

detection in the retina and eye colourings of birds<sup>7,8</sup>, this is the first time it has been reported in land animals. This is perhaps less surprising when one considers that locusts are quite closely related to the Crustacea, the typical carotenoid of which is astaxanthin.

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T. W. GOODWIN  
S. SRISUKH

Department of Biochemistry,  
University, Liverpool.  
Dec. 22.

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### Isolation in Chick Embryos of a Filtrable Agent possibly related etiologically to Lumpy Skin Disease of Cattle

THE widespread occurrence of lumpy skin disease<sup>1</sup> in dairy herds of the Cape Peninsula offered suitable material for investigations on the etiology of this disease.

Skin nodules or lymph gland excised under local anaesthesia from cases in the early stages of the disease as well as tissues obtained at post mortem were made available to us. Emulsions of these tissues, treated with antibiotics or freed of bacteria by filtration through 'Gradocol' membranes<sup>2</sup>, were used for the inoculation of laboratory animals and chick embryos.

Inoculation of laboratory animals by the usual routes has so far proved unsuccessful.

One series of experiments using chick embryos has, however, resulted in the isolation of a virus. The starting material was obtained from a calf which died at a stage of the disease when active skin and lymph gland lesions were still present. An emulsion was made in broth of a lymph gland and a skin nodule. After light centrifugation and removal of the supernatant, penicillin (100  $\mu$  per ml.) and streptomycin (50  $\gamma$  per ml.) were added, and the emulsion then filtered through a 'Gradocol' membrane with an average pore diameter of 600  $m\mu$ . The filtrate was used for the inoculation of six nine-day-old chick embryos by the combined amniotic and chorio-allantoic routes.

The eggs were returned to an incubator and maintained at 35–36° C. Four of the embryos were dead on or before the sixth day after inoculation. One of the survivors, opened on the sixth day, showed a marked gelatinous oedema of the chorio-allantois and numerous hæmorrhages into the shafts of the feather follicles.

Serial passages of embryo and chorio-allantois have been maintained since then for more than a year. Nine-day embryos are usually employed, and lesions developed by them are constant. On the fourth day after inoculation, the embryos present a characteristic shrivelled appearance—tightly rolled up in a dry, thickened, amniotic membrane. Feather development is usually absent, or only irregularly swollen cystic or hæmorrhagic feather follicles are present. The

chorio-allantoic membrane almost invariably shows gross gelatinous oedema. That the agent responsible for these lesions is a virus is suggested by: (a) the presence of numerous inclusion bodies in histological sections of affected embryos; and (b) the development of lesions following the inoculation of filtrates through 'Gradocol' membranes with A.P.D. of 52  $m\mu$  or more; filtrates through 21  $m\mu$  membranes have regularly failed to elicit the characteristic lesions.

Successful neutralization experiments have been carried out in eggs, with immune sera from adult fowls that had received repeated injections of the virus cultivated in chick embryos, as well as sera from convalescent bovines. Results of neutralization tests have, however, not always been conclusive, because positive results have been obtained with the serum of occasional apparently normal bovines. The possibility that these apparently normal animals had suffered subclinical infections could not, however, be excluded. Also, one serum from a bovine convalescent after a typical attack of lumpy skin disease contained no demonstrable neutralizing antibody.

A small number of normal bovines obtained from areas where the disease was not endemic have been tested for their susceptibility to the virus cultivated in eggs. None of these has developed lumpy skin disease. The experimental transmission of the disease in its natural host has, however, proved uncertain even when presumably infective inocula obtained from other bovines have been used. Furthermore, it would not be surprising in the light of experience with other animal viruses if serial transfer in eggs has resulted in a loss of virulence for the original host.

Although the identity of the virus has not yet been definitely established, it is felt that the successful neutralization by the majority of lumpy skin convalescent sera tested indicates the possible etiological relationship of this virus to lumpy skin disease.

Full details of the experiments will be published elsewhere.

M. VAN DEN ENDE  
P. DON  
A. KIPPS

R. ALEXANDER

Department of Pathology, Veterinary Institute,  
University of Cape Town. Onderstepoort, Pretoria.  
Dec. 19.

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### Effect of Calcium Ions on the Charge of Myosin

DURING a preliminary investigation of the electrophoretic behaviour of the crystalline myosin prepared according to Szent-Györgyi<sup>1</sup> we found, in a Tiselius electrophoresis cell, that myosin was negatively charged when dissolved in potassium chloride at pH 7.1.

Based upon these data, Szent-Györgyi suggested that myosin dissolved in an excess of calcium chloride at the same pH ought to be positively charged. Our present experiments confirm this suggestion.

Some data of our preliminary work are summarized in the accompanying table.

ONCE-CRYSTALLIZED MYOSIN DISSOLVED IN THE GIVEN BUFFER.  
CONCENTRATION OF MYOSIN 0.3 PER CENT

	pH	Mobility
0.28 M KCl	7.1	-2.5
0.12 M K-veronale-acetate-HCl buffer of Michaelis	5.6	+1.6
0.22 M CaCl <sub>2</sub>	7.5	+1.27
0.04 M K-veronale-acetate-HCl buffer of Michaelis	8.7	+1.28