A PARTIALLY PURIFIED LIVER EXTRACT THERAPEUTICALLY EFFECTIVE IN PERNICIOUS ANEMIA*

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IN the course of experiments on the isolation of the hematopoietically active liver material it was found, in April, 1933, that filtrates of charcoal adsorption on therapeutically active liver extracts were inert in pernicious anemia. Attempts to extract the active material from the charcoal with a variety of eluents were begun in June, 1933. None of the eluents studied, among them isopropyl, butyl, and amyl alcohols, yielded products from different batches of crude liver extract that were consistently therapeutically active. Since September, 1934, elution by means of ethyl alcohol has always resulted in active extracts. Mention of this procedure has already been made in a previous communication[1]. The preparation and biological activity of this material are described in detail in this paper.

Patient	J. T.	C. H.	F. W.	A. T.	J. D.	C. H.
Date	9/27/34	1/16/35	1/3/35	10/1/35	2/20/35	2/1/35
Red blood cells in millions per c. mm. at beginning of experimental period	3.51	1.18	2.47	1.07	2.10	1.36
Red blood cells in millions per c. mm. at termination of experimental period	4.07	1.69	2.82	2.42	2.68	2.28
Reticulocyte peak, *per cent*	5.6	10.6	7.8	31.8	11.2	26.6
Length of experimental period, *days*	10	9	9	10	8	11
Total amount of fresh liver, from which administered extract, derived, *grams*	67	72	88	100	103	200
Total amount of nitrogen administered, *milligrams*	8.3	9.4	13.4	12	14	24

The starting point in the preparation is a commercial liver extract‡, in a concentration of 3 cc. derived from 100 Gm. of fresh liver. One hundred and fifty cc. of this extract are dissolved in one liter of water. The solution is brought to pH 8 with NaOH and is then acidified to pH 6 with HCl. Fifty Gm. of norit are added and the mixture is stirred mechanically for one hour, and filtered. The charcoal, washed repeatedly with water until the washings are colorless, is then suspended in one liter of 65 per cent ethyl alcohol, the mixture is brought to the boiling point, stirred mechanically for five minutes, and filtered hot. The elution is repeated once. Both elutes are combined and concentrated under diminished pressure at 40°C. to a volume of 150 cc. (3 cc. per 100 Gm. of liver).

Different batches of the commercial liver extract contain 140 to 180 mg. of total nitrogen, per 100 Gm. of liver, and exhibit a biological activity of approximately 328,000 guinea pig units, per 100 Gm. of liver[2]. The light-brown-colored ethyl alcohol elute, on the other hand, contains from 12 to 15 mg. of total nitrogen per 100 Gm. of the fresh liver from which it is derived, and a biological activity of approximately 164,000 guinea pig units. The evidence for the therapeutic efficacy of this elute in pernicious anemia is presented below.

The elute was sterilized by boiling and was administered to the patients by intramuscular injection.

The preparation of the elute described above, and referred to in a previous publication[1], bears similarities to the procedure recently reported by Kyer[3].

SUMMARY

Charcoal adsorption is utilized in the preparation of a partially purified liver extract that is therapeutically effective in pernicious anemia.

REFERENCES

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‡Lederle Solution Liver Extract Parenteral Refined and Concentrated, N.N.R. This material was generously furnished by the Lederle Laboratories, Inc., through the courtesy of Dr. Guy W. Clark.

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