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Lack of Effect of Secondary Liver Extract (No. 55) on Absorption of Radioactive Iron.*

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Introduction. It has long been recognized that in anemias due to chronic hemorrage, either experimental or pathological, iron is efficient in the restoration of the normal blood picture. An inability on the part of the body to form enough of the porphyrin fraction of the hemoglobin molecule has never been established, even under the most stringent conditions. It is with extreme difficulty[1] that one can demonstrate the limitation of hemoglobin production under experimental conditions through the lack of protein for the formation of the globin part of hemoglobin. That other factors are involved, however, in the production of hemoglobin or the stimulus to its production, is suggested by the fact that Whipple, Robscheit-Robbins, and Walden were able to show[2] that a liver fraction other than that most active in the treatment of pernicious anemia was capable of causing new hemoglobin formation. That the effect was not due to the iron content alone was suggested by the observation that there seemed to be "summation response" when this extract and iron were given in combination as contrasted to the administration of either alone.

This liver fraction, called the secondary anemia fraction, or No. 55 fraction, contains that part of the liver material insoluble in 70% alcohol as distinguished from the "pernicious anemia fraction" (No. 343 fraction).

It seemed of interest to determine whether or not there was a possibility that such a fraction might be involved in the augmentation of iron uptake from the gastro-intestinal tract. With the availability of the sensitive means of determining uptake, using iron tagged with the radioactive isotope Fe^{59}, one is provided with a tool with which such an effect could easily be tested. That this "summation effect" is due to an increase in the absorption of iron from the gastro-intestinal tract seems to be pretty well ruled out by the following experiments.

Methods. The subjects studied were 11 normal women, laboratory technicians or secretaries, ranging in age from 21 to 45 years. In addition, 1 patient, who had had repeated massive hemorrhage due to duodenal and gastric ulcers, was included. The subjects were divided into 2 groups, half receiving at the first feeding iron tagged with the radioactive isotope alone and the other half receiving the same dosage level of tagged iron, plus 5 g of liver extract No. 55.† Two weeks after the feeding a single sample of whole blood was drawn into ammonium and potassium oxalate, and this was divided into duplicate samples for estimation of radioactivity content of the red blood cells. At this time those subjects who had received iron alone were then given the same dosage of iron plus the liver extract, and that group which had received both iron and extract were given iron alone. Two weeks later the blood was sampled again, and feeding reverted to the original program as of the first experimental period. In this way each subject acted as his own control.

The radioactive iron was purified as described elsewhere[3] in order to eliminate contamination due to traces of radioactive cobalt, manganese, zinc, nickel, copper, indium, etc. The iron isotope used was a cyclotron

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1 Hahn, P. F., and Whipple, G. H., *J. Exp. Med.*, 1939, **69**, 315.

2 Whipple, G. H., Robscheit-Robbins, F. S., and Walden, G. B., *Am. J. Med. Sci.*, 1930, **179**, 628.

† The No. 55 Liver Extract fraction was provided through the courtesy of Dr. W. W. Davis of the Eli Lilly Company.

3 Hahn, P. F., *Ind. and Eng. Chem.*, 1945, **17**, 45.

TABLE I
Effect of Concomitant Feeding of Secondary Liver Extract (No. 55 Fraction) on Absorption of Iron Tagged with the Radioactive Isotope Fe^{59}. Dose of Fe = 39 mg.*

Subject	Wt kgm	RBC Hct.%	Uptake of tagged iron in % of administered dose when fed			
			Fe Alone	Fe + Liver	Fe Alone	Fe + Liver
E. C.	48.6	43	9.1	7.8	7.9	
P. J.	48.2	42		4.1	6.4	0.7
B. R.	53.5	44	1.4	1.4	3.9	
M. C.	50.4	44		5.3	9.9	7.1
D. R.	49.2	38	5.0	3.0	3.7	
J. C.	44.6	41		2.1	1.7	1.2
I. L.	39.7	43	5.1	1.2	4.2	
J. A.	55.8	44		1.5	7.4	2.3
M. G.	58.9	41	3.6	1.1	3.6	
B. P.	57.6	43		8.9	9.8	6.8
H. T.	52.2	42			4.7	7.8
C. P.	73.6	22	49.0	47.0		

* Except Subject C.P. who received 25 mg.

prepared product, Fe^{59}, made by the d-p reaction on Fe^{58}. The blood withdrawn was centrifuged in graduated 15 ml tubes in a type 1 International standard 8 unit head for 35 minutes at 2800 r.p.m. or more. The plasma was discarded following reading of the hematocrit value, and the red cells washed into Pyrex beakers, dried, ashed in a muffle furnace at 625°C, and the resulting iron electroplated as described elsewhere.[3] Radioactivity measurements were made, using a thin mica window, argon filled bell type Geiger counting tube, in conjunction with M.I.T. counting rate meter. The assumption was made that all iron absorbed was utilized, and the calculation of uptake was based on calculations described previously.[4]

Experimental Observations. In Table I, below, are summarized the results of the experiments carried out. The iron uptakes as measured with the radioactive tracer varied in these women from 1½ to 10% of the administered dose. The series involved is too small to allow one to speculate effectively on the meaning of such variation. It is to be noted, however, that with such a wide range of uptake, one must interpret carefully the absorption of single doses of iron in attempt ing to use this technique for diagnostic purposes.[3] It was for this reason that the procedure was carried out as described above, where each individual would furnish a control for each particular experiment. It is obvious on inspection of Table I that there is no enhancement of the uptake of the tagged iron resulting from the concomitant administration of the secondary liver extract. If anything, there is a suggestion that the liver extract might conceivably, to a small extent, inhibit the absorption of iron, although this may not be statistically significant. The rationale of such an inhibition might be related to the presence of phosphates or related material, which act to precipitate iron in the gastro-intestinal tract and prevent its absorption before it has passed beyond the stomach and duodenum.

Discussion. It is of interest to note that the one individual who had a marked iron deficiency type of anemia related to repeated acute and chronic blood loss, patient C.P. as shown in the table, showed the typical high efficiency of absorption and utilization of iron which one associates with such a condition, and this contrasts very markedly with the uptakes in the normal laboratory workers and secretaries who made up the remainder of the experimental group of subjects.

It is necessary to point out that these experiments, in spite of the sensitivity of quantitation attainable through the use of the radioactive isotopes over the technique used by Whipple and Robscheit-Robbins, differed

[4] Hahn, P. F., Bale, W. F., Ross, J. F., Balfour, W. M., and Whipple, G. H., *J. Exp. Med.*, 1943, **78**, 169.

in one possibly important matter in that they represented the feeding of single small doses of tagged iron; whereas, the work done on dogs by the other investigators represented daily feedings of iron and liver extract over several weeks periods. Recently Granick[5] has shown that the ferritin of the gastro-intestinal tract increases following the feeding of iron. Thus we must keep in mind the possibility that the body may adapt itself to the absorption of larger amounts of iron through some as yet unknown mechanism for the production of the material which itself is probably concerned with iron absorption.

It is important also to keep in mind the fact that the experimental standard anemic dogs used by Whipple and Robscheit-Robbins in their classical experiments on factors involved in hemoglobin regeneration are not simple "iron deficiency" experimental animals. The iron content of the salmon-bread diet fed these animals is adequate for normal growth and maintenance, but probably not sufficient to provide for the increased demands imposed by the necessity of removal of large amounts of blood occasioned by feedings of active supplements. Since it has been shown, as mentioned earlier,[1] that under certain circumstances restriction of proteins may cause a lowered ability to form sufficient hemoglobin in experimental hemorrhagic anemia in dogs, it is possible that under the conditions of the experiments cited by Whipple, Robscheit-Robbins, and Walden that there was a more complicated deficiency involved, in which more than one factor or two interrelating factors were involved.

Whatever the explanation may be as to the different results obtained under these widely various sets of conditions, it is apparent that any influence on hematopoiesis exerted by liver extract No. 55 is not one which acts through its ability to augment the absorption of the iron from the gastro-intestinal tract.

Summary. Iron tagged with the radioactive isotope Fe^{59} was fed with and without supplements of liver extract No. 55 to a group of 11 normal adult women. There were no obvious differences in the absorption and utilization of iron when this material was given alone or in conjunction with the "secondary anemia fraction of liver." In one case of human iron deficiency due to multiple acute and chronic hemorrhage there was also no difference in the absorption of iron as indicated by this method.

The "summation response" described by Whipple, Robscheit-Robbins and Walden is apparently not related to the effect of the secondary liver extract on *absorption of iron* in the gastro-intestinal tract.

5 Granick, S., *J. Biol. Chem.*, 1946, **164**, 737.

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The Influence of Oral Saccharin on Blood Sugar.

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The purpose of this investigation was to determine whether or not sweet taste as a nervous sensation has an effect on carbohydrate metabolism. It was found that saccharin, a substance of intensely sweet taste, can cause a decrease in blood sugar, presumably through the reflex liberation of insulin.

Methods. Saccharin (Sodium salt o-benzoic-acid-sulfimide) was given to human subjects in doses of 0.05 g in 80 ml water. In one group of experiments the effect of single doses, and in another group the effect of repeated doses of saccharin solution was followed.

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