



The present method has been employed for 2 years and the extract has been found to be potent both in inducing remissions in patients with pernicious anemia in relapse and in the maintenance of normal red blood cell levels.

Five pounds of fresh liver are ground and suspended in 5 liters of water. The mixture is heated in a water bath to 80°C and filtered. The residue is washed with hot water and the combined filtrates are concentrated to about one liter. Ethyl alcohol, 95%, is then added to a final concentration of 70% and the mixture is filtered. The alcohol is removed from the filtrate by distillation *in vacuo* and to the water extract, after filtration, is added a solution of 27.5 g of anhydrous CaCl<sub>2</sub> followed by the addition of 26.5 g of anhydrous Na<sub>2</sub>CO<sub>3</sub>. The formation of the CaCO<sub>3</sub> brings down a considerable amount of material and appears to accomplish the same result as the use of lead acetate in our previous method. After filtration the clear yellow solution is adjusted to pH 5 with hydrochloric acid and 25 g of norit charcoal are added. The mixture is shaken frequently over a period of an hour and the norit is filtered off by suction. After washing with a small amount of water, 10 g of norit are added to the filtrate and the mixture is shaken for a short time. After filtration the norit residues are eluted with 400 cc of hot 50% alcohol. The alcoholic extract is filtered off by suction, and the elution in hot alcohol is repeated. The combined alcoholic extracts are concentrated *in vacuo* until 3 cc represent 100 g of fresh liver. This light yellow solution is allowed to stand over night in the ice box and is then filtered, bottled, and sterilized.

The data in Table I show the reticulocyte response in a patient with pernicious anemia in relapse, receiving one 3 cc injection of the extract.

Normal red blood cell levels have been maintained in several patients by treatment with the concentrated extract for varying

TABLE I.

Days after Treatment	Red Blood Cells (millions per mm <sup>3</sup> )	Reticulocytes %
0	1.53	2.0
1		1.5
2		5.9
3		15.4
4		32.5
5		39.6
6		44.4
7	1.87	20.3
8		21.1
9		9.9
10		5.3

periods up to 2 years. One patient has received a 3 cc injection on an average of once every 15 days for 9 months. Two patients have had one 3 cc injection every 2 weeks, one for over a year and the other for about 6 months. It has been found necessary to administer 3 cc weekly to 2 patients to maintain a normal red blood cell level.

In the investigation of some of the properties of the extract determinations of total solids and total nitrogen were made on 12 individual lots derived from 5 pounds of fresh liver and one lot derived from 10 pounds. A marked difference may occur between individual lots of liver without apparent variation in potency and the total solid content has varied from 3.9% to 14.6% while the total nitrogen values have ranged from 0.41% to 0.91%. The average of the individual determinations gave a total solid content of 6.3% or 189 mg per 3 cc, and a total nitrogen of 0.62% or 18.6 mg per 3 cc.

The extract reduces alkaline copper solution, is precipitated by phosphotungstic acid, and gives a positive biuret reaction. Saturation with ammonium sulfate produces a precipitate which is active in inducing a reticulocyte response but is not so potent as the original solution.

The material filtered off after concentration of the alcoholic elute and cooling in the ice box gives a positive murexide test and is difficultly soluble in cold water. This substance resembles the complex purine described by Subbarow, Jacobson and Fiske<sup>2</sup> and classified by Jacobson and Subbarow<sup>3</sup> as an "accessory" factor which augments the action of a primary factor. This material has always been discarded in our preparations since the concentrate from which it is filtered has shown satisfactory hematopoietic activity.

Tyrosine, which has also been called one of the accessory factors,<sup>3</sup> has been found in charcoal filtrates of liver extract by Subbarow, Jacobson and Fiske.<sup>2</sup> Using both the Millon reaction and Folin-Marenzi procedure, tyrosine was found in our final product which is a charcoal adsorbate, not a filtrate. The charcoal filtrates have also been tested and no tyrosine could be detected. This is in disagreement with the original work of the above authors<sup>2</sup> but may be due to the use of a particular type of charcoal. We have observed that 0.5 g of our norit adsorbs all the tyrosine from 10 cc (10 mg tyrosine) of the standard tyrosine solution used in the Folin-

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<sup>2</sup> Subbarow, Y., Jacobson, B. M., and Fiske, C. H., *New Eng. J. Med.*, 1935, **212**, 663.

<sup>3</sup> Jacobson, B. M., and Subbarow, Y., *J. Clin. Invest.*, 1937, **16**, 573.

Marenzi procedure, while with the use of a greater amount of another type of charcoal tyrosine could be detected in the charcoal filtrate.

By use of the method of preparation here described the concentrated liver extract contains tyrosine, which Subbarow, Jacobson and Fiske<sup>2</sup> postulate as one of the factors necessary for blood formation.

*Summary.* 1. A method for the preparation of a concentrated liver extract is described in which the precipitation of  $\text{CaCO}_3$  by the interaction of  $\text{CaCl}_2$  and  $\text{Na}_2\text{CO}_3$  is used to remove protein, and the active principle is subsequently adsorbed on norit. Of this extract 3 cc are derived from 100 g of fresh liver. 2. The extract is effective in the treatment of pernicious anemia in relapse and in the maintenance of a normal red blood cell level. 3. Some properties of the extract are discussed. 4. Tyrosine is present in the final product.

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#### Determination of "Hormone Iodine" in 5 cc. Blood.\*

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In a series of 100 determinations of blood iodine by the McCleendon-Bratton method it appeared as if a long time was required to eliminate a dose of Lugol's solution from the blood.

This suggested the desirability of developing a method for fractionating quantitatively the iodine of blood. We found when a sample of blood is divided into 2 parts and to 1 part KI is added and then the blood samples are subjected to the following procedure that the KI does not appreciably raise the blood iodine.

Five cc of blood is spurted through a fine opening into 100 cc of methanol in a glass-stoppered flask of capacity of about 115 cc to the stopper. This is violently shaken and then allowed to settle. The methanol is decanted and 100 cc of acetone introduced and the shaking repeated. An 8-inch length of  $\frac{3}{8}$ -inch Visking sausage casing is closed at one end by any method, and the other end tied onto a 100 cc burette without stop-cock. The acetone suspension is shaken and poured into the burette. The acetone filters through

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