

rats might be attributed to an increase in the pituitary follicular stimulating hormone (FSH) without concomitant increase in the interstitial-cell stimulating hormone (ICSH). Such an interpretation would be in conformity with the well known fact that vitamin-E deficiency causes degenerative changes of the germinal epithelium without evident histological or functional changes in the interstitial cells in the testes. It is possible that differences in the quantities of the two gonadotrophins could be revealed if several different dose levels were to be used for the comparisons. However, no conclusion can be drawn from the experiments reported in Table II.

That the anterior pituitary of the vitamin-E deficient female rats also contained an increased amount of gonadotrophic hormone is of interest because of the controversy concerning ovarian function in vitamin-E deficiency. Although the ovaries of old, E-deficient rats showed a greater accumulation of connective tissue and pigmentation than those of controls, observations of ovarian function, such as the production of ova followed by fertilization, revealed no abnormalities. It remains to be investigated, however, whether the increased

gonadotrophin content of the pituitary gland indicates a disturbance of ovarian function or whether a different explanation must be sought.

Summary. The gonadotrophin content of the anterior pituitary taken from vitamin-E deficient rats, 315 to 469 days of age, was determined. The weights of testes, seminal vesicles and anterior prostates of groups of male rats hypophysectomized at 21 days of age and subsequently injected with suspensions of frozen-dried anterior pituitaries were compared. The testes of the hypophysectomized rats which received the pituitary suspension from vitamin-E deficient male or female rats were significantly heavier than those of rats which received pituitary powder from the controls. There was no difference in the weights of the seminal vesicles or anterior prostates.

It is concluded that the gonadotrophin content of the anterior pituitary gland is elevated in older vitamin-E deficient male and female rats. The relationship of these findings to the changes in the gonads in vitamin-E deficiency are discussed.

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A Probable Acid Mucopolysaccharide Present in Granulation Tissue.* (17487)

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(Introduced by M. Heidelberger)

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This paper is a preliminary report of observations made on the healing of experimental fractures in the rabbit. Certain histological and chemical reactions of the reparative tissue have led us to the belief that there is present in the early granulation tissue of bone repair, an acid mucopolysaccharide which may play a role in tissue healing. Tissue sections of rabbit callus from one to 10 days

after fracture were stained metachromatically with a 1% solution of toluidine blue. Early specimens take up only a small quantity of the dye. As the granulation tissue forms, however, an increasing metachromasia appears, and as the fibrous connective tissue becomes more abundant and differentiated, the ability to stain metachromatically with toluidine blue diminishes. Lison(1) attributed this ability to stain metachromatically to the

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1. Lison, L., *Soc. de Biol. Compt. Rend.*, 1935, v118.

presence of sulphate radicals. Wislocki, *et al.*(2) have shown, however, that hyaluronic acid, a mucopolysaccharide which contains no sulphate radical, also stains with toluidine blue. This gradual change in staining characteristics toward toluidine blue probably indicates a change in the nature of the ground substance.

Micro sections of the rabbit callus from the first to the sixteenth day after fracture were stained with the Hale technic. Hale(3) feels that this stain is specific for acid mucopolysaccharides. An acid polysaccharide such as hyaluronic acid combines with dialyzed iron in an acid solution but a neutral polysaccharide or a protein does not. The bound iron can be demonstrated as Prussian Blue by treatment with potassium ferricyanide and hydrochloric acid. If the slides are counterstained with a red dye such as paracarmine, the acid polysaccharides appear blue against the red background of other tissue structures. The positive reaction for acid polysaccharides parallels those obtained with toluidine blue. Recently injured tissue had little affinity for the dialyzed iron, but by 7 days the formed granulation tissue stained a deep blue strongly suggesting the presence of a high concentration of acid polysaccharides. Staining was most intense at 7 to 10 days, declined thereafter, and was almost disappearing by the tenth to fourteenth day.

Qualitative studies of the 7 day rabbit callus showed a high concentration of sulphate and phosphate ions. A test for the presence of amino sugars with Ehrlich's reagent was strongly positive. Believing that possibly we were staining one of the known acid mucopolysaccharides we attempted chemical isolation. A chemical analysis for hyaluronic acid by viscosimetric methods gave a negative result. Since great loss is entailed in these methods, we incubated tissue sections of the 7 to 10 day rabbit callus with increasing concentrations of hyaluronidase for varying periods of time. The tissue's staining did not change. Viscosimetric analysis by means of

an Ostwald viscosimeter showed no depolymerization of the ground rabbit callus by hyaluronidase.

Following Meyer's procedure(4) attempts were made to isolate chondroitin sulphuric acid from the cartilagenous 7 day rabbit callus. The results were negative although this mucopolysaccharide might logically be present since it is seen so abundantly in mature cartilage. Repeated attempts at identification for chondroitin sulfuric acid gave negative results. A possible explanation may be that procedures for the isolation of mucopolysaccharides entail great loss and as yet, we have not had available, the necessary large quantities of tissue.

In the course of analysis for chondroitin sulfuric acid a granular substance precipitated out of slightly alkaline alcohol. This substance prolonged the Lee White clotting time of blood almost fifteen times. The addition of both protamine and toluidine blue would inhibit this action. An analysis for heparin following Howell's(5) original method yielded a small quantity of protein contaminated crystals which possessed mild anticoagulant powers.

The 7 day rabbit callus was also incubated with lysozyme. Histochemically and viscosimetrically no depolymerization of the ground substance took place. These studies indicate the probable absence of hyaluronic acid and the substrate of lysozyme. The enzyme lysozyme is present in the granulation tissue of the rabbit callus, however, in a concentration of about 84 units after the fifth day after fracture.(6) The presence of chondroitin sulfuric acid is still unsettled, but much larger quantities of tissue must be studied.

We have studied the healing cycle in the rabbit and have sought to identify by histochemical and chemical methods an acid mucopolysaccharide which appears as an intermediary metabolite in the transformation of the interfibrillar ground substance. The studies are incomplete but contribute to the clarification

2. Wislocki, G., Bunting, G. H., and Dempsey, E. W., *Am. J. Anat.*, 1947, v81, 1.

3. Hale, C. W., *Nature*, London, 1946, v157, 802.

4. Meyer, Karl, and Smyth, Elizabeth M., *J. Biol. Chem.*, 1937, v119, 507.

5. Howell, W. H., *Am. J. Phys. Proc.*, 1922-1923, v63, 434.

6. Prudden, J. F., Lane, N., and Meyer, Karl, in press.

tion of the changes during healing in the organic matrix. To integrate these observations with present knowledge, a working hypothesis must take into account enzyme action, hydrogen ion concentration, and the concentrations of ions such as calcium, carbonate, and phosphate as well as other electrolytes participating in the transformation of the organic matrix. Further studies are indicated and are in progress. Because of the

difficulties involved in obtaining large quantities of healing callus, the identification of the acid mucopolysaccharide is incomplete.

Summary. Chemical and histochemical observations concentrate on a mucopolysaccharide which appears to be important in bone healing.

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Effect of Dietary Restriction on Susceptibility of Mice to Infection with Theiler's GDVII Virus.* (17488)

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That nutritional deficiencies are frequently accompanied by anorexia is well known. Numerous reports(1-7) have been published which concern the relation of nutritional inadequacies both to bacterial and to viral infections, but the evidence as to whether or not the course of experimental infections is influenced by caloric restriction, or by restriction of an adequate diet, inanition, seems equivocal. Different infective agents, different host species, genetic status of host and of pathogen, different routes of infection, and many other important variables frequently make direct

comparison impossible and analysis of conflicting results exceedingly difficult. The experiments here reported, query further the relative effects of reduced food intake resulting from loss of appetite and of a simple decrease in caloric intake on the course of Theiler's GDVII encephalomyelitis in mice.

Materials and methods. Mice: Webster Swiss mice, from our own colony, were used in all experiments. When 3 to 4 weeks old, they were removed from the colony, weighed and distributed in individual screen-bottom cages. Each group contained approximately equal numbers of male and female mice of similar weight and litter mates in any one group were kept to a minimum.

Diets: the diet referred to as "optimum" has the following percentage composition; casein 18† sucrose 72.2, salts IV 4,(8) vitamins 0.5,‡ choline chloride 0.3 and corn oil 5.§ The vitamins were made up with sucrose so

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3. Rasmussen, A. F., Jr., Waisman, H. A., Elvehjem, C. A., and Clark, P. F., *J. Inf. Dis.*, 1944, v74, 41.

4. Foster, C., Jones, J. H., Henle, W., and Dorfman, F., *J. Exp. Med.*, 1944, v79, 221.

5. Foster, C., Jones, J. H., Henle, W., and Dorfman, F., *J. Exp. Med.*, 1944, v80, 257.

6. Weaver, H. M., *Am. J. Dis. Child.*, 1945, v69, 26.

7. Metcalf, J., Darling, D., Wilson, D., Lapi, A., and Stare, F. J., *J. Lab. Clin. Med.*, 1949, v34, 335.

† A hot alcohol-extracted, vitamin test casein (General Biochemicals, Inc., Chagrin Falls, Ohio).

‡ We are indebted to Merck & Co., Rahway, N.J., for the crystalline vitamins.

§ Lichstein, H. C., McCall, K. B., Kearney, E. B., Elvehjem, C. A., and Clark, P. F., *Proc. Soc. Exp. Biol. and Med.*, 1946, v62, 279. In these studies the salt mixture was modified by using ZnSO₄ insted of Zn Cl₂.