

The augmentation of pregnant mare serum gonadotropin by the pituitary follicle-stimulating hormone can be considered to be of a specific nature, since it has been shown that non-specific augmenting substances, such as copper or zinc salts, inert proteins, adrenalin, etc., which synergize pituitary extracts, do not augment the hormone from pregnant mare serum.³

Summary. An augmentative effect was observed when pregnant mare serum gonadotropin was administered in combination with a pituitary follicle-stimulating preparation. On the other hand, the addition of chorionic gonadotropin under the same experimental conditions, did not potentiate the gonadotropic effect of the pregnant mare serum preparations.

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Electrophoretic Analysis of Anti-Papilloma Rabbit Serum.*

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Serum from domestic rabbits bearing the growths of infectious papillomatosis¹ and those injected with extracts of domestic or cottontail rabbit growths or with the purified papilloma virus protein may contain immune bodies demonstrable by complement fixation, neutralization or precipitation.² Recently, studies have been made in this laboratory to learn whether these immunological characters are paralleled by abnormal electrophoretic behavior of such serum.

Sera from individuals of 6 groups of rabbits were examined: (1) domestic rabbits injected intraperitoneally with 0.5 mg of purified papilloma virus protein, once a week for 6 weeks, and serum taken for study every 7 days (for a total of 51 days); (2) domestic rabbits injected intravenously with 0.5 mg of purified papilloma virus protein, twice a week for 6 weeks, and the serum examined after 21 and 50 days; (3) domestic rabbits injected intraperitoneally with

³ Evans, J. S., Hines, L. R., Ceithaml, J. J., and Koch, F. C., *Endocrinol.*, 1940, **26**, 1012.

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¹ Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

² Bryan, W. R., and Beard, J. W., *J. Nat. Cancer Inst.*, 1941, **1**, 607.

1.0 cc of a 7.5% suspension of domestic rabbit warts, twice weekly for 6 weeks; (4) domestic rabbits bearing experimentally-induced growths for 39 to 72 days; (5) domestic rabbits carrying transplanted growths³ in abdominal organs for 115 days; (6) cottontail rabbits bearing experimentally-induced growths for 37 and 40 days. The serum was diluted with 2 volumes of buffer solution of pH 7.8 containing 8.7675 g NaCl, 2.6274 g Na₂HPO₄, and 0.2071 g NaH₂PO₄ · H₂O per liter and dialyzed for 4 to 6 days at 2 to 9°C. For electrophoresis the moving boundary method of Tiselius was used and the boundary gradients were recorded as previously described.⁴ The index of serological change was complement-fixing titer.

In the sera of animals receiving the virus intravenously or intraperitoneally, the complement-fixing titer was 1:16 to 1:256. No abnormality either qualitative or quantitative, was seen in the electrophoretic behavior of the sera. The serum from animals receiving suspensions of domestic rabbit growths intraperitoneally fixed complement in 0 to 1:4 dilution, and no change occurred in the electrophoretic pattern.

Definite changes were seen, however, in the sera of animals bearing growths in the skin (complement not fixed in 1 case, but fixed in dilutions of 1:2 to 1:8 in 5 cases) or growths transplanted to abdominal organs (complement fixed in 0 dilution). The electrophoretic diagram of such a serum is seen in Fig. 1. For comparison and to illustrate the significant characters, the diagram of a serum from an animal injected intraperitoneally with papilloma virus protein is given in Fig. 2. The diagram of Fig. 2 shows no difference from that of normal rabbit serum described elsewhere. The significant abnormality of Fig. 1 was the high, relative and absolute, quantity of the complex represented in the beta boundary, both of which were well beyond the limits of variation in normal sera.⁵ The beta complex of Fig. 1 represented 30.3% of the total protein in contrast with 15% as the mean for normal rabbit serum. The significance of the enhanced beta boundary of serum from rabbits carrying warts is not yet apparent. Enhanced beta boundaries have been seen⁶ in human sera from patients with obstructive jaundice,

³ Rous, P., and Beard, J. W., *J. Exp. Med.*, 1934, **60**, 701.

⁴ Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W., *J. Biol. Chem.*, 1942, **142**, 193.

⁵ Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W., *J. Immunol.*, 1942, in press.

⁶ Longsworth, L. G., Shedlovsky, T., and MacInnes, D. A., *J. Exp. Med.*, 1939, **70**, 399.

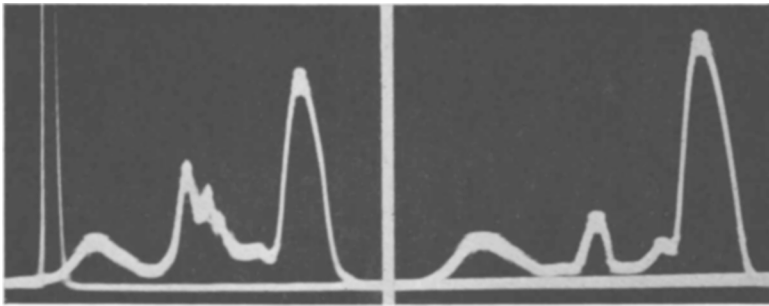


FIG. 1.

FIG. 2.

FIG. 1. Refractive index gradient curve of serum from a domestic rabbit bearing cutaneous papillomas. The curves were photographed at 0 and 276 minutes by the method of crossed slits. The potential gradient was 4.73 volts/cm at 0.2 ionic strength and 1°C.

FIG. 2. Refractive index gradient curve of serum from a domestic rabbit inoculated intraperitoneally with purified papilloma virus. The curve was photographed after 311 minutes' migration. The potential gradient was 4.77 volts/cm at 0.2 ionic strength and 1°C.

multiple myeloma and nephrosis. In these cases portions of the complex responsible for the beta boundary were ether-soluble. Such proved to be the case in the present work since the beta component could be reduced to normal proportions by extraction of the serum with ether. None of the beta component was absorbed by purified virus (9.0 mg virus to 6.0 cc of serum) nor by suspensions of domestic rabbit growths. Only one of 6 sera examined showed no enhancement of the beta boundary and this was from an animal in which retrogression of the growths was in progress. It thus appears that the abnormal component associated with the enhanced beta boundary represents a lipoidal material unrelated to the papilloma antibodies.

In the sera of 2 cottontail rabbits bearing warts, complement-fixing titer 1:16 and 1:32, the findings differed from those with domestic rabbits. In both, change in the beta globulin component was questionable but there appeared to be a definite increase in the gamma globulin. Serological changes after immunization are usually paralleled by changes in the gamma globulin component, but it is noteworthy that the complement-fixing titers of these cottontail rabbit sera were less than those seen in domestic rabbits immunized with purified virus. The lack of increase in the beta boundary may be related to biological phenomena concerned with the growths themselves as reported in an accompanying paper.⁷

⁷ Taylor, A. R., Sharp, D. G., Beard, D., and Beard, J. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, submitted for publication.