmeal	*Avena sativa*	4·15		96	
Rice	*Oryza sativa*	0·45		85	

Table A (*cont.*).

Foodstuff	Systematic name	Total iron (mg./100 g.)	Ionisable iron (as % of total iron)	
Miscellaneous:				
Beer, Bitter. Watneys	—	0·04	100	
Burton. Watneys	—	0·06	100	
Cocoa	—	14·20	93	
Chocolate, Milk	—	1·67	84	
Plain	—	3·28	89	
Treacle, Golden syrup	—	1·68	95	
Black	—	9·17	100	

Table B. *Total and ionisable iron in flesh foods.*

Foodstuff	Systematic name	Total iron (mg./100 g.)	Ionisable iron (as % of total iron)	
Fish:				
Cod, raw	*Gadus morrhua*	0·34	100	
Haddock, steamed	*Gadus aeglefinus*	0·75	100	
Halibut, raw	*Hippoglossus vulgaris*	0·44	100	
Herring, fried	*Clupea harengus*	1·02	74	
(Bloater) fried	—	1·29	31	
raw	—	1·02	39	
(Kipper) boiled	—	1·38	58	
Mackerel, fried	*Scomber scombrus*	1·17	64	
Plaice, steamed	*Pleuronectes platessa*	0·68	97	
Prawns, boiled	*Leander serratus*	0·62	81	
Rock salmon (catfish), raw	*Anarrhichus lupus*	0·36	44	32
Roe (herring), raw	—	0·63	99	
Salmon, "Shipmate" tinned	—	0·89	94	
Sardines "Skipper" tinned	—	3·44	65	
Skate, raw	*Raja batis*	0·33	100	
Sole, steamed	*Pleuronectes microcephalus*	0·45	100	
Winkles, boiled	*Littorina littorea*	10·4	58	
Muscular organs. Animal and Bird:				
Beef, raw	—	3·54	11	9
roast	—	5·20	22	15
corned, tinned	—	3·34	35	
Chicken, white flesh, roast	—	1·60	31	
red flesh, roast	—	2·70	24	
Heart, baked	—	5·83	63	
Mutton, roast	—	5·10	24	
Pig products, Bacon, raw	—	2·50	29	
Chop, fried	—	1·40	47	
Ham, cooked	—	4·45	15	
Rabbit, stewed	—	1·89	42	
Tongue, cooked	—	5·80	19	
Veal, raw	—	0·93	45	35
roast	—	1·35	55	
Glandular organs:				
Kidney, Ox, stewed	—	4·92	66	
Pig, fried	—	9·50	58	
Liver, Calf's, raw	—	13·30	100	
Lamb's, fried	—	2·76	100	
Ox, raw	—	6·70	78	100
Pig's, raw	—	20·00	80	
Sweetbreads, raw	—	1·47	71	
Miscellaneous:				
Egg, raw	—	2·50	100	
Sausage, Beef, fried	—	4·18	72	
Pork, fried	—	3·39	81	

Table II. *Comparison of methods for ionisable iron.*

Nature of material	Weight taken for analysis g.	Original method: Ionisable iron in sample mg.	Original method: Iron added to sample mg.	Original method: Iron recovered mg.	Modified method: Ionisable iron in sample mg.	Modified method: Iron added to sample mg.	Modified method: Iron recovered mg.
Currants, Black	5	0·025	0·05	0·01	0·05	0·05	0·05
,, Red	5	0·012	0·05	0·033	0·028	0·05	0·049
,, Dried	2	0·012	0·05	0·025	0·019	0·05	0·051
Loganberries	5	0·017	0·05	0·035	0·038	0·05	0·05
Raspberries	5	0·025	0·05	0·043	0·035	0·05	0·05
Cocoa	1	0·016	0·05	Traces only	0·135	0·05	0·05

certain proteins and will not react with $\alpha\alpha'$-dipyridyl. Hydrosulphite reduces the iron, and the ferrous iron, which does not form these complexes, will then react with the dipyridyl.

(*f*) The p_H was always tested before the hydrosulphite was added, and if not between p_H 5·4 and 5·8 was brought within these limits by the necessary additions. Sherman *et al.* [1934, 2] have reported that they have experienced some difficulty in this respect with animal tissues, but we have only had to reinforce the buffer in the case of some acid fruits.

(*g*) *Impurities.* The hydrosulphite has already been discussed. Ordinary filter-papers were found to contain iron, sometimes in large amounts, but Whatman papers No. 541 were reasonably satisfactory. Reagent blanks were carried out from time to time, filtered and placed in the comparator when matching unknowns. These blanks were generally less coloured than the 0·0025 mg. standard.

(*h*) *Interference with colour development.* As judged by recoveries this only occurred twice. Blackberries were found to contain a substance which prevented ferrous iron from reacting with $\alpha\alpha'$-dipyridyl. The natural pigment of the fruit was completely decolorised by the hydrosulphite, so that no difficulty was experienced in matching the solutions. The filtered juice behaved in a similar manner, but the interference observed was variable in amount. The figure given in Table A for the available iron in this fruit was obtained from a sample which gave very slight inhibition of colour development, but the result may still be too low. It was also impossible to obtain satisfactory recoveries from walnuts, and the figure given for the ionisable iron is probably too low.

Determination of total iron.

Samples were incinerated according to the method of McCance and Shipp [1933]. Such foodstuffs as liver, raw sweetbreads and fruits with a high sugar content tend to ash badly, and in these cases the ash was moistened with a little water, two or three drops of concentrated hydrochloric acid were added, the whole was placed on a hot plate until dry, and the ash was then again incinerated. When only traces of carbon were left, the ash was cooled, moistened with water and heated with 2·5 ml. of concentrated A.R. HCl. 10 ml. of approximately $N/2$ HCl were added, the contents of the crucible brought to the boil and filtered into a 100 ml. graduated flask. Three further extractions of the small charcoal residue (if any) were made using 10 ml. of $N/2$ acid each time. The filter-paper was washed with boiling water until the contents of the flask were near the graduation mark. When cool, the volume was made up to 100 ml. with distilled water. The iron in an aliquot of this solution was determined by means of thiolacetic acid [Lyons, 1927]. The presence of pyrophosphate does not interfere with the development of the colour. The incineration and determination were always carried out in duplicate. $\alpha\alpha'$-Dipyridyl was also used to determine the total iron in some foodstuffs by a method somewhat similar to that used by Hill and Keilin [1933]. In general the results obtained by both methods were in close agreement,

but with meat αα′-dipyridyl tended to give low results. Since the determination of total iron by αα′-dipyridyl was originally undertaken only as a further test of the value and scope of this reagent, the iron figures obtained by incineration have been preferred in all cases of difference and are the only ones given in this paper. The low results obtained by the αα′-dipyridyl method are due to the incomplete decomposition of haemin compounds.

Sources of materials.

Material to be analysed was purchased from the Amalgamated Fruiterers, the Army and Navy Stores and local shops. Many of the figures were obtained on mixed samples from all three sources, but this has not always been possible and some determinations have been made on material purchased at one shop only. This applies particularly to rare fruits and to estimations of common materials which have been carried out for confirmatory purposes. The figures given for meat and fish apply in most instances to samples from one source.

Results.

Tables A and B contain the figures which have been obtained for total and ionisable iron. The figure for total iron is the mean of the results found on the separate occasions on which the material was analysed. The ionisable iron is given as a percentage of the total iron, and the results for different occasions are given separately. The materials analysed have been classified under vegetables, fruits, nuts, fish and meats, and are arranged in alphabetical order within each group.

Discussion of the results.

These figures for ionisable iron, depending as they do upon two determinations with independent errors, cannot be claimed to have a high degree of accuracy, even though each determination was carried out in duplicate and with the greatest care. When cloudy solutions were obtained, which made matching in the comparator difficult, an error of 10–15 % was possible, but this is not incompatible with valuable results for dietetic purposes. Percentages of the same order have been obtained when individual members of a group of similar foodstuffs have been estimated, or when the same foodstuff has been analysed more than once. There are many instances of this in Table A. The results for the cereal group, the dried and fresh apricots, the figs, and the plums and prunes illustrate this. The results for the herring family and the gadoid group of demersal fish are equally good examples. This is the more interesting because the total iron may vary considerably from one occasion to another, and it would seem from the results that the percentage of the total iron in ionisable form is perhaps a more characteristic feature of any particular material than the total amount of iron in it. This is illustrated by the figures shown in Table III. It will be seen that gross fluctuations in the total iron are not accompanied by variations in the percentage of that iron which reacts with αα′-dipyridyl. It is for this reason that the figures for total iron in Tables A and B have been averaged. This communication is not concerned with the variability of the total iron in food materials, or with the best average figure for dietary purposes. The figures in Table A will be found in some instances to be the same as those given for total iron by McCance *et al.* [1936].

(1) It would seem therefore that the ionisable iron of groups of foodstuffs, as determined by these methods and expressed as a percentage of the total, may be accepted with considerable confidence. Individual variations from the group

Table III. *Constancy of ionisable iron as percentage of the total iron.*

Foodstuff	1		2	
	Total iron (mg./100 g. wet weight)	Ionisable iron (as % of total iron)	Total iron (mg./100 g. wet weight)	Ionisable iron (as % of total iron)
Cauliflower	0·91	99	0·52	100
Cranberry	1·11	61	0·25	79
Egg plant	0·32	50	0·27	53
Fennel	9·06	45	4·10	49
Kidney	4·92	66	9·50	58
Mushroom	0·95	100	0·35	98
Mustard and cress	1·80	50	4·70	45
Pomegranate juice	0·15	33	0·24	33
Potato	0·85	95	0·40	100
Raisin	3·80	97	5·40	94
Tomato	0·47	66	0·20	74

figure have always been confirmed at least once before being included in Table A, so that they also may be accepted as having significance.

(2) It will be observed that a number of values for available iron are stated in Table III as being 100 % of the total. If cytochrome is a constituent of every aerobic cell this cannot be strictly true. It is certain also that some blood must have been included with liver and white fish, so that in these cases also a figure of 100 % should be accepted with reserve. The reason for obtaining figures of 100 % may be inaccuracies inherent in the method, the destruction of cytochrome by cooking, or by the heating for 10 min. at 100° to which raw materials were subjected. It is, however, probable from the experiments with haemin and the small percentage of ionisable iron found in meat, that under our experimental conditions negligible quantities of haematin are decomposed. Destruction by heating does not matter, for most foods are cooked before being eaten, so that for dietetic purposes the figures may be accepted.

(3) There are very few previous figures with which to compare the present ones. Elvehjem *et al.* [1933] reported that 47 % of the iron in wheat was "available" and 57 % of that in oats. The present results are higher, but our experience has been that the method used by Elvehjem may give, with plant products, results which are far too low (Table II). Rose *et al.* [1934; Vahlteich *et al.*, 1935] moreover, have found that wheat and bran are excellent sources of available iron. Sherman *et al.* [1934, 2] found that more than 60 % of the iron in liver was available and 50 % of that in beef muscle. According to the present findings the former of these seems rather low and the latter definitely too high. The figure obtained by these authors for soya bean agrees with the present group figure for legumes, but the present figure for spinach is higher than their figure obtained with $\alpha\alpha'$-dipyridyl and agrees with their figure for available iron determined by an acid extraction method.

(4) Considering the present series of results it has been found that: (*a*) At least 75 % of the iron in cereals reacts with $\alpha\alpha'$-dipyridyl. The average is much higher and may be taken to be about 90 %. (*b*) Only some 10–25 % of the iron in beef and mutton so reacts. Other flesh foods contain a variable but higher percentage of their iron in reactive form. Almost the whole of the iron in liver seems to be in ionisable form. (*c*) All the iron in most white fish reacts with $\alpha\alpha'$-dipyridyl, but there are exceptions, *e.g.* rock salmon (catfish). Herrings, mackerel and sardines contain only about 60 % of their iron in inorganic form. (*d*) The iron in most nuts is mainly ionisable but there are exceptions, *e.g.* Brazil nuts. (*e*) Green-leaf vegetables contain 60–70 % of their iron in inorganic form.

Cauliflower and globe artichokes, which are generally classed with the green vegetables but which are not really green leaves, contain nothing but inorganic iron. (*f*) Root and stem vegetables give variable results. Some contain only inorganic iron. Potatoes contain 90–100 % of their iron in this form. (*g*) Fresh and dried legumes contain 70–80 % of their iron as inorganic iron. Tinned beans and peas were found to contain all their iron in reactive form and this difference may be due to destruction of the organic compounds during the preserving. It is perhaps worth noting that tinned meat also contained a much higher percentage of its iron in the inorganic state than fresh or domestically cooked meat. Compare also the results for cocoa, which have been confirmed on several occasions, with the average figure for the legumes. (*h*) 50–100 % of the iron in most fruits is ionisable, and percentages of the same order have generally been found within each species. In plums, prunes and greengages, for example, about 75 % of the iron was found to be in ionisable form. In cherries, apricots and peaches, however, nearly 100 % of the iron was ionisable. 80–100 % of the iron in apples and pears was found to be ionisable. Tomatoes contained only 50–70 % of their iron in this form.

SUMMARY.

1. The general applicability of $\alpha\alpha'$-dipyridyl to the determination of ionisable iron in foodstuffs has been investigated, and methods have been described for its use with plant and animal materials.

2. The ionisable iron in 155 British foodstuffs has been determined by means of $\alpha\alpha'$-dipyridyl. Total iron has also been determined in these foods.

3. The percentage of the total iron in ionisable form may be a more characteristic feature of any particular material than the total amount of iron in it.

One of us (L. S.) is indebted to the Medical Research Council for a personal grant.

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