

flora of other antirachitic agents, such as cod liver oil and viosterol. It seemed best to begin with a test of the effect of ultra-violet irradiation, as this measure did not necessitate the introduction of any curative substance into the intestinal canal.

## 6071

### Hemoglobin Regeneration in the Anemic Albino Rat with Dietary Supplements of Spinach, Apricot and Liver.

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Waddell, Elvehjem, Steenbock and Hart<sup>1</sup> showed that anemic rats on a milk diet receiving a supplement of 0.5 mg. iron daily failed to materially increase their hemoglobin levels. Acid extracts of ash residue from liver, lettuce and corn when fed in amounts sufficient to supply 0.5 mg. iron daily, however, induced hemoglobin regeneration. Subsequently, Hart, Steenbock, Waddell and Elvehjem<sup>2</sup> presented evidence that the response elicited by vegetable and meat tissue ash is due to the presence of copper in small but sufficient amounts to act as a supplement to the iron. They also stated that their experiments failed to demonstrate the existence of an organic factor necessary for hemoglobin synthesis. Later work by Elvehjem, Steenbock and Hart<sup>3</sup> failed to substantiate the claim of Drabkin and Miller<sup>4</sup> that glutamic acid may serve to stimulate hemoglobin regeneration in anemic rats on a diet of whole milk with additional iron.

Myers and Beard<sup>5, 6</sup> report hemoglobin regeneration with but 0.25 mg. iron unsupplemented by other metals when fed with a milk diet. Larger doses, and supplemental metals as copper, manganese, arsenic, etc., gave more rapid hemoglobin regeneration.

This paper reports results obtained in experiments on rats with

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<sup>1</sup> Waddell, J., Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1928, **77**, 777.

<sup>2</sup> Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *J. Biol. Chem.*, 1928, **77**, 797.

<sup>3</sup> Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1931, **93**, 197.

<sup>4</sup> Drabkin, D. L., and Miller, H. K., *J. Biol. Chem.*, 1931, **90**, 531.

<sup>5</sup> Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 71.

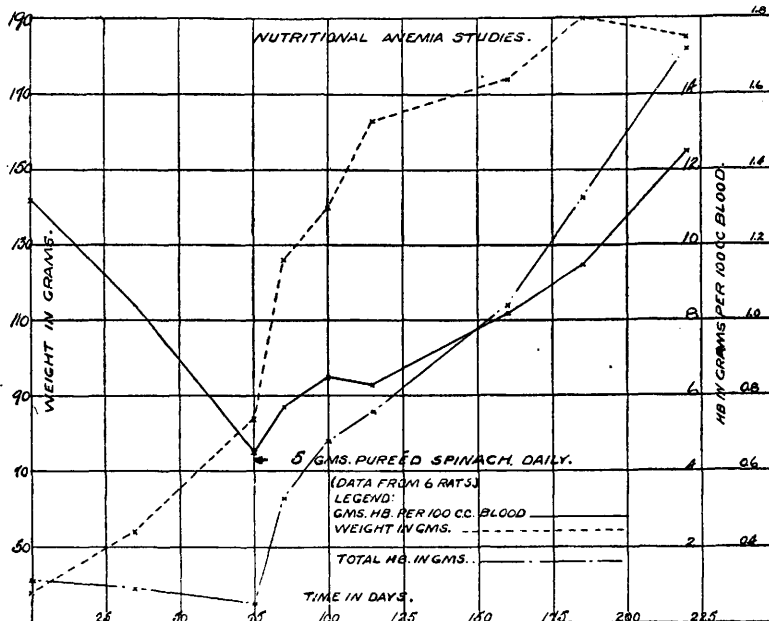
<sup>6</sup> Myers, V. C., and Beard, H. H., *J. Biol. Chem.*, 1931, **94**, 89.

nutritional anemia when a milk regimen is supplemented with spinach, apricot, or liver.

Albino rats at the time of weaning (28 to 30 days old) were placed in individual glass cages<sup>7</sup> on a diet of whole raw cow's milk (collected in glass) for a period of 70 to 80 days. During this interval the hemoglobin content decreased from an average value of 11 to 12 gm. per 100 cc. blood to 3.6 to 4.8 gm. At this time a definite quantity of the supplement was fed daily in a small glass dish, the completeness of consumption being noted. Hemoglobin regeneration was obtained with 5 gm. of pureed spinach daily as shown in Graph I.

GRAPH I.

Hemoglobin Regeneration on Daily Supplement of 5 gm. Pureed Spinach. (Typical Hemoglobin Depletion is Shown During 75 Day Period Prior to Spinach Feeding.)



The iron content is about one-fifth (0.0910 mg.) of the generally accepted optimum. Larger amounts of this supplement (10 and 20 gm. daily) gave more rapid regeneration. The maximum for 20 gm. is, however, but 0.364 mg. iron. The copper content is in all cases equal to or above the accepted minimum.

In experiments conducted with acid solutions of the spinach ash, obtained from 5 gm. of pureed spinach, hemoglobin regeneration was insignificant. A slow response to spinach ash as compared with an equivalent portion of concentrated aqueous spinach extract has been reported by Mitchell and Miller.<sup>8</sup>

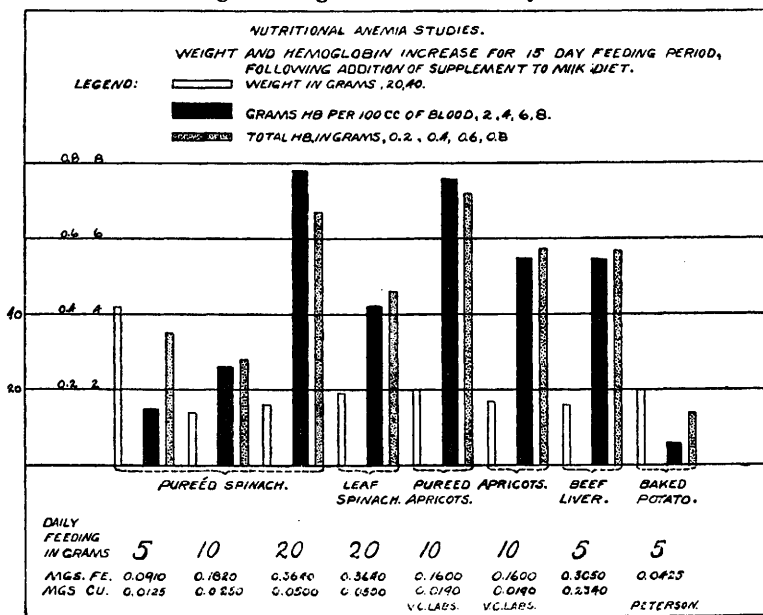
<sup>7</sup> Nevins, W. B., and Shaw, D. D., *Science*, 1930, **72**, 250.

<sup>8</sup> Mitchell, H. S., and Miller, L., *J. Biol. Chem.*, 1929, **85**, 355.

It has been reported by Zih<sup>9</sup> that anemic rabbits will regenerate hemoglobin on optimum doses of chlorophyll, while an overdose causes hemoglobin destruction. In order to check up on the hematopoietic function of the chlorophyll and mineral factors, extracted chlorophyll from 5 gm. of spinach was fed daily. We were unable to get a favorable response. Our results rather indicate a toxic action with chlorophyll at this level, when fed alone or with the ash equivalent of 5 gm. of pureed spinach.

Robscheit-Robbins and Whipple<sup>10</sup> have recently reported experiments on the relative hematopoietic efficiency of liver, apricot, and spinach in dogs rendered anemic by bleeding. From their results, these substances can be graded in a 4-2-1 ratio. We have found a close approximation to their ratio with the anemic rat as shown in Graph II.

GRAPH II.  
Hemoglobin Regeneration in 15 Day Period.



(The pureed and unpureed materials were from the same respective factory batches.)

It should be observed that supplements of pureed spinach or apricot give a greater hemoglobin response during a 15-day period than equal quantities of the non-pureed materials. Since the min-

<sup>9</sup> Zih, A., *Arch. ges. Physiol.*, 1930, **223**, 728.

<sup>10</sup> Robscheit-Robbins, F. S., and Whipple, G. H., *Am. J. Physiol.*, 1930, **92**, 400.

eral components of these respective materials are the same, our experiments would suggest a better utilization of the finely divided (pureed) material by the animal.

We wish to acknowledge the kindness of Dr. G. A. Fisher of the Van Camp Packing Company in supplying the spinach and apricots for this investigation. All material was sealed in lacquered tin cans. No evidence of corrosion could be detected nor was it indicated by the following analysis:

June 23, 1931, Canned Pureed Spinach	Fe = 0.001820%
Feb. 20, 1932, Canned Leaf Spinach	Fe = 0.001852%
Feb. 20, 1932, Above Spinach Hand Pureed	Fe = 0.001829%
Oct. 4, 1931, Canned Pureed Spinach	Cu = 0.000250%
Feb. 25, 1932, Canned Leaf Spinach	Cu = 0.000236%
Feb. 25, 1932, Above Spinach Hand Pureed	Cu = 0.000246%

All material was from the same original factory batch.

## 6072

### Nutritional Value of Proteins as Influenced by Exposure to Ultra-violet Irradiation.\*

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The experiments described below include (A) *in vitro* digestion experiments on irradiated and non-irradiated proteins with pepsin and trypsin respectively and (B) growth and metabolism studies on rats kept on low protein diets where the protein was either irradiated or non-irradiated.

#### A. *In vitro* Digestion Experiments on Irradiated and Non-irradiated Purified Proteins with Pepsin and Trypsin, Respectively.

1. Tryptic Digestion of Casein. The irradiated product was prepared by exposing dry casein (Merck according to Hammarsten) for 2½ hours at a distance of 18 inches to the mercury quartz lamp in a current of air. The digestion experiments with irradiated and non-irradiated casein were carried out as described by Helmer.<sup>1</sup> At the end of the digestion period the undigested protein was precipitated and the refractive indices of the filtrates read.

\* These researches were supported in part by the Quaker Oats Company.

<sup>1</sup> Helmer, Oscar, Doctor of Philosophy Dissertation, September, 1927, 10, University of Chicago.