oric content of diets by adding tallow did not give a growth response comparable to that obtained by simply cooking the peas. Alkali-insoluble fractions of peas depressed chick growth but alkali-soluble fractions did not. It is suggested that peas (*P. sativum*) contain a growth inhibitor for birds that is inactivated by heating treatments employed.

1. Woods, E., Bolin, D. W., *J. Nutr.*, 1943, v26, 327.
2. Peterson, C. F., Lampman, C. E., Bolin, D. W., Stramberg, O. E., *Poul. Sci.*, 1944, v23, 287.
3. Cooney, W. T., Butts, J. S., Bacon, L. E., *ibid.*, 1948, v27, 828.
4. Ham, W. E., Sandstedt, R. M., Mussehl, F. E., *J. Biol. Chem.*, 1945, v161, 635.
5. Heuser, G. F., Norris, L. C., McGinnis, J., *Poul. Sci.*, 1946, v25, 130.
6. Milligan, J. L., Bird, H. R., *ibid.*, 1951, v30, 651.
7. Borchers, R., Ackerson, C. W., *Arch. Biochem. Biophy.*, 1937, v13, 291.
8. Schilling, E. D., Strong, F. M., *J. Am. Chem. Soc.*, 1954, v75, 2848.
9. Geiger, B. G., Steenbock, H., Parsons, H. T., *J. Nutr.*, 1933, v6, 427.
10. McKay, G. F., Lalich, J. J., Schilling, E. D., Strong, F. M., *Arch. Biochem. and Biophy.*, 1954, v52, 313.
11. Armbruster, G., Murray, H. C., *J. Nutr.*, 1951, v44, 205.

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Electrophoretic Migration of Serum Lipoproteins in Starch Gel.* (25134)

HENN KUTT, FLETCHER MCDOWELL AND JAMES H. PERT†
(Introduced by H. G. Wolff)
Neurological Service, Cornell Med. Division, Bellevue Hospital and Dept. of Medicine, Cornell University Medical College, N. Y.

Electrophoresis in starch gel(1), is being increasingly used mainly because of its good resolution of protein fractions. During our studies of serum and cerebrospinal fluid proteins(2) with this method we found it advantageous to stain half of the gel electrophoretogram for lipoproteins. Oil red O was selected as coloring agent in staining the lipoproteins in starch gel as described by Smithies (personal communication). Initially considerable difficulties were encountered with reproducibility of the pattern using this method; later, however, we determined that these variations were mostly produced by inconstant composition of commercial oil red O which contains components that stain albumin and globulins as well as lipids. Partial purification has reduced these inconsistencies and has enabled relatively constant lipoprotein patterns in the starch gel to be obtained. To interpret the patterns obtained with oil red O, we thought it important to identify the bands that were seen. Serum lipoproteins were fractionated by starch granule electrophoresis and by sedimentation in the preparative ultracentrifuge. The isolated fractions were submitted to electrophoresis in starch gel and the results described below.

Materials and methods. Human serum was separated by centrifugation, pooled and stored at 4°C no longer than 48 hours before ultracentrifugation or electrophoresis. All chemicals were of reagent grade and the solvents were redistilled. Double glass-distilled water was used in all experiments to reduce contact of lipoproteins with copper ions. Starch gel electrophoresis was carried out as described by Smithies(1). Coloring of lipoproteins with oil red O was performed as previously reported(2) and crude oil red O was partially purified eliminating the protein staining components with a technic we developed(3). Spinco Model L preparative ultracentrifuge was used to prepare high and low density lipo-

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† Present address: Research Lab., Am. Nat. Red Cross, Washington, D. C.

proteins. Specific gravity of serum was adjusted to 1.063 g/ml with a saturated solution of KBr and $NaNO_3$ in equal parts(4). In Rotor 40 the specimen was spun at 105.400 x G for 24 hours. Floating lipoproteins were collected and designated as low density lipoproteins. The bottom of the 1.063 tubes was aspirated and adjusted to specific gravity of 1.21 g/ml and centrifugation repeated. Floating lipoproteins were collected and designated as high density lipoproteins. Alpha-1, alpha-2 and beta lipoproteins were obtained by starch granule electrophoresis. The starch blocks were prepared as described by Kunkel(5), with minor modifications. Barbital buffer of pH 8.6 and ionic strength of 0.06 was used. After completion of electrophoretic separation, the block was cut in segments, suspended in 0.9% saline and a protein curve was prepared by the method of Folin-Ciocalteu. Tubes of beta, alpha-2, and alpha-1 (containing a large proportion of albumin) zones were pooled into the 3 respective fractions and concentrated by negative pressure dialysis in collodion bags according to the method of Mies(6). The concentrated lipoprotein fractions were then subjected to electrophoresis in starch gels. The strips were stained with purified oil red O and scanned with a recording photoelectric densitometer.‡

In another block, segments were extracted with methylal : methanol 4 : 1 mixture and phospholipid and cholesterol analyses were made by methods of Fiske and Subbarow and Sperry and Schonheimer respectively, to determine the position of lipoproteins in the starch block.

Results. In starch gel electrophoresis, the beta lipoproteins prepared by starch granule electrophoresis migrated close to the origin forming a wide band, which spread from the origin toward the anode forming a plateau (Fig. 1 A). The alpha-2 lipoproteins also remained close to the origin similar to the pattern of beta lipoproteins; however, there was a rising front present towards the anode (Fig. 1 B). The alpha-1 lipoproteins migrated in the postalbumin area as a band, which had a rapidly rising limb and a somewhat gradually falling limb towards the anode (Fig. 1 C). This asymmetrical leading edge of the alpha-1 component was occasionally resolved into an additional band.

To confirm the identity of lipoproteins eluted from the starch block and rule out the possibility that the beta lipoproteins might have extended into the area of alpha-2, the following experiment was performed. All tubes of a starch block were separately concentrated and submitted to electrophoresis in starch gel. The slow lipoprotein band was seen in concentrates from both beta and alpha-2 segments and little, if any, lipid-containing material was seen in segments that constituted the valleys between peaks of starch block electrophoresis. The fast band was found in the concentrate from albumin-alpha-1 segments. These patterns were in agreement with the phospholipid curve from a block of the same serum (Fig. 2).

Low density lipoproteins prepared by ultracentrifugation remained close to the origin as did the beta lipoproteins from the starch block, but they also showed a rising front towards anode similar to the alpha-2 lipoproteins. The high density lipoproteins showed a band similar in shape and position to that given by alpha-1 lipoproteins eluted from the starch block.

When whole serum was applied, patterns were obtained which showed a band close to the origin in the position of the low density lipoproteins and another band in the postalbumin region, where the high density lipoproteins migrated (Fig. 1 D). It was noted that the band close to the origin showed considerable variations among individual patients. This band also decreased in intensity more rapidly than the faster band when serum was stored, and repeated runs performed or when concentration by lyophilization rather than pressure dialysis was employed.

Before purification of oil red O was undertaken, the number of bands seen on the gel strips where whole serum has been separated, was not constant and at times all serum proteins were stained. Some bands in addition appeared to be reddish-brown or even yellowish-brown. This occurred whenever a new

‡ Generously supplied by Airborne Instrument Co., Mineola, L. I., N. Y.

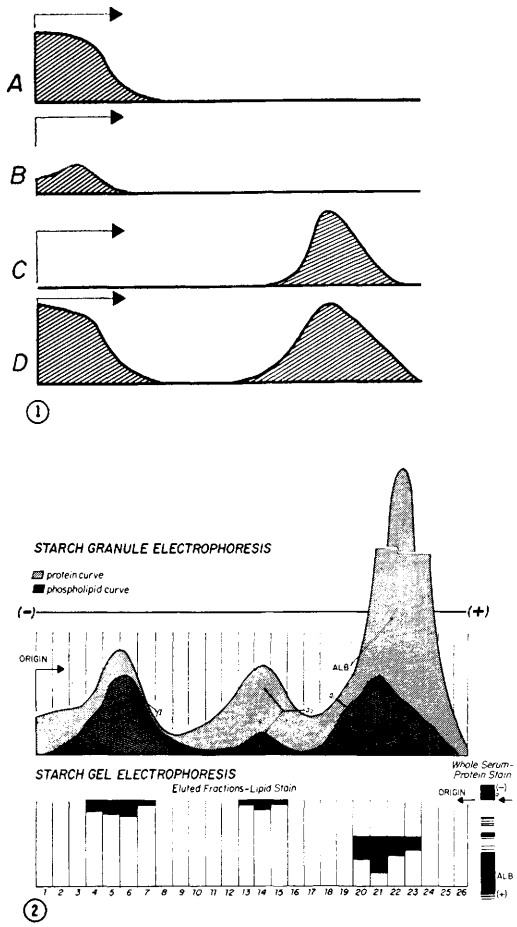

FIG. 1. Photoelectric scan patterns of starch gel electrophoresis strips. A. Beta lipoprotein; B. Alpha-2 lipoprotein; C. Alpha-1 lipoprotein; D. Full serum lipoprotein pattern. Arrow marks point of application and points toward anode.

FIG. 2. Comparison of migration of serum lipoproteins by starch granule electrophoresis and starch gel electrophoresis. Protein curve (lightly shaded) and phospholipid curve (heavily shaded) of starch granule electrophoresis are shown in upper part of Figure. Twenty-six segments were eluted and run in starch gel. Location of lipoprotein bands in starch gel is illustrated in lower part of Figure. Gamma section of starch granule block has been omitted.

batch of staining solution was prepared, which happened to contain a large amount of yellow-brown protein staining components. Following use of purified oil red O, only the slow and fast bands were consistently seen.

Discussion. Electrophoretic mobilities of alpha-1 and beta lipoproteins in starch gel could be anticipated. They were similar to zone electrophoresis patterns obtained with other supporting media such as filter paper, starch granules or agar, and confirm the findings of Silberman(7) and Poulik and Smithies (8). The behavior of alpha-2 lipoproteins, however, is of interest. The work of Kunkel and Trautman(4) indicated that some alpha-2 lipoproteins are lighter than serum and possibly contain chylomicra. Our results tend to support that evidence, in that migration of alpha-2 lipoproteins in starch gel is similar to the beta, low density, lipoproteins. It appears therefore that when whole serum is submitted to starch gel electrophoresis, the slow band includes beta and alpha-2 lipoproteins, whereas the band in the postalbumin region contains only high density lipoproteins.

When fat dyes are used which contain protein staining components for demonstration of lipoproteins, the starch gel method offers some advantages over paper electrophoresis. Lipoproteins in gel migrate in areas, where no major protein component is present and the co-staining of proteins is therefore less disturbing in the interpretation of patterns. The major disadvantage of the gel method is that quantitative estimates from gel strips can be obtained only by scanning devices and these are less accurate than data obtainable with dye elution technics.

Summary and conclusions. 1. Alpha-2 and beta lipoproteins in starch gel migrated close to the origin, whereas alpha-1 lipoproteins migrated in the postalbumin region. 2. Some of the causes of inconsistencies of lipoprotein patterns in starch gel are discussed.

1. Smithies, O., *Biochem. J.,* 1955, v61, 629.

2. Pert, J. H., Kutt, H., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 181.

3. Kutt, H., Tsaltas, T. T., *Clin. Chem.,* 1959, v5, 149.

4. Kunkel, H. G., Trautman, R., *J. Clin. Invest.,* 1956, v35, 641.

5. Kunkel, H. G., *Methods of Biochemical Analysis,* 1954, v1, 155.

6. Mies, H., *Klin. Wschr.,* 1953, v31, 159.

7. Silberman, H. J., *Biochim. Biophys. Acta,* 1957, v24, 647.

8. Poulik, M. O., Smithies, O., *Biochem. J.,* 1958, v68, 636.

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