N-ethyl-l-methyl butyl barbiturate (nembutal), and sodium N-ethyl-l-methyl butyl thiobarbiturate (pentothal). In each case, a 5% solution was freshly prepared and injected slowly intravenously until the lid reflex disappeared. The doses varied between 30 and 60 mg per kilo body weight. An endotracheal tube was introduced by the oral route as soon as surgical anesthesia supervened in order to assure a patent airway and obviate the effect of the ensuing anoxemia.

In every instance and with all the different barbiturates used the immediate effect, lasting from 5 to 15 minutes following the injection, consisted of a marked reduction in the amplitude of intestinal contractions and a fall in general tonus. Immediately following this primary depressing effect, however, there was a gradual rise in general tonus to above normal and increased rhythmic intestinal contractions which were maintained until the animal awakened. This secondary period during which anesthesia was still evident lasted from 10 minutes to 2 hours depending upon the rapidity of action of the particular barbituric acid derivative used. Fig. 1 shows the typical picture obtained with nembutal.

*Conclusion.* The short-acting barbituric acid derivatives studied when injected intravenously cause an immediate depression of rhythmic intestinal contractions and tonus. This primary effect, however, is transient and is succeeded by a more prolonged phase characterized by increase in both intestinal contractions and tonus.

**10329**

## A Hematopoietic Substance in Liver.

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In two previous communications[1] the authors have shown that liver yields a peptide in some respects resembling a typical albumose capable of causing blood regeneration in pernicious anemia. Clinically active preparations were obtained which were completely free from pentose or other carbohydrate, purines, phosphoric acid, and sulphur. Other workers have stressed the presence of some or all of these groups or have postulated the accessory action of a variety of other chemical types which are absent from our material. Karrer,

[1] Dakin, H. D., and West, R., *J. Biol. Chem.*, 1935, **109**, 489; 1936, **115**, 771.

Frei and Fritzsche,[2] without reference to our previous work, stated that the clinical activity of their material was proportional to the phosphoric acid content and that adenine and pentose were also present. More recently, they describe the active substance as free from these substances and regard it as a biuret negative peptide with an elementary composition closely resembling that of our product. No experimental data are furnished but some amino acids contained in our product are stated to be absent, while other amino acids of unspecified type are said to be present. We have recently assured ourselves of the absence of a nicotinic acid group in our peptide.

The following note consists of the results of a few experiments on the precipitation of our substance with typical albumose precipitants including nucleic acid, bile, taurocholic, and other bile acids and the barium carbamate reaction of Seigfried. We find that the first 3 reagents yield precipitates containing much potent material. Glycocholic acid and the carbamate reaction gave unsatisfactory results.

The precipitation with nucleic acid was carried out as follows: Thymonucleic acid (4 g) was dissolved in decinormal sodium hydroxide (50 cc), diluted to 200 cc, and acidified with glacial acetic acid (2 cc). This was added with stirring to 50 cc of a 10% solution of our peptide. An immediate precipitation occurred, and the mixture was placed in the refrigerator overnight. The heavy precipitate was washed with water, acetone, and alcohol. Approximately half of the peptide was precipitated. Dilute hydrochloric acid decomposed the precipitate only incompletely, but it was easily soluble in disodium hydrogen phosphate solution and was tested clinically in this form. (Cases 552,986 and 553,894.) Thirty milligrams gave a reticulocyte increase from 1.8 to 24.2%, and the R.B.C. count rose from 1.5 to 2.9 million. Twenty milligrams gave a good positive but submaximal response. The precipitation by nucleic acid of some but not all the albumoses in Witte's peptone was observed by Löbisch[3] many years ago.

Filtered bile was adjusted to pH 3.5 by addition of dilute sulphuric acid and added by degrees to a solution of the peptide (1 g) in water (10 cc). About 20 cc bile was needed for maximal precipitation. After washing the oily precipitate with water it was dissolved in 60% alcohol (5 cc), made alkaline with alcoholic ammonia, and the clear solution precipitated with absolute alcohol (25

[2] Karrer, P., Frei, R., and Fritzsche, H., *Helv. chim. Acta*, 1937, **20**, 622; 1938, **21**, 314.

[3] Löbisch, W., *Beitr. z. chem. Phys. u. Path.*, 1906, **8**, 205.

cc). A significant amount of bile pigment was retained by the precipitate which was dissolved in water and tested clinically (Case 540,130). About 60% of the peptide was precipitated by the bile and its potency appeared to be slightly less than that of the original material.

Sodium taurocholate was dissolved in 10 parts of water and brought to pH 3.5 with dilute hydrochloric acid. The peptide (10 g) was dissolved in 20 parts of 3% acetic acid and the taurocholic acid added as long as precipitation occurred. The amount of taurocholic acid necessary was about one-third the weight of peptide. The finely divided precipitate was gently warmed to coagulate it and then after cooling, filtered off and washed with water. To regenerate the peptide, the precipitate was dissolved in a minimum of 60% alcohol, pyridine added (3 cc) to combine with the taurocholic acid, and then a large excess of acetone added. The recovered peptide only represented about 20% of the starting material. The precipitation of the peptide with taurocholic acid is apparently a reversible reaction between the 2 colloids. The very complete precipitation of proteins by taurocholic acid in contrast to its failure to precipitate the simpler peptones was observed by Maly and Emich[4] more than 50 years ago. On testing the material precipitated by taurocholic acid and that left in the filtrate it was found that both chemically and clinically there was no significant difference. (Cases 539,788, 543,019, 546,989, 481,608, 550,302.)

*Summary.* It has been shown that bile, taurocholic acid, and nucleic acid precipitate liver material active in pernicious anemia from aqueous solution.

## 10330 P

## Changes in Human Blood Preserved for Transfusion.

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The purpose of the present studies was to determine the rate and mode of disintegration of human blood in various preservatives suitable for transfusion.

[4] Maly, R., and Emich, F., *Monatschrift f. Chemie*, 1883, **4**, 89.