U.S.P. chemical requirements for desiccated thyroid —although biologically substandard—may thus have been used for the manufacture of defective U.S.P. thyroid tablets. It has not been possible to ascertain with certainity the primary source of the nongenuine thyroid material.

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Determination of Iron Content in Mice

By THEODORE H. EICKHOLT* and WALLACE F. WHITE

A colorimetric method was employed in the estimation of the normal iron content of mice. An estimate of the total amount of iron present per mouse was 1.457 ± 0.152 mg. This represented an average of 52 ± 6 mcg. per Gm. body weight of mouse with approximately 11.5 per cent of the total iron per mouse present in the gastrointestinal tract and 88.5 per cent in the remainder of the carcass.

AN ESTIMATION of normal iron content of mice, the total amount contained therein compared with the amount contained in the separated entire gastrointestinal tract, is indicated in studies of iron absorption following oral administration.

There are numerous methods available for estimating iron content; *o*-phenanthroline, α,α'-dipyridyl, and benzidine methods (1–13). A sodium sulfocyanate or potassium thiocyanate method was described by Kennedy (14), improved and simplified by Farrar (15), Andes and Northup (16), and Wong (17), resulting in the method for the determination of iron in blood and hemoglobin published by the Fisher Scientific Company (18).

Employing a modification of the Fisher method, it was the purpose of this investigation to determine an estimate of the normal amount of iron present in a whole mouse carcass and that present in the separated gastrointestinal tract. This latter method of separation of carcass and gastrointestinal tract is essentially the method described by Cori *et al.* (19–21).

EXPERIMENTAL

The method employed was a colorimetric analysis with the Fisher model AC electrophotometer. The procedure as published (18) was followed except for the elimination of the tungstate solution. Since the tungstate solution was only involved in precipitating protein, it was unnecessary in the procedures employed in this investigation.

The iron, after being liberated and oxidized with sulfuric acid and potassium persulfate, was treated with potassium thiocyanate. The electrophotometer scale reading of the resulting colored solution was determined, and the iron concentration was calculated from this using a calibration curve.

Following the determination and calculation of the standard iron concentration curve, as described in the Fisher method, iron determinations were done on whole mice fasted at least 20 hours but not longer than 24 hours. Six B4BC male mice, 5 weighing 27 Gm. and one weighing 26 Gm., were sacrificed and digested in a Kjeldahl flask using concentrated sulfuric and nitric acids. The complete digestion resulted in a clear solution of sulfuric acid with or without a small amount of white precipitate (calcium salts) in the bottom of the flask.

The sulfuric acid solution was cooled and slowly diluted to 100 ml. with distilled water and was used as the sample solution for iron determination using the modified Fisher method.

At all times, precautions were taken to use glass distilled water and carefully cleaned glassware.

A separate determination on the gastrointestinal tract and on the remainder of the mouse carcass was done on three 32.5-Gm. B4BC male mice employing the same procedure.

In experiments done to observe the method and obtain more preliminary control figures for the amount of iron present, mice in groups of 6 were fasted, sacrificed, separated as to carcass and entire gastrointestinal tract, digested, and an aliquot amount obtained for sample determination. In a number of animals equaling that of the controls, the same procedure was employed, except that 1 hour prior to sacrificing the animals received a total oral dose of 1, 2, or 4 mg. of ferrous sulfate in solution, the concentration of which was varied to provide near equal volumes orally administered.

Ordinarily, the feces had not been dealt with. In two of the above groups of animals, one a con-

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TABLE I.—IRON DETERMINATIONS ON A SERIES OF INDIVIDUAL MICE

Mouse No. and Wt. in Gm.	% Absorption	Sample Size, ml.	Total Iron Present, mg.	mcg. Iron per Gm. wt. of Mouse (Av. of Samples)
1, 26	8.0	1	1.5	
	30.0	4	1.425	56
2, 27	9.5	1	1.75	
	34.5	4	1.64	63
3, 27	9.0	1	1.65	
	32.0	4	1.53	59
4, 27	8.0	1	1.5	
	30.5	4	1.45	55
5, 27	34.5	5	1.32	
	35.0	5	1.34	
	34.5	5	1.32	
	34.5	5	1.32	50
6, 27	33.5	5	1.28	
	33.5	5	1.28	
	33.0	5	1.26	
	33.0	5	1.26	47

TABLE II.—SEPARATE IRON DETERMINATIONS OF INDIVIDUAL MOUSE CARCASS AND GUT

Mouse, No.	Sample Size, ml.	% Absorption	Total Iron Present, mg.	mcg. Iron per Gm. wt. of Whole Mouse
1, Body	5	33.0	1.26	
1, Gut	10	10.0	0.185	44
2, Body	5	38.0	1.44	
2, Gut	10	5.0	0.09	47
3, Body	5	37.0	1.40	
3, Gut	10	12.0	0.22	50

trol group and the other an iron-treated group, receiving a total oral dose of 1 mg. ferrous sulfate, the feces were digested and the iron content determined along with the respective determinations on the carcasses and gastrointestinal tracts.

RESULT AND DISCUSSION

The values read from the electrophotometer for each initial whole mouse tested were based upon the values obtained from the standard iron concentration curve. The values tabulated in Table I by observation show the variability in the method to be relatively small, largely attributed to differences in the mice. The different and replicate sample sizes were employed simply to check the method of procedure without applying any statistical analyses on this number of animals employed.

The following averages were calculated from the data in Table I: average iron present per mouse, 1.470 mg.; average weight per mouse, 26.83 Gm.; average mcg. iron per Gm. mouse, 56 mcg.

The values obtained from the separate gastrointestinal and carcass determinations are recorded in Table II. The sample size, absorption reading, and the amount of iron present are given. The amount or size of the sample, the 1/2 ml. ratio of the sample sizes for the mouse and gut samples, respectively, was arbitrarily chosen. The two separate determinations would be necessary as controls for use in absorption studies where the gut and rest of the body could be treated separately in the attempt to determine whether alteration in absorption had taken place. Knowing the amount of iron administered, the amount in the gut, and the amount in the rest of the body, it would be possible to ascertain whether absorption had been changed by any experimental procedure employed.

The average iron per mouse from the data in Table II was 1.532 mg. (1.470 mg., Table I); the average mcg. iron per Gm. mouse was 47 (56 mcg., Table I).

From these data, an average percentage of approximately 11% of the total iron present was found in the gastrointestinal tract, leaving approximately 89% in the remainder of the carcass.

In the expanded experiments on mice in groups of six, the averages of iron present per mouse carcass and gut, separate and combined, are noted in Table III. Although there is some individual overlapping, the average figures observed indicate a reliable positive trend toward the differences between the control and iron treated groups. However, the finding of less iron in the animals treated with the larger doses of iron is apparently due to the colorimetric method employed, the test solution being too concentrated over extending Lambert-Beer's law. The amount of iron apparently unaccounted for here was linearly related to the amount of iron that had been added, again indicating the overextension of the Lambert-Beer's law. In the groups of mice treated orally with the 2- and 4-mg. iron doses, the animals apparently had an inherent low iron content prior to treatment, thus showing a low carcass iron content. However, when compared on a microgram iron content per gram weight basis the figure shows the expected increase, although no

TABLE III.—AVERAGES FROM MICE IN GROUPS OF SIX OF IRON PRESENT PER MOUSE CARCASS AND GUT, SEPARATE AND COMBINED[a]

Group	Av. Iron Content in Carcass, mg.	Av. Iron Content in Gut, mcg.	Av. Iron Content per Mouse, mg.	Av. Iron Content per Gm. Mouse, mcg.
Controls	1.45	210	1.660	47
	1.20	192	1.392	55
	1.12	110	1.230	56
Combined Av.	1.26	171	1.427	53
With total oral dose of 1 mg. ferrous sulfate	1.61	342	1.952	61
	1.43	410	1.840	59
	1.26	380	1.635	68
Combined Av.	1.43	377	1.809	63
With total oral dose of 2 mg. ferrous sulfate	1.265	425	1.690	68
With total oral dose of 4 mg. ferrous sulfate	1.380	530	1.910	73

[a] Single figures indicate averages of mice in groups of six with combined averages of these where more than one group was tested.

apparent increase in carcass iron (iron absorption) was observed to occur in the limited (1 hour) time period prior to sacrificing and determination in any of the iron treated groups.

From these data on the control groups, an average percentage of approximately 12% of the total iron present was found in the gastrointestinal tract, leaving approximately 88% in the remainder of the carcass. The average iron content per mouse was 1.427 mg.; the average iron content per gram mouse was 53 mcg.

From the determination of the iron content in the feces from two groups of mice, the average iron content of the feces per mouse in the control group was 27 mcg. and in the iron treated group 13 mcg. This would appear to indicate that estimates of fecal iron could be ignored in selected studies.

SUMMARY AND CONCLUSIONS

As modified in this laboratory, the Fisher method was observed to be relatively accurate within concentration limits and comparatively easy to accomplish. The digestion of the animal was the most tedious part of the technique.

An average estimate of the total amount of iron present per mouse was 1.457 ± 0.152 mg. This represented an average of 52 ± 6 mcg. per Gm. body weight of mouse with approximately 11.5% of the total iron per mouse present in the gastrointestinal tract (168 ± 54 mcg.) and 88.5% in the remainder of the carcass (1.31 ± 0.14 mg.).

In the animals treated orally with 1, 2, and 4 mg. of iron 1 hour prior to sacrificing and determination, the average percentages of iron present in the gastrointestinal tracts were 21, 25, and 28, with 79, 75, and 72% in the remainder of the carcasses, respectively. Low inherent carcass iron content must be considered even when comparisons are made on a microgram iron content per gram weight basis as no apparent increase in carcass iron (iron absorption) was observed to occur in the limited (1 hour) time period prior to sacrificing and determining in any of the iron treated groups.

Employing this method, the quantity of iron apparently unaccounted for in the animals treated orally with iron was in direct linear relationship to the amount of iron administered or added and due to the inherent properties of the colorimetric method itself; therefore, the quantities of iron determined are relative and indicative of the true quantities of iron present.

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3-Bromopyruvic Acid, a Highly Selective Antimicrobial Agent Against Certain Yeasts

By WALTER A. ZYGMUNT and TELLIS A. MARTIN

3-Bromopyruvic acid has been shown to be a highly effective growth inhibitor of certain yeasts. Wide differences were noted in the inhibitory activity of the compound among different species and genera of yeast.

THE USE of halogens as antimicrobial agents is well documented, and certain halogenated metabolites possess potent antimetabolite activity. Among such compounds are fluoroacetic acid (1), halogenated phenylalanines (2), 5-halogenated pyrimidines (3), and many others.

Busch *et al.* (4) have reported that β-halogenated pyruvic acids have pharmacological activity as cholinesterase inhibitors. However, there appears to be only limited information in the literature on the activity of such compounds as possible antimicrobial agents. 3-Bromopyruvic acid was selected as a representative compound. This report summarizes our findings on its inhibitory activity against a variety of microorganisms.

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EXPERIMENTAL

Since significant growth inhibition was noted only with certain yeasts and yeast-like fungi, description of the testing techniques employed will be restricted to this group of microorganisms. Yeast nitrogen base medium (Difco), supplemented with 1.5% dextrose and an initial medium pH of 4.5, were used. Solutions of 3-bromopyruvic acid and—where applicable—of reversal agents were adjusted to pH 4.5, sterilized by Seitz filtration, and portions added to previously autoclaved flasks containing sterile distilled water and basal medium. Only freshly prepared solutions of test compound and reversal agents were used. The final volume was 10 ml. per 50-ml. conical flask. In the preparation of inocula, all cells were carefully washed and standardized to a constant absorbance reading. All flasks were agitated on a rotary shaker during incubation for 20 hours at 28°. Growth was measured turbidimetrically as absorbance using a Coleman Junior spectrophotometer at a wavelength of 620 mμ.

All yeast cultures tested, except for *Candida albicans* (ATCC 10231), were kindly supplied by Dr. L. J. Wickerham, Northern Utilization Research