count of the detailed structural organization already described, we may hope that the insight, which has so beautifully ordered the multiple reactions of the visual opsins, will eventually weave in with function the amazing complexity of structure in which these remarkable molecules are constrained to work.

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Of the interesting points raised by Talbot, I should like at present to comment upon only one. This is the relationship between the microscopic and the submicroscopic layering of the outer segments of the rods and cones, and the location of their proteins and lipids.

The microscopic layers, which exhibit periods of 300 to 700 mμ, are not visible in fresh intact preparations. They appear only after outer segments have lain for a time in the suspension medium or have swelled in acid or alkali (*1, 2*). It has been assumed that they indicate a preexisting submicroscopic layering of unknown dimensions.

The evidence that the outer segments are composed of alternate layers of protein and lipid rests upon their behavior in plane-polarized light. They exhibit lamellar birefringence, attributable to a regular alternation of layers of material, one component of which is extractable with fat solvents (*1*). The conditions for this type of birefringence, formulated by Wiener (*3*), are that the layers differ in refractive index and be thin relative to the wavelength of light. The microscopic layers of the rods and cones are too coarse to satisfy the latter condition. The presence of lamellar birefringence itself indicates a much finer submicroscopic layering.

Sjöstrand has now revealed this in the electron microscope (*4*). The outer segment of a rod or cone presents a pile of regularly spaced, parallel membranes, varying in thickness from about 40 to 160 A and separated by spaces about 65 to 130 A wide (guinea pig rods; perch rods and cones). There is no evidence of a coarser structure. In a personal communication, Sjöstrand expresses much the same opinion as Schmidt, that the microscopic cross-striation, when visible, probably represents some effect of swelling of the normal structures.

We have recently observed in the electron microscope that the coarse cross-striation exhibited by occasional outer segments arises by the deterioration of their fine structure. In such rods the submicroscopic layers have lost their integrity and regular spacing and have coalesced to form an intricate meshwork. This presents at times the appearance of surprisingly regular cross-striation (*5*).

I think it is clear that the microscopic layering under all conditions represents an artifact and that only the submicroscopic layers need to be considered. The method of preparing the ultrathin sections of retina so far examined removes lipids; the lamellar structure that remains appears to be composed primarily of protein. The visual pigments are probably located in these layers, in the thicker layers perhaps at their interfaces. The location of the lipids is still uncertain. Some lipids may be bound to protein and located either in the protein or lipid layers. The outer segments, however, exhibit a strong positive intrinsic birefringence which appears to be associated with highly oriented layers—probably double layers—of lipid, the carbon chains of which lie parallel with the long axes of the rods and cones (*1*). Such material presumably occupies, at least in part, the interstices between the protein membranes. It is significant that the narrowest interstices yet observed—about 65 A in guinea pig rods—could just accommodate a double layer of mixed myelin sheath lipids—63.7 A in the dry state (*6*).

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Water-Soluble Chelates in Histochemical Staining

Histochemical stains for metals have depended on the precipitation of an insoluble compound that was colored or could otherwise be made visible. In the case of the metal stains, such as the calcium stains, this has required removing the metal from its naturally bound state and reprecipitating it (or the anion associated with it) as the desired compound.

The use of water-soluble chelates provides another approach to the microscopic visualization of metals. Chelating compounds bind metals in a ringlike configuration through the formation of at least one coordinate bond and frequently one or more ionic bonds. For several years these agents have been used in analytic determinations of various metals (*1*). Weak chelating compounds that change color in the presence of the metal being analyzed have often been employed as titrimetric indicators.

This communication reports preliminary studies (*2*) on water-soluble, colored chelating agents as used in two types of histochemical stains. In type 1, the colored compound coordinates with the metal and is selectively bound only where the metal is present. In type 2, the agent impregnates the tissue as a whole but

changes color only in the presence of the metal. In neither type does the histochemical demonstration of the metal require reprecipitation; thus, it may be possible to stain the metal without removing it from its naturally bound site.

Meurexide is a colored chelating agent that has been used as an indicator in the analysis of calcium in aqueous solution (*3*). Both the free compound and the calcium chelate have similar solubilities in water, and when calcium is added to a saturated solution of meurexide, no precipitation occurs. If used as a calcium stain, the meurexide reaction is of type 1; that is, it is adsorbed and bound only in the presence of the metal, staining the calcium an orange-red and leaving the background a nearly neutral color. No diffusion is apparent with periodic visual examination for at least 1 hr.

Procedure. (i) Remove paraffin from section with xylol; (ii) hydrate by bathing in two solutions of absolute alcohol, then 95-percent alcohol, and then 80-percent alcohol; (iii) stain 5 min in a solution that is both 0.1*N* in NaOH and 0.1*N* in KCN and is saturated with meurexide (*4*); (iv) wash, briefly either in distilled water or in 50-percent alcohol, so as not to decolorize excessively; (v) dehydrate and mount in Harleco Synthetic Resin (*5*).

Specificity. The stability constants of most of the meurexide-metal chelates are unknown (*1, 3*). Comparisons of serial sections stained for iron and calcium by various methods indicate that iron is complexed by the cyanide in the staining solution and does not itself stain. It seems likely that copper might be similarly bound, since it is known that present analytic determinations of calcium in water and serum using meurexide and a cyanide solution are not interfered with by the afore-mentioned metal in appreciable amounts.

Comparison with other methods. Areas stained by the Von Kossa method (silver stain for phosphate) stain with meurexide (except in areas where the phosphate is known to be present as iron phosphate). Areas that did not appear colored in the meurexide or silver-treated sections were stained by the alizarin precipitate method (*6*), but the precise role of greater specificity as opposed to less sensitivity has not been investigated. Conclusions as to the sensitivity of the meurexide stain based on the use of meurexide as an indicator in solution were not thought justified. Quantitative data on the color change of the indicator in solution may not be related to the adsorption of the dye on the metal in tissue section and may be misleading.

In addition to the afore-mentioned type-1 stain, preliminary work indicates that a substance such as Eirochrome Schwarz T may be usable as a type-2 histologic stain (*7*). Although areas of calcification appeared a midnight blue and the background remained a bright green with Eirochrome Schwarz T, this particular agent was less satisfactory than the meurexide for histologic work since the cellular detail was obliterated by the depth of the background stain. Work is in progress, however, for testing it as a magnesium stain for the magnesium ribonucleate complex of gram-positive organisms, and thus an alternate for the gram stain.

The use of water-soluble chelating agents in histochemical staining may eliminate some of the theoretical disadvantages of methods employing reprecipitation and may permit the use in histochemistry and pathology of a whole group of relatively unexplored compounds, of which meurexide is only one. Perhaps similar agents may make the localization of many different metals possible and aid the study of their metabolism and distribution.

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Aureomycin and the Thyroid Gland

A recent report (*1*) suggested that the growth-stimulating properties of antibiotics (*2*) were attributable to an antithyroid effect. The present study constituted a repetition and extension of this report (*1*) and failed to confirm any antithyroid effect of the antibiotic Aureomycin (*3*) chlortetracycline. After our manuscript had been submitted, findings similar to ours by Libby and Meites appeared (*4*).

In a preliminary trial (Table 1), Aureomycin, 1 mg/kg of the diet, and propylthiouracil, 2 percent of the diet, were used with two different diets to repeat

Table 1. Effect of Aureomycin on thyroid weight.

Diet	Addition per kg of diet	Thyroid weight (mg/100 g body weight)	"p" values
1	None	7.5* ± 0.3	
1	1 mg Aureomycin	6.6 ± .5	0.3
1	20 mg Aureomycin	9.2 ± .3	.05
2	None	9.8 ± .4	
2	1 mg Aureomycin	11.1 ± .2	.1
2	20 mg Aureomycin	9.4 ± .1	.6

* Mean ± S.E.