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Nature of the Factor Concerned in Loss of Blood Coagulability of Bile Fistula Rats.

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In previous papers,¹ a marked increase in the coagulation time of the blood of the bile fistula rat was demonstrated. Analogous observations on dogs have been reported by Hawkins and Whipple.² The condition became evident when the animals had been maintained on a vitamin E free, low fat diet (See previous paper) or on the stock diet,[†] which was also low in fat, for a period of 30 to 50 days. The condition was not shown by unoperated rats when they were maintained on either of these diets. Small scratches or clipping of the tail in these bile fistula rats resulted in prolonged bleeding. At times this led to the death of the animal unless the bleeding was stopped by the application of collodion.

The content of prothrombin in the blood of the bile fistula rats decreased with time. The decrease after the prothrombin level had fallen to a certain point was roughly parallel to the hemorrhagic tendency. The method of Quick³ was employed to estimate the prothrombin. Blood and brain tissues of normal rats were used to prepare the solutions. The normal serum as well as the serum which was treated with aluminum hydroxide were obtained from the pooled blood of a group of 8 to 10 normal rats. One-half cc. of blood was obtained by heart puncture from the bile fistula rats at intervals of 10 to 14 days. The prothrombin content of these animals was reduced to 20 to 30% of the normal before the coagulation time of the blood was markedly increased. Histological sections made from livers of some of the bile fistula rats when the blood coagulation time was markedly increased showed no evidence of liver damage.

* E. R. Squibb and Sons Fellow. Technical assistance was supplied by the Works Progress Administration.

¹ Greaves, J. D., and Schmidt, C. L. A., *Am. J. Physiol.*, 1936, **116**, 456. Also accompanying paper.

² Hawkins, W. B., and Brinkhous, K. M., *J. Exp. Med.*, 1936, **63**, 795.

[†] The low fat stock diet contained 30 lbs. of wheat, 10 lbs. of whole milk powder, 1 lb. of alfalfa meal, and 100 gm. each of sodium chloride and calcium carbonate. The diet was supplemented with vitamin A and D.

³ Quick, A. J., *Am. J. Physiol.*, 1937, **118**, 260.

All of the known dietary factors, such as vitamins A, D, E, B, and G, as well as the essential fatty acids, when added to the diet, had no effect on the hemorrhagic condition. Lemon juice, when fed at levels of 2 cc., likewise had no effect. On the other hand, when 2 to 3 cc. of beef bile were fed daily, a marked rise in the prothrombin content of the blood was noted within a period of 2 to 4 days after the bile was administered. The clotting time of the blood decreased. An ether extract of acidified bile was also effective. The activity of the factor was not destroyed by boiling the bile. Crude extracts of vitamin K (prepared from alfalfa), which had proven effective in preventing the hemorrhagic syndrome in chicks, as well as a highly purified preparation of vitamin K,[‡] when fed to the bile fistula rat, were effective in decreasing the coagulation time of the blood and increasing the prothrombin level. The vitamin K preparations, after boiling with dilute alkali, were ineffective.

Similar loss in the coagulability of the blood with concurrent decrease in prothrombin was shown by rats which were maintained on the stock diet and were made icteric by ligation of the bile duct. When the flow of bile into the intestines was reestablished in these animals, the prothrombin content of the blood increased to the normal level within a short time.

The factor which is essential in preventing the increased coagulation time and the decrease in the prothrombin content of the blood of the bile fistula and the icteric rat appears to be vitamin K or a substance which is closely associated with it. The question arises whether the absence of bile from the intestinal tract of the rat leads to failure in the absorption of the essential factor or whether the factor is excreted through the bile, or both. The low fat stock diet contained sufficient alfalfa meal to provide the vitamin K needs of the chick. Nevertheless, bile fistula rats developed loss in blood coagulability when maintained on this diet. Only when massive doses of vitamin K, without bile salts, or bile itself were fed did the coagulation time of the blood return to normal. These results appear to point to the possibility that bile acts as a carrying agent for the factor across the intestinal tract. Some absorption of the factor can, however, take place when massive doses of vitamin K are administered. Added evidence that certain of the bile salts favor utilization of the factor is gained from the fact that feeding crude bile salts, or bile which had been treated with alkali and heated, to bile fistula rats maintained on the low fat stock diet led to an increase in the prothrombin level and decrease in the coagulation time of the blood.

[‡] Kindly supplied by Dr. H. J. Almquist.

Further work on this problem is in progress.

Summary. 1. Bile fistula rats show loss in blood coagulability and a decrease in the prothrombin level. 2. This condition can be relieved by administration of a vitamin K concentrate.

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The Loss of Specific Substance in Washing Phase I *H. pertussis* Vaccines.*

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Recent field studies of immunization against pertussis have yielded conflicting evidence. This is not altogether surprising in view of the fact that there is no standard method of preparing vaccines of *Hemophilus pertussis*. Recently isolated, toxic, Phase 1 strains¹ of *H. pertussis* have been used in the majority of recent trials.²⁻⁵ Stock strains, however, are still used in preparing vaccine.⁶ Although the newer knowledge of the antigenic changes of *H. pertussis* contra-indicates the use of stock strains, it is obvious that a proposed immunizing agent must be judged only by careful clinical trial. Such trials as have been made with stock strain vaccines^{7, 8} have not been sufficiently comprehensive to permit comparison.

Vaccines made from recently isolated, toxic, Phase 1 strains have differed principally in the amount of washing to which they were subjected. It is a current view⁹ that the efficiency of any bacterial vaccine depends on (a) its production from virulent, smooth bacteria, and (b) the presence of the surface antigens of the virulent,

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¹ Leslie, P. H., and Gardner, A. D., *J. Hygiene*, 1931, **31**, 423.

² Sauer, L., *Am. J. Pub. Health*, 1935, **25**, 1226.

³ Kendrick, P., and Eldering, G., *Am. J. Pub. Health*, 1936, **26**, 8.

⁴ Daughtry-Denmark, L., *Am. J. Dis. Child.*, 1936, **52**, 587.

⁵ Doull, J. A., Shibley, G. S., and McClelland, J. E., *Am. J. Pub. Health*, 1936, **26**, 1097.

⁶ Mishulow, L., Mowry, I., and Orange, R., *J. Pediat.*, 1936, **9**, 492.

⁷ Park, W. H., *J. Pediat.*, 1935, **7**, 690.

⁸ Shorr, E. Y., *J. Pediat.*, 1936, **9**, 49.

⁹ Topley, W. W. C., and Wilson, G. S., *The Principles of Bacteriology and Immunity*, 2nd Edition, Baltimore, 1936, p. 831.