determined under conditions of high background illumination (0.28 mlam). Also, these animals were thoroughly light adapted prior to the experiment by exposing them for 2 hours to the light from a 60-watt bulb suspended over their home tank.

The spectral sensitivity of animals in group 2 was carried out under mesopic conditions (0.01 mlam). In each case the background illumination was provided by the room's fluorescent lights and stray light from the projector. The level of background illumination was determined for groups 1 and 2 by measuring with an exposure photometer (Salford Electrical Instruments Ltd., London) the amount of light reflected from the white cardboard base upon which the container was placed.

During a threshold run the stimulus light was left on for 5 minutes before being interrupted by a mechanical shutter. Ten minutes were allowed between trials, and on successive trials the intensity of the stimulus beam was reduced in steps of approximately 0.2 log unit. The animal rarely, if ever, responded to the onset of illumination, and the adequate stimulus appeared always to be the sudden diminution in light intensity. However, once the animal had responded, it seemed to enter into a refractory phase for a period of at least 5 minutes, during which no change in light condition would produce a closure response, although it was still possible to elicit such a response by mechanical stimulation. Because of this, it was necessary to allow at least 10 minutes to elapse between trials. The threshold at a particular wavelength was determined by taking the lowest intensity at which the animal would respond twice in the course of three successive trials.

The spectral sensitivity curves are shown in Fig. 1. Individual results within each group were pooled; each of the experimental points in Fig. 1 represents an average threshold. In each case the appropriate correction factor has been added to compensate for the difference in light energy transmitted by different interference filters. Standard deviations were computed for the data within each group and are represented in Fig. 1 by the vertical lines on the curves.

It is immediately apparent that at least two different types of photoreceptors with different spectral sensitivities contribute to the overall spectral sensitivity of this animal. One of these appears to be maximally sensitive at approximately 475 to 480 mμ, and the other at approximately 540 mμ. In the absence of direct spectrophotometric measurements on the photopigments of this eye we cannot conclude that the two peaks in the behavioral sensitivity curve represent two visual pigments, since the data might equally well be explained by assuming the existence of one receptor pigment and a screening pigment with an appropriate absorption. For the sake of comparison the points of Dartnall's (*12*) nomogram for a visual pigment with absorption maximum at 475 mμ are also displayed in Fig. 1.

Comparing now the spectral sensitivity curves between groups 1 and 2, we see that when the level of background illumination is low the relative increase in log sensitivity of the 475-mμ region is several times greater than the corresponding increase in sensitivity of the 540-mμ region. This suggests that at low background levels of illumination the photoreceptors which mediate sensitivity in the 540-mμ region may have a high-response threshold relative to the 475-mμ photoreceptors. The possibility that under conditions of high background illumination sensitivity in the 475-mμ region may be depressed by active inhibition of other photoreceptors must also be considered. In general, the situation appears to be similar to that which occurs in the vertebrate retina, where it has been shown in goldfish that the rod portion of the photopic visibility curve shows a considerable increase in sensitivity, relative to the cones, when the background illumination is reduced (*9*). These considerations lead one to suspect that the eyes of *Pecten* may possess photoreceptors that are functionally similar to the rods and cones of the vertebrate retina, and that under suitable light conditions the spectral sensitivity of this animal is likely to display a Purkinje shift.

Throughout the preceding discussion it has been assumed that the spectral sensitivity which has been measured represents the sensitivity of the eye. However, a note of caution is required in this interpretation, since the possibility is not excluded that other light-sensitive structures, perhaps located directly within the nervous system itself, may be contributing to the spectral sensitivity of this animal.

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19 November 1965

Lipoprotein Patterns in Acrylamide Gel Electrophoresis

Abstract. *Lipoproteins of serum stained before electrophoresis gave distinct patterns in acrylamide gel of 3-percent concentration. Samples compared in parallel on gel slabs showed qualitative differences in migration rates of β-lipoprotein bands.*

Human serum lipoproteins have not been extensively examined by electrophoresis in either starch gel or acrylamide gel, presumably because of inadequate technical methods (*1*). The technique described here provides (i) low gel concentration to permit migration of large lipoprotein molecules, (ii) subsequent stiffening of the gel for further processing of the pattern, (iii) an improved stain for use prior to electrophoresis, (iv) direct comparison of multiple samples on a single gel slab. The technique reveals variations in electrophoretic mobility of β-lipoproteins from different individuals and from the same individual in different clinical conditions.

Serum samples were selected at random from the routine laboratory; they were from healthy individuals and patients with various diseases, some of the patients having hyperlipemic status. Samples stored for several days

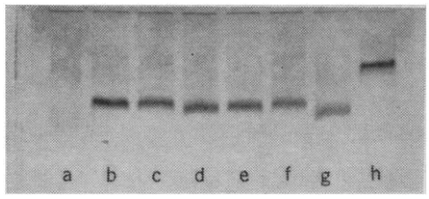

Fig. 1. (*a* to *g*) Serum specimens from subject B.S. taken at different times: (*a* and *b*) specimens 3 weeks apart; (*c* to *g*) samples taken during a glucose tolerance test, at half-hour intervals (*g* is the fasting specimen); (*h*) serum specimen from subject T.C. Electrophoresis in tris-EDTA buffer *p*H 9.0, 3-percent polyacrylamide gel, at 150 volts for 2.5 hours at 26°C; preliminary run for 15 minutes.

in the refrigerator or for weeks in the freezer gave qualitatively identical results. Hyperlipemic serums produced streaked patterns unless subjected to high-speed centrifugation before electrophoresis.

The serum samples were stained, prior to electrophoresis (*2*), with a dye solution consisting of 1-percent lipid crimson (Gurr, London) in diethylene glycol; the dye was filtered before use. The combined serum (200 μl) and dye solution (100 μl) were agitated for 5 minutes and centrifuged (Spinco 152 Microfuge) for 20 minutes. A small amount of precipitated dye was usually visible at the bottom of the tube. The clear supernatant (20 μl) was placed for electrophoresis in a slot 1 cm wide. The supernatant was reddish-purple; if lipoprotein was absent from the sample the supernatant was similarly colored, although no stained band appeared in the pattern.

The vertical cell described by Raymond (*3*) was used. The electrophoresis buffer at *p*H 9.0 contained 50 g of ethylenediaminetetraacetate (EDTA) and 460 g of tris in 45 liters of water. All gel solutions were made up in this buffer. The cell was supported at an angle of 30°, and 50 ml of an 8-percent gel solution [including 0.1 percent each *N,N,N′,N′*-tetramethylethylenediamine (TMED) and ammonium persulfate] was poured into the cell, forming a layer 3 cm deep at the bottom of the gel space. The 8-percent gel supported the 3-percent gel subsequently poured, but did not form part of the electrophoresis pattern. After the supporting gel was fully polymerized, the cell was returned to the horizontal position and 180 ml of 3-percent gel solution was poured into it. The 3-percent gel was allowed to polymerize for 2 hours, the excess gel above the slot form was removed, and the cell was then placed in the upright position. The buffer compartments were filled with buffer, and the slot form was removed. Circulation of the buffer facilitated removal of the slot form.

After a 15-minute preliminary run at 150 volts, 20 μl of the stained serum was placed in each 1-cm slot. Current was then applied at 150 volts with buffer recirculation. The temperature of the cooling plates was controlled by circulating tap water (26° to 30°C). After 2.5 hours, the visible pattern included one or more red bands in the β-lipoprotein region and two diffuse bands in the region of α-lipoprotein and albumin.

For subsequent operations a 3-percent gel was difficult to handle. Therefore a technique was developed to stiffen the gel before removing it from the electrophoresis cell. After completion of the electrophoresis, the buffer was drained, the cell was placed in the horizontal position, the upper cooling assembly was removed, and the excess gel was trimmed from the pattern. The remaining gel was covered with 120 ml of buffer containing 10 percent of cyanogum and 0.1 percent of TMED, the persulfate being omitted. After 45 minutes, 30 ml of 0.5-percent ammonium persulfate was mixed with the gel solution. A cover was placed over the pattern to exclude air and permit the stiffening gel to polymerize uniformly. By this procedure, a gel with strength close to that of an 8-percent gel was produced.

The stained lipoproteins did not migrate satisfactorily in gels of greater than 3.75-percent concentration. In 3-percent gel the pattern exhibited a zone visible about 1 to 2 cm from the origin which reacted with antiserum to β-lipoprotein. In addition there were two diffuse stained zones about 7.5 cm from the origin, one of which was identified as albumin by staining with amido black; the other, slightly slower, was presumed to be α-lipoprotein, which occurs in this position in other electrophoresis media.

In this procedure the β-lipoprotein bands exhibited variations in mobility not heretofore reported. Figures 1 and 2 show these variations in serum specimens from a single individual, and between specimens from different individuals.

In separate experiments with 5- and 8-percent gels in the techniques of Peacock (*4*) diethylene glycol either alone or with lipid crimson did not visibly alter the serum protein pattern in tris-EDTA buffer. However, in buffers containing borate, diethylene glycol caused the appearance of an extra amido-black-staining band near that of transferrin.

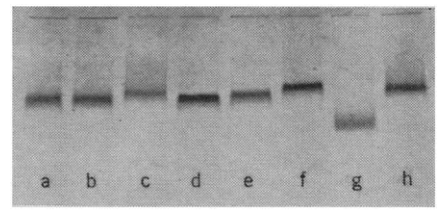

Fig. 2. (*a* to *e*) Serum specimens taken at 2-day intervals from subject N.S.; (*f* to *h*) serum specimens taken at 3-day intervals from subject M.A. Electrophoresis as described for Fig. 1.

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Chloroquine: Mode of Action

Abstract. *The drug chloroquine is bactericidal for* Bacillus megaterium; *it inhibits DNA and RNA biosynthesis and produces rapid degradation of ribosomes and dissimilation of ribosomal RNA. Inhibition of protein synthesis is also observed, evidently as a secondary effect. Inhibition of DNA replication is proposed as a general mechanism of the antimicrobial action of chloroquine.*

The antimalarial drug, chloroquine (Resochin, *1*), forms a complex with DNA (*2*, *3*) and inhibits in vitro reactions catalyzed by DNA polymerase (*4*), RNA polymerase (*4*), and deoxyribonuclease (*3*). It also inhibits incorporation of P^{32}-orthophosphate into the nucleic acids of two plasmodia (*5*). Although bacteria are relatively insensitive to the drug (*6*) we have found a strain of *Bacillus megaterium* to be susceptible to chloroquine (*7*) and