different destruction factors have been obtained. Other differences may be indications of 'microheterogeneity'[5]. The tyrosine values are in good agreement with the spectrophotometric data of Léonis and Li[6] for bovine growth hormone. The chromatograms did not reveal the presence of any new or unusual amino-acids.

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Starch Gel Electrophoresis of Hen Egg White, Oviduct White, Yolk, Ova and Serum Proteins

The Smithies[1] starch gel technique has recently been successfully applied by Lush[2] to demonstrate genetic polymorphism in proteins of the white of a hen egg.

Lush[2] referred to two electrophoretically distinct components of crystalline hen ovalbumin described by me[3]. Three—and not two—components were reported in this work, which was later briefly reported[4]. The electrophoretic technique used in an examination of hen serum and egg proteins is now described in more detail. Suitable starch gels containing 15–17 per cent w/v starch were prepared from B.D.H. soluble starch and buffered as recommended by Smithies. The 'Perspex' troughs containing the starch gels were placed on a metal sheet in contact with an ice block in a cold room at 0°. The run was continued for 8 hr., at 400 V. and 10 m.amp.

The components of the 'thin', 'inner thin' and 'thick' layers[5] of hen egg white proteins were compared with those of the viscous fluid obtained from the magnum region of the oviducts of laying hens. The protein composition appeared to be identical by comparison of pairs of these fluids on a single gel in all four cases. Sixteen well-defined bands were observed (Fig. 1*a*). The fast anodic components 16 : 15 : 14 were shown to be identical with three components of a sample of five-times crystallized ovalbumin, $A_1 : A_2 : A_3$ respectively, as described by Cann[6].

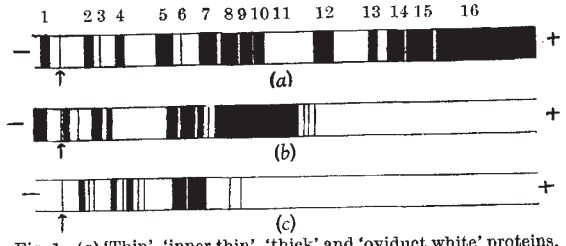

Fig. 1. (*a*) 'Thin', 'inner thin', 'thick' and 'oviduct white' proteins. Ovalbumins A_1, A_2, A_3 corresponding to bands 16, 15, 14, respectively. (*b*) Proteins of laying-hen serum. (*c*) Hen egg yolk and ova proteins. In each diagram the origin is indicated by the arrow

For comparative purposes the protein patterns of hen serum (Fig. 1*b*), egg yolk and ova isolated from the ovary (Fig. 1*c*) are given. The actual position of the bands in the figures cannot be directly compared since these patterns were obtained from different gels. It can be seen that the high resolving power of starch electrophoresis is well suited to the study of species differences in complex protein and lipoprotein mixtures.

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Electrophoretic Separation of Conjugated Bilirubin on Paper

In previous experiments[1] the 0·02 *M* phosphate buffer of *p*H 6·8 was used for electrophoresis of bile pigments on paper. Under these conditions (in the absence of proteins) the conjugated bilirubin migrating anodically separates from the unconjugated bilirubin which remains on the starting line. The presence of bile salts clearly influences this separation. Purified preparations[2] of conjugated bilirubin which are devoid of bile salts migrate in the form of a tail beginning just from the starting line. If bile salts are present a sharply defined zone of conjugated bilirubin separates, migrating in front of the bile salts. The velocity of migration of this zone in the presence of bile salts is considerably higher than the rate of migration of pure preparations of the pigment.

For example, the purified preparation, containing about 80 per cent of pigment II (as determined according to Billing[3]) after paper electrophoresis (1 m.amp./1 cm. of Whatman No. 1 paper, potential gradient about 12·5 V./cm., 2 hr. 15 min.) in 0·02 *M* phosphate of *p*H 6·8, yielded a diffuse zone beginning at the starting line and tailing to 9 cm. The same preparation in the presence of about 10-fold excess (on the weight basis) sodium taurocholate (and taurodeoxycholate) migrated simultaneously as a relatively narrow zone between 12 and 13 cm. from the start. The separation of both fractions of conjugated bilirubin (pigment I and pigment II[4]) could not be observed. Analogous results were obtained with the use of sodium acetate–acetic acid buffer of *p*H 5·2; only the rate of migration was slightly lower. Barbital buffer of *p*H 8·6 cannot be recommended owing to the instability of conjugated pigments in the alkaline range. The migration of unpurified preparations in this buffer proceeds in the same way as in phosphate buffers.

For the purpose of electrophoretic separation of purified preparations of conjugated bilirubin (that is, those which were devoid of excess bile salts) the systems containing pyridine were tested. Such systems were described by Grassmann *et al.*[5] (*p*H 3·9) and by Runeckles and Krotkov[6] (*p*H 6·0). Both yielded better results than mere aqueous solutions; systems containing more pyridine caused less tailing. The system (*p*H 6·1) was selected containing 5 ml. of glacial acetic acid, 70 ml. of pyridine in 1 litre. Under