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Electrophoretic Analysis of Extracts of Rabbit Papillomas.*

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The serum of rabbits bearing the growths of infectious papillomatosis and of rabbits immunized with purified virus or wart extracts has been studied¹ by the electrophoretic procedures of Tiselius. Differences from the normal electrophoretic patterns were seen only in the serum of animals actually carrying growths. In the present work studies were made on saline extracts of the virus-induced growths of domestic and cottontail rabbits.

The growths had been present for 39 to 115 days and, except for retrogressing warts in one animal, were exuberant and fleshy. Growths were removed from rabbits killed by air injection and, freed of connective tissue, were pulped in a Waring Blender and extracted for 24 hr at 2-8°C in 20% suspension in 0.9% NaCl solution. The extracts were filtered with celite and dialyzed against buffer prepared as in the serum studies.¹

In Fig. 1 is shown the electrophoretic diagram of a filtered extract of domestic rabbit papillomas showing 3 distinct migrating peaks, the mobilities of which were 5.73, 3.07, and 1.22 cm²/sec. volt, respectively. These values corresponded fairly closely to the mean mobilities found for albumin, beta globulin and gamma globulin of normal rabbit serum,² namely 5.13, 2.98 and 0.92, respectively. The greatest difference was seen with the peak in the relative position of the gamma globulin of serum diagrams. It has been noted that when filtered extracts are heated to 56°C for 30 min some of the protein is coagulated, a phenomenon not observed when serum is so

heated. The diagram obtained with extract of Fig. 1 after being heated in this way is shown in Fig. 2. The protein coagulated and removed was 43% of the total present in the unheated extract. In Fig. 2 are seen again 3 chief components, the mobilities of which were, 5.20, 2.78, and 1.04 cm²/sec. volt.

These findings suggest that a large portion of the protein of the domestic rabbit papilloma extracts may be very similar to, if not identical with, the protein of rabbit serum. That much of it differed from serum protein was indicated by the amount coagulated at 56°C for 30 min. After heating, the residue of the 3 components, designated as A, B, and C, resembled more closely in mobility the albumin, beta globulin and gamma globulin components of serum. The serum of the animal furnishing the growths of Fig. 1 contained albumin plus alpha globulin 55.1%, beta globulin 30.3%, and gamma globulin 14.6%. In the unheated extract the percentage composition was 28.7%, 39.8% and 31.5% for the A, B, and C components, respectively. For the heated extract, the values were 48.0%, 30.1% and 21.9%, a distribution more closely approximating that of the albumin, beta globulin and gamma globulin of serum. The protein loss due to heat was slight for the A component, 3.9%, most of it being distributed between the B, 57%, and C, 60%, components.

Extracts from cottontail rabbit growths differed greatly from those of domestic rabbit growths both in composition and behavior. Again 3 peaks were noted in the diagrams of the filtered extracts, an example of which showed a percentage composition with respect to the A, B, and C components of 37.8%, 41.0% and 21.2%. Heating resulted in the removal of only 10% of the total protein distributed approximately equally among the A, B, and C components.

Further work is necessary to establish the

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¹ Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **50**, 358.

² Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W., *J. Immunol.*, 1942, **44**, 115.



FIG. 1

FIG. 2

FIG. 1. Electrophoretic diagram of a filtered extract of domestic rabbit papillomas. The curve was photographed after migration for 206 minutes. The potential gradient was 4.66 volts/cm at 0.2 ionic strength and 1°C.

FIG. 2. Diagram obtained after heating extract of Fig. 1 at 56°C for 30 minutes. The curve was photographed after migration for 0 and 176 minutes. The potential gradient was 4.78 volts/cm at 0.2 ionic strength and 1°C.

origin and nature of the proteins in the papilloma extracts. No evidence was seen of a component behaving electrophoretically as the papilloma virus.³ The protein coagulated at 56 C for 30 min was probably of tissue origin though it may have been altered serum protein. After heating, the protein of the extract still differed in its distribution among the 3 components from the analogous distribution in serum between albumin, beta globulin and gamma globulin. From hemoglobin determinations the wart tissue extract of Fig. 1 and 2 did not contain more than 0.049 cc of whole blood per g of warts extracted. If the protein of the heated extract is assumed to be of serum origin, the amount present per g of warts would represent 0.45 cc of serum. It is possible that some tissue protein indistinguishable from serum protein electrophoretically remained after heating, an indication of which was seen in the distribution among the A, B, and C components. From calculations based on the assumption of the identity of the A component with serum

albumin, the equivalent amount of serum possibly present was 0.39 cc per g of warts. In the instance of cottontail rabbit warts similar calculations gave values of 0.91 cc and 0.68 cc per g of warts.

The data demonstrate the presence of protein in rabbit papilloma extracts similar in electrophoretic behavior to the protein of serum and provide an estimate of its quantity. The evidence indicated that the protein was located in the wart substance which it probably entered during the period of growth. The papilloma is virus-induced and the serum of animals carrying it contains virus immune bodies. It is possible that serum immune bodies might exert an influence either on the growth itself or on the virus present in the growth.⁴ No evidence for this was seen since more protein was found in fleshy warts than in those growing less well. Further, contrary to expected results, more serum protein was found in cottontail rabbit warts which generally yield much virus than in domestic rabbit growths which seldom yield active virus.

³ Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W., *J. Biol. Chem.*, 1942, **142**, 193.

⁴ Kidd, J. G., *J. Exp. Med.*, 1939, **70**, 583.