of maturation and release of reticulocytes would also account for the data but it seems questionable to assume that adrenalectomy would increase the degree of immaturity in leucocytes and hasten maturation of erythrocytes. Another, and seemingly a more likely, possibility would be a decreased rate of maturation of the circulating reticulocytes thus allowing for their accumulation in the blood.

Summary. Adrenalectomy resulted in an increase in total count and increased absolute numbers of mononuclear and eosinophilic cells in the circulating blood of adrenalectomized mice. The increases were progressively greater with increases in the length of the interval after adrenalectomy from 4-5 days to 17-30 days. There was no consistent effect on neutrophiles. Reticulocyte percentages increased in the circulating blood. The number of mitotic figures in the bone marrow was reduced, while the ratio of myeloid to erythroid elements was increased. All the differences noted were found to be significant. The results suggest a decreased rate of activity and of maturation of cells in the bone marrow and an increase in the length of period of maturation and longevity in the circulating blood.

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Toxicity Studies on Subtilin. (19124)

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The possibility that it may become desirable to use antibiotics in the normal diet either for growth promotion or as food preservatives requires that adequate knowledge be available on their long-term effects. The present study dealing with the continued feeding of subtilin-containing diets to rats was prompted by the observation that subtilin appeared to have possibilities as a food-preserving substance(1).

Wilson, Humphreys, Reynolds and Lewis (2) have reported on the absorption of subtilin in acute experiments and commented on accompanying toxic reactions. Consideration of that data and the *in vitro* studies of others (mentioned below) leads to the presumption that chronic oral ingestion would be harmless. Subtilin is a polypeptide(3) inactivated and presumably hydrolyzed by proteolytic enzymes(4). Destruction in the gastrointestinal tract could be expected. In the acute toxicity studies(2) it was mentioned that a single rabbit received 1 g of subtilin/kg by stomach tube. After 5 hours only 2 μg were found in the urine, 15 mg in the intestine and 0.5 g in the stomach, or a total of 13% of the administered 4 g. The highest blood level was 0.1 ppm. This would indicate rapid inactivation or destruction of the subtilin entering the intestine. Subtilin is but sparingly soluble in physiological saline solutions and in body fluids(4). This insolubility accounted for all observed acute toxic effects* and might limit chronic oral toxicity. Andersen and Michener(1) suggested that "although *B. subtilis* is often found in foods, where it may well have produced appreciable quantities of subtilin, no cases of food poisoning have ever been attributed to it." They suggested further that the predominant intestinal microflora would not be adversely affected since subtilin is not active against Gram-negative bacteria:

1. Andersen, A. A., and Michener, H. D., *Food Technology,* 1950, v4, 188.

2. Wilson, R. H., Humphreys, E. M., Reynolds, D. M., and Lewis, J. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 700.

3. Jansen, E. F., and Hirschmann, D. J., *Arch. Biochem.,* 1944, v4, 297.

4. Dimick, K. P., Alderton, G., Lewis, J. C., Lightbody, H. D., and Fevold, H. L., *Arch. Biochem.,* 1947, v15, 1.

Methods. The rats were weanling male albinos of the strain used in this laboratory and previously described(5). The animals were caged in groups of 5, furnished water and food *ad libitum,* and body weights and food intakes were determined weekly. The subtilin was a refined sample obtained from the Western Regional Research Laboratory. It was ground to a powder in a mortar and then mixed with successive additions of basal diet, first in the mortar and finally in a mixing machine. Diets with lower concentrations of subtilin were made by serial dilutions of the most concentrated diet with basal diet. All subtilin-containing diets were refrigerated until offered to the animals. The basal diet was the one usually used in this laboratory for stock animals and for continued feeding experiments. It consisted of: yellow cornmeal 73%, linseed oil cake meal 10%, alfalfa meal 2%, casein 10%, cod-liver oil 3%, bone ash 1.5%, and sodium chloride 0.5%. The diets fed contained 0, 0.0062, 0.0125, 0.025, 0.05 and 0.1% of subtilin. The highest level (0.1%) is 100 times the concentration used by Andersen and Michener(1) in their original experiments. Each diet was fed to 5 animals, with 10 rats serving as controls, for 220 days. At the close of the feeding period the rats were autopsied; the organs were weighed and representative sections of tissue were fixed in 4% formaldehyde. Paraffin sections stained with hematoxylin and eosin were prepared for histopathological examination.

Results. The appearance and activity of the animals were normal throughout the course of the experiment, except for those suffering with respiratory infection. The incidence and severity of these infections were no greater than in the colony as a whole. An epidemic in the colony of fulminating bronchopneumonia led to an earlier termination of the experiment than was originally planned, to avoid possible loss of the animals through this disease.

Growth of the rats was not adversely affected. There was a definite suggestion of augmented growth in most of the groups receiving subtilin. For example, after 190 days, animals receiving diets containing 0.1, 0.05 and 0.0125% subtilin averaged 25 to 30 g heavier than their controls. Because of individual variations within the small groups, the differences in body weight were not shown to be statistically significant. The possibility of more rapid growth should be checked carefully, since there are reports that oral ingestion of certain antibiotics increases the rate of growth of chicks(6) and pigs(7).

At autopsy, organs appeared normal except for a few instances, scattered among the groups, of partially consolidated lungs, and one hyperemic kidney in a control animal. Weights of the organs did not differ significantly from normal, although there was a suggestion of increased weight of adrenals and testes. The gross observations were confirmed

* In our earlier paper(2) on absorption and acute toxicity of subtilin, gross evidence was given of deposition in the tissues of precipitated subtilin, with formation of chronic abscesses if the deposits were in the subcutaneous or muscular tissues. Histopathological examination of some of these lesions now has been made. That precipitation of subtilin at the site of injection or in the blood stream does occur is indicated by the following examples: In a rabbit, injected intramuscularly 24 hours earlier, polynuclear cells, many of which were eosinophiles, infiltrated widely into the muscle. In one zone the muscle fibers were displaced by clumps of eosinophilic material showing some irregularity of staining and with finely-basophilic strand-like threads scattered throughout. Five weeks after subcutaneous injection in a guinea pig, the cavity of the resulting abscess was bordered by a wall about 2 mm thick, composed of closely packed multinucleated giant cells of the foreign body type. Many of these contained clumps of amorphous, homogeneous, eosinophilic material which evidently was the source of the persistent reaction. In a rabbit which was killed by the intravenous injection of 50 mg of subtilin kg, the right heart was filled, the left heart was empty and the lungs were pale. Microscopic examination showed the right heart to be filled with red cells and irregular masses of homogeneously eosinophilic material. The large and small vessels of the lung were distended, as if by an injection mass, by homogeneously eosinophilic material of similar nature.

5. Wilson, R. H., De Eds, F., and Cox, A. J., Jr., *Cancer Research,* 1941, v1, 595.

6. Whitehill, A. R., Oleson, J. J., and Hutchings, B. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 11.

7. Jukes, T. H., Stokstad, E. L. R., Taylor, R. R., Cunha, T. J., Edwards, H. M., and Meadows, G. B., *Arch. Biochem.,* 1950, v26, 324.

by the microscopic examination, with no morphological changes attributable to the subtilin being observed. Tissues studied in this manner included liver, kidney, spleen, pancreas, adrenal, testis, heart, lung and thyroid.

Conclusions. The feeding to rats of diets containing as high as 0.1% of subtilin for 220 days was not detrimental as judged by growth, activity, organ weights and macroscopic and microscopic appearance of the tissues. The previously reported evidence for precipitation of parenterally-administered subtilin has been substantiated by microscopic examination.

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Hemagglutination Reaction in Streptococcal Infections and Acute Rheumatic Fever.* (19125)

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A hemagglutination test described by Middlebrook and Dubos(1) is being widely studied in tuberculous infections in experimental animals and in man. Preliminary evidence indicates that this reaction has greater specificity than other serological procedures such as the complement fixation test, and that it tends to be positive only during active tuberculous infections(1,2).

These promising results suggested the possibility that hitherto undetected antibodies might be discovered during the active phase of rheumatic fever with the hemagglutination technic. The present study was undertaken, therefore, to determine whether various streptococcal extracts or products could be adsorbed onto sheep erythrocytes, and to observe the behavior of the sensitized cells in the presence of sera from patients with streptococcal infections and acute rheumatic fever.

Materials and methods. The following streptococcal filtrates and extracts were prepared for hemagglutination tests: A. *Heated filtrate of a culture of whole streptococci.* A Group A, non-typable streptococcus recently isolated from a patient with acute rheumatic fever was grown overnight in 500 cc of Todd-Hewitt broth. The culture was placed in a boiling water bath, and the total volume was reduced from 500 cc to 100 cc by evaporation. Following Seitz filtration, the filtrate was diluted 1 to 6 in normal saline for attempts at sensitization of sheep erythrocytes. When negative results were obtained, the filtrate was further concentrated from 100 cc to 20 cc by evaporation. Even when diluted 1 to 6 this thick, syrupy material was unsatisfactory for sensitization because it caused hemolysis of most of the red cells. B. *Hydrochloric acid extract.* The streptococcus used was the same as for preparation A. The crude streptococcal extract, containing both the group-specific C substance and the type-specific M antigen was prepared in the usual fashion(3) by boiling the organisms with HCl at a pH of 2.5, discarding the cellular residue, and neutralizing the supernatant. The extract was diluted 1 to 4 in normal saline for attempts at sensitization. C. *Formamide extract.* The method of Fuller(4) was used to extract the C substance. The streptococcus was the same as that used for preparations A and B. For sensitization the extract was diluted 1 to 4 in saline. D. *Streptolysin O.* Streptolysin O was prepared from the Richards strain according to the method of Rantz and Randall(5), and was stored in the refrigerator in the oxidized state.

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2. Rothbard, S., Dooneief, A. S., and Hite, K. E., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 72.

3. Swift, H. W., Wilson, A. T., and Lancefield, R. C., *J. Exp. Med.*, 1943, v78, 127.

4. Fuller, A. T., *Brit. J. Exp. Path.*, 1938, v19, 130.