

In the 18-month litter the initial group average was 2.4 (minimum 2.3, maximum 2.7). On an adequate diet plus ammonium chloride (2 dogs) the final phosphatase values decreased to 1.5 and 1.8. On a low calcium diet, on the contrary, both with and without ammonium chloride treatment, the downward trend was not shown, the final phosphatase values remaining practically unchanged at 2.6 and 2.5, respectively.

The results of these experiments may be summarized as follows:

In growing animals the serum phosphatase normally decreases with age. On an experimental regime involving loss of appetite, further decrease of serum phosphatase would be expected. Therefore the serum phosphatase rise in the two younger litters is all the more convincing evidence of the greater susceptibility of young animals to the effects of experimental calcium deficiency and ammonium chloride acidosis. In older dogs, ammonium chloride is relatively ineffective in producing decalcification and serum phosphatase changes when an adequate calcium supplement is given.

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Pernicious Anemia: Method Whereby Therapeutic Efficacy of Liver and Liver Fractions May be Substantially Increased.

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Following the discovery of Minot and Murphy that raw liver is highly effective in the treatment of pernicious anemia, liver fractionation was undertaken by Cohn, Minot and their associates. The Cohn liver fraction G, in which approximately two-thirds to three-quarters of the potency of the original liver is recovered in 3 to 4% of its weight, has been generally employed in the oral treatment of pernicious anemia. Use of this preparation, involving the consumption of 10 to 14 gm. of material daily, entails some inconvenience to the patient. In attempting to isolate and identify the active principle Cohn, West and others have made very highly concentrated preparations but the procedures employed have not found practical application on account of their complexity and the losses of active principle entailed.

We here describe a method whereby the bulk of material administered orally to pernicious anemia cases to produce a given thera-

peutic effect may be substantially reduced, not by employing fractional methods as heretofore, but by so treating the raw liver or liver fractions (Cohn Fraction G) as to increase their therapeutic efficacy several fold.

These experiments were started immediately after Castle's demonstration that a thermolabile agent derived from the normal human stomach could interact with beef muscle to produce an active product which when administered to pernicious anemia cases induced remission with reticulocyte rise and increase in red cell count, while control pernicious anemia cases receiving normal human gastric juices alone and other control cases receiving beef muscle alone exhibited no such effects. Since fresh untreated muscle exerted no effect whatever, while a given weight of fresh muscle previously treated with normal stomach juice exerted a very substantial effect approximately equal to that exerted by an equal weight of fresh raw liver, it appeared important to determine whether a similar pre-treatment with normal stomach juices or stomach tissues would cause a corresponding rise in the therapeutic efficacy of the already active raw liver or liver fractions.

Fresh hog liver was ground and admixed with ground fresh hog stomachs, with and without the addition of acid, and held for varying periods of time, at varying temperatures, and the resulting products or their extracts evaporated *in vacuo* and defatted or subjected to alcoholic fractionation. Considerable difficulty was experienced in the conduct of these preliminary experiments but certain of the preparations obtained when tested on pernicious anemia cases exhibited definite gains in potency as compared with the original raw liver.

Subsequently preparations were obtained exhibiting a potency at least 3 to 4 times that of the raw liver from which they were derived.

Furthermore, the Cohn Fraction G (Liver Extract No. 343) when similarly treated with fresh ground hog stomachs yielded a product which showed a 3 to 4 fold gain in potency. The tests were conducted on pernicious anemia cases in relapse, the value of a given preparation being determined by the reticulocyte peak, the gain in red corpuscles and the general improvement of the patient as compared with the effect exerted by 225 to 300 gm. of raw liver or 3 to 4 vials of Liver Extract No. 343.

This increase in therapeutic efficacy of raw liver and of the Cohn Fraction G as a result of admixture and interaction with a moderate amount of fresh stomach tissues and products derived therefrom,

can not be explained as a purely additive effect. The clinical results indicate that one-third to one-seventh of a unit dose of liver or liver products treated with one-tenth to one-thirtieth of a unit dose of raw stomach or stomach products will exert an effect equal to that of a unit dose of liver or a unit dose of stomach.

These results find support in the report of Reimann¹ that the efficacy of raw liver in the treatment of pernicious anemia is markedly increased by admixture with normal human gastric juice prior to administration.

We desire to express our indebtedness to Dr. John H. Warvel, to Dr. L. G. Zerfas and Dr. P. J. Fouts, and to Dr. Howard L. Alt for conducting tests on pernicious anemia cases in relapse with the above described materials. The detailed clinical results will be reported in the near future.

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Value of Desiccation and Identification of Blood Typing Serums.*

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The accuracy of the determination of the blood group depends essentially upon the sterility of the typing serums and the freedom from formation of precipitates. The usual method of preserving stock typing serums is in a liquid form with the addition of 0.5% phenol. Lattes¹ has shown that serums preserved with phenol as well as many other preservatives, become turbid and form precipitates, and advises the use of sterile serums. The length of time that these aseptically collected serums will remain uncontaminated is variable and indefinite. Prati,² Grove and Crum³ have demonstrated that liquid typing serums are very subject to contamination by a mustard bacillus and thus develop in the contaminated serums a non-specific property of human blood cell agglutination.

Desiccation may offer a desirable means of preservation. The value of desiccation is dependent upon its effect in time on the

¹ Reimann, Von, Dr. F., *Med. Klinik*, June 12, 1931.

* Jr. article No. 94 (n.s.) from the Mich. Agr. Exp. Sta.

¹ Lattes, L., *L'individualite du sang*, Paris, 1929, Masson.

² Prati, Z. f. *Immunitätsf.*, 1928, **57**, 1.

³ Grove and Crum, *J. Lab. and Clin. Med.*, 1930, **16**, 259.