ent, it is meaningful to test the significance of the differences between reading 1 or 3 and the feedback reading 2. For all three intensity conditions $t$ values proved to be significant at the 1% level of confidence or beyond. Therefore we are free to reject the hypothesis that reading 1 and reading 2 are the same. It would seem likely, therefore, that the delayed side-tone produces a significantly retarded reading rate for all three intensity conditions. It is well to note that in no case, despite the instructions, did a subject read the delayed reading at a more rapid rate.

3. It is of interest to note that the magnitude of the differences between readings 1 and 2 increases with increases in intensity of the feedback over the ranges studied (35, 55, and 75 db). The mean difference between readings 1 and 2 for the 75-db intensity was 11.3 sec, for 55 db intensity 7.6 sec, and for 35 db intensity 2.7 sec. To determine whether these mean differences were different, an $F$ test was applied. This revealed that the null hypothesis could be rejected at the .1% level of confidence.

It appears probable that reading rate is a positive function of the intensity of the delayed side-tone.

The important finding of this study would seem to be that, despite all efforts of the sophisticated subjects to overcome the effect of delayed speech, even at intensity levels which were reported by listeners as not distracting, differences exceeding normal variation in rate were found for our sample.

Shortly after completion of this study a case of possible psychogenic deafness was presented to the authors for evaluation by the technique described. This was the case of an 11-year-old girl reporting to the Department of Otolaryngology at the State University of Iowa Hospitals complaining of severe hearing loss. Initial audiometric studies revealed losses for pure tones of between 75 and 85 db bilaterally. Her response to speech appeared inconsistent with these findings. This fact, coupled with a case history revealing emotional trauma, led the examining physician and psychologist to suspect a possible psychogenic etiology for the apparent hearing loss. So as not to reveal the actual purpose of the test, the girl was told that she would read from a picture story-book and make a record so that hospital personnel could determine what effect, if any, her hearing loss had on her speech. After establishing a normal reading pattern, the following technique was employed. Having no accurate knowledge of the loss for speech, we used an intensity of about 50-db feedback for a first reading. The abrupt and radical change in the speech pattern was so marked that no close measure was necessary to realize that the patient was responding to the feedback. From this point on the intensity level was reduced to a loudness level within the normal range of hearing acuity. It was found finally that this girl had, as far as this test was concerned, normal hearing for speech.

In view of these findings and the technique employed, it would appear that the use of the delayed side-tone or the feedback speech phenomena is feasible in the detection of malingering, and/or psychogenic deafness.[1]

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[1] The authors would like to point out that during the early discussion of this technique as a test for auditory malingering, it was discovered that other investigators, including E. G. Witting, of the Signal Corps Engineering Laboratories, cited by Lee (*1*), and S. R. Silverman, of Central Institute for the Deaf, St. Louis, had also proposed a similar notion in the course of informal conversations reported to the present writers by Scott N. Reger.

## Cis-trans Isomers of Vitamin A and Retinene in Vision


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We have recently described the synthesis of rhodopsin *in vitro* in a system of four components: vitamin A,[2] the precursor of the rhodopsin chromophore; opsin, the protein moiety of rhodopsin; and liver alcohol dehydrogenase and cozymase, the enzyme and coenzyme that oxidize vitamin A to retinene (*2, 3*). The vitamin A in our original experiments was a concentrate from fish liver oils. When this was replaced with an equivalent amount of crystalline vitamin A, the system no longer synthesized rhodopsin.

It seemed at once that this difference in behavior must have the following significance: Vitamin A, like other carotenoids, exists in a number of stereoisomeric forms, depending upon whether the groups adjacent to its double bonds are in cis- or trans- arrangement (*4, 5*). For steric reasons, only four different isomers are likely to occur in appreciable quantities (*6, 7*). Their structures are shown in Fig. 1.

The usual preparations of crystalline vitamin A probably represent the most stable, di-trans isomer. A second isomer, called neovitamin A, was crystallized by Robeson and Baxter (*5*); it probably is the $\Delta_5$-cis form. A third modification, synthesized by Graham, van Dorp, and Arens (*8*), is probably the $\Delta_3$-cis isomer. The di-cis isomer has not yet been identified with certainty. Fish liver oils contain mixtures of


[1] This research was supported in part by a grant from the Medical Sciences Division of the Office of Naval Research. Portions of the data were presented at the spring meeting of the Society of Biological Chemists in Cleveland in 1951 (*1*), and at the Symposium on Vitamins and Trace Elements held in honor of E. V. McCollum at The Johns Hopkins University Sept. 10–11, 1951. A detailed account of this investigation will be published in the *Journal of General Physiology*.


[2] Throughout this paper the terms vitamin A and retinene refer to vitamin $A_1$ and retinene$_1$. There is every reason to suppose that a similar situation involves vitamin $A_2$ and retinene$_2$ in the porphyropsin system, but this has not yet been explored.

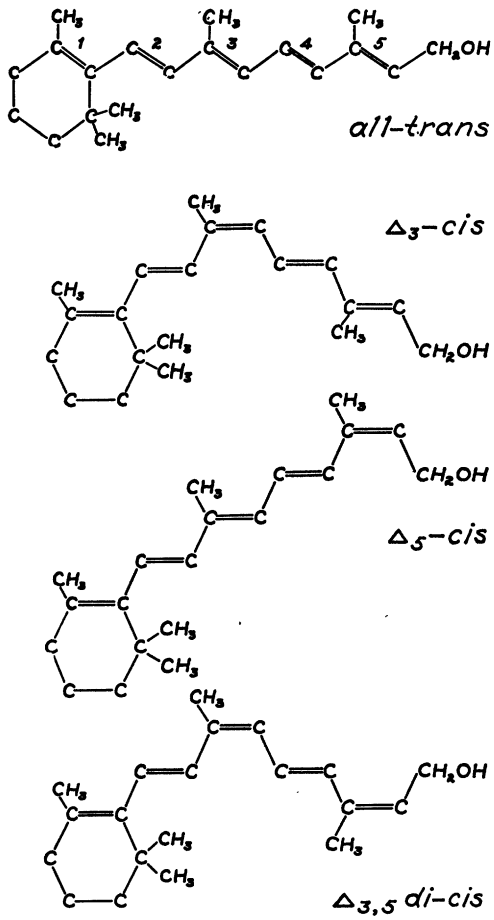


FIG. 1. Stereoisomers of vitamin A. The only forms which probably need to be considered are the di-trans isomer, two mono-cis isomers, and one di-cis isomer. Other forms probably occur only in negligible amounts (*cf.* 6, 7).

di-trans and cis-isomers in various proportions (*5, 9*). Our observation implied, therefore, that di-trans vitamin A is ineffective in the synthesis of rhodopsin, and that some cis-isomer present in liver oil is the precursor of the visual pigment.

Like other carotenoids, vitamin A is readily converted by light in the presence of a trace of iodine to an equilibrium mixture of cis- and trans- isomers (*4, 5*). When a solution of crystalline vitamin A is treated in this way, it becomes as efficient a precursor of rhodopsin as the liver oil concentrate (Fig. 2). A sample of crystalline neovitamin A, for which we are indebted to J. G. Baxter, proved to be as ineffective as the ordinary crystalline vitamin. This again, on isomerization with iodine in the light, became an effective source of rhodopsin (Fig. 2).

It can be concluded that the precursor of rhodopsin is some cis- isomer of vitamin A other than neovitamin A. The most probable such form is the $\Delta_3$-cis isomer. We have had no opportunity as yet to test a pure sample of this material.

Such structural specificity as this is usually associated with protein reactions. The rhodopsin system includes two proteins: alcohol dehydrogenase and opsin. Which of these is isomerspecific?

We have found that crystalline liver alcohol dehydrogenase[3] acts effectively upon crystalline vitamin A and upon the natural mixture of isomers. This is hardly surprising, for liver alcohol dehydrogenase is a relatively unspecific enzyme, which accepts as substrates primary alcohols from ethanol up to vitamin A, and seems to be concerned primarily with the presence of the terminal alcohol or aldehyde group.

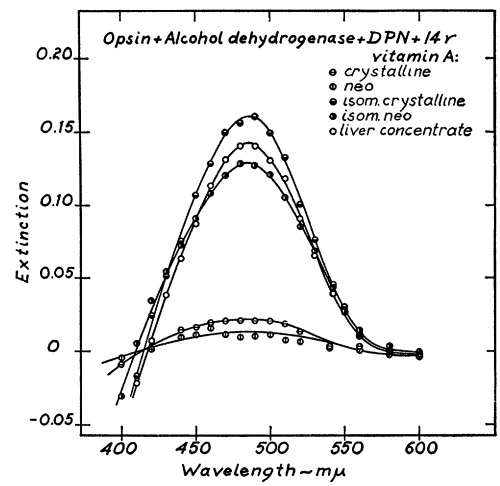


FIG. 2. The synthesis of rhodopsin *in vitro* from various configurations of vitamin A. Five solutions contain equivalent mixtures of cattle opsin, crystalline alcohol dehydrogenase from horse livers, cozymase (DPN), and vitamin A. After incubation in the dark for 15 hr at 23° C, pH 6.8, each mixture was examined for the presence of rhodopsin. The figure shows the difference spectra of rhodopsin—the differences in absorption spectrum before and after bleaching with light in the presence of hydroxylamine. From liver oil vitamin A, which contains a mixture of isomers, considerable rhodopsin had been formed. From crystalline vitamin A and crystalline neovitamin A almost no rhodopsin was formed; but after these preparations had been isomerized with light in the presence of iodine, they were about as active as liver oil concentrate.

The dominant isomer-specific component in our system is opsin. Retinene is easily prepared by the oxidation of vitamin A on a short column of solid manganese dioxide (*10*). Retinene prepared in this way from liver oil vitamin A condenses readily with opsin to form rhodopsin, whereas retinene prepared from crystalline vitamin A is almost wholly ineffective (Fig. 3). Special precautions must be taken in such experiments, for retinene is rapidly isomerized by light even in the absence of iodine. It is necessary, therefore, to shield the retinene from light throughout the course of the procedure.

Robert Gregerman, in our laboratory, has crystallized the presumptive di-trans and neoretinene prepared, respectively, from crystalline vitamin A and from neovitamin A. They differ slightly in absorption spectrum, retinene possessing an absorption maximum in ethanol at 383 mμ, neoretinene at 377 mμ. Both isomers proved to be almost entirely inactive in

[3] We are indebted to R. K. Bonnichsen for a gift of crystalline alcohol dehydrogenase prepared from horse livers.

rhodopsin synthesis. After several minutes' exposure to light, both preparations formed rhodopsin efficiently. The immediate precursor of rhodopsin, therefore, is a cis-retinene other than neoretinene. It may well be the $\Delta_3$-cis isomer.

Which configuration of retinene is liberated in the bleaching of rhodopsin? To answer this question, we bleached rhodopsin in the dark with alcohol, to shield the newly formed retinene from isomerization by light. The retinene obtained from rhodopsin in this way is relatively *ineffective* in rhodopsin synthesis.

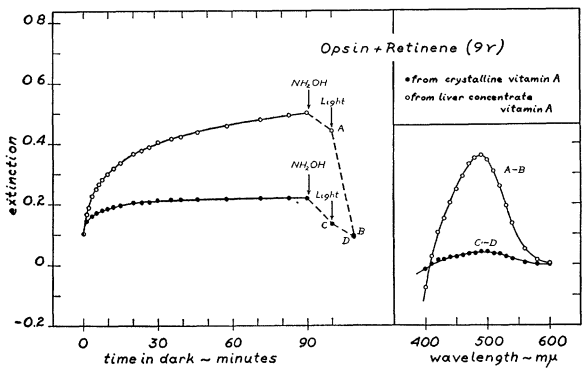


FIG. 3. The synthesis of rhodopsin *in vitro* from various configurations of retinene. Two solutions contain cattle opsin and equal amounts of retinene, prepared either from crystalline vitamin A or from the natural mixture of vitamin A isomers found in liver oil. Both solutions were incubated in the dark for 90 min at 22° C, pH 7. Then hydroxylamine was added to both, and they were tested for the presence of rhodopsin by measuring the absorption spectra before and after bleaching with light. The left-hand portion of the figure shows changes in the extinction at 500 mμ, the absorption maximum of rhodopsin; the right-hand portion shows the difference spectra of the rhodopsin which had been synthesized. Only a trace of rhodopsin was formed from the retinene derived from crystalline vitamin A, whereas the retinene derived from liver oil vitamin A yielded a large synthesis of rhodopsin.

Some years ago Chase, and Chase and Smith (*11*), reported that solutions of rhodopsin bleached with light containing blue and violet components of the spectrum regenerate rhodopsin slightly on subsequent incubation in the dark, whereas rhodopsin bleached with yellow light fails to regenerate. They noted also that blue light caused a special fall in absorption at low wavelengths (450 mμ), not found with yellow light. They concluded that their preparations contained some yellow substance, the decomposition of which by light is necessary for the regeneration of rhodopsin.

It is now clear that this substance is retinene, and that the action of light is not to decompose but to isomerize it. The absorption spectrum of retinene, maximal at about 385 mμ in water solution, extends into the violet and blue, and falls to negligible values in the green and beyond. Only light that is absorbed by retinene can isomerize it. Blue light, therefore, isomerizes retinene efficiently, but yellow light has no effect upon it. Both blue and yellow light bleach rhodopsin and yield as the immediate product an inactive isomer of retinene. Only blue light goes on to

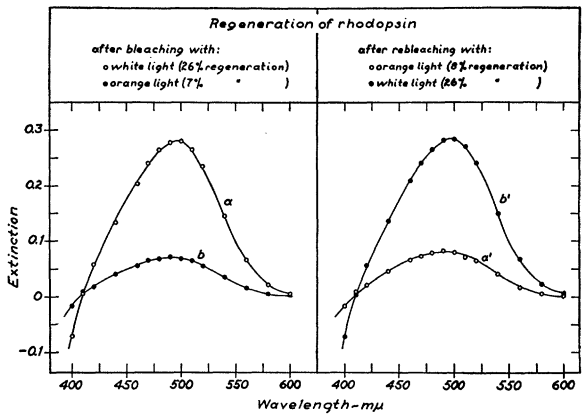


FIG. 4. The regeneration of rhodopsin after bleaching with white light (400–700 mμ) and with orange light (540–700 mμ). Two samples of a solution of cattle rhodopsin were bleached completely by irradiating for 20 min with these sources of light; then were incubated in the dark for 1½ hr at 23° C. The left-hand portion of the figure shows the difference spectra of the regenerated rhodopsin. Then samples of both solutions were rebleached as before, but with the lights reversed: the solution bleached the first time with white light was rebleached with orange light, and vice versa. Both were again incubated in the dark as before. The right-hand portion of the figure shows the rhodopsin regenerated after the second bleaching. In both cases the solutions had regenerated only 7–8% after bleaching in orange light, 26% after bleaching in white light.

isomerize this product, permitting the rhodopsin to regenerate.

These relations are illustrated in Fig. 4, which repeats under more favorable conditions an experiment first performed by Chase and Smith (*11*). The bleaching of rhodopsin by a source of light containing only wavelengths longer than 540 mμ is followed by a regeneration of 7–8 %; whereas, after bleaching with light containing all wavelengths above 400 mμ, 26% of the rhodopsin regenerates. In the experiment shown, half the rhodopsin was subjected to a second bleaching in which these lights were reversed; the sample first bleached with long wavelengths was rebleached with the entire spectrum, and vice versa. It is clear that the extent of regeneration is governed simply by the degree to which the light used to bleach rhodopsin can also isomerize retinene.

During this isomerization the absorption spectrum of the retinene liberated from rhodopsin shifts several mμ toward shorter wavelengths—from about 387 mμ to about 383 mμ—and falls somewhat in height. These changes account for the special decrease of absorption in the violet observed by Chase and Smith when rhodopsin is bleached with blue light. Such changes in spectrum are regularly associated with the stereoisomerization of all-trans carotenoids to cis-configurations (*4, 6*). We have observed them on irradiating crystalline di-trans retinene in solution with white light. They indicate that the inactive form of retinene derived from rhodopsin is probably the di-trans isomer.

Since the retinene that emerges in the bleaching of rhodopsin is a different isomer from that which enters its synthesis, the stereoisomerization of retinene or of

the corresponding vitamin A is an integral and necessary component of the rhodopsin cycle. Its place in the cycle may be expressed in some such diagram as Fig. 5.

In the eye, part of this isomerization may be accomplished by light, but this is probably not the most important way in which the retina obtains the active isomer. For one thing, it should be noted that vision continues very well in yellow, orange, and red light, in which no isomerization takes place. Furthermore, there is little opportunity for light to isomerize retinene in the eye, since ordinarily retinene is removed almost as fast as formed, by reduction to

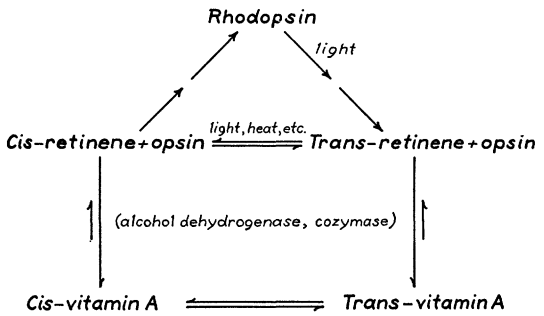


FIG. 5. The place of geometrical isomers of retinene and vitamin A in the rhodopsin cycle. Retinene enters rhodopsin as a cis-isomer, and emerges apparently as the all-trans isomer. This can be reisomerized to the active form by light or heat. More generally *in vivo* new supplies of the corresponding vitamin A are withdrawn from the circulation. On a long-term basis all the isomers of vitamin A are in equilibrium in the body.

vitamin A. For these reasons it seems probable that the eye must be continually supplied with the active isomer of vitamin A from the blood circulation, which in turn takes it from stores in the liver and, ultimately, from the nutrition. In the process of seeing, the retina continuously withdraws the active isomer of vitamin A from the circulation and returns to the blood the inactive, di-trans isomer. In this way the visual process is connected intimately with the metabolism and transport of vitamin A throughout the body.

It is probably not necessary to feed the active isomer to make it available in vision, for vitamin A apparently isomerizes in the body. After feeding either crystalline vitamin A or neovitamin A to rats, Robeson and Baxter (*5*) found that mixtures of both isomers were deposited in the liver. The rate of isomerization *in vivo* is not known, yet it seems to keep pace at least with such long-term processes as growth, since vitamin A, neovitamin A (*5*), and the cis-isomer of retinene prepared by Graham *et al.* (*8*) all are reported to yield comparable bioassays in growth tests in the rat.[4]

[4] Amending the original conclusion of Robeson and Baxter (*5*) that all-trans and neovitamin A have "substantially the same" biological potency in rats, Harris, Ames, and Brinkman (*12*) have recently reported that neovitamin A is 80.7% as potent as the all-trans isomer in promoting rat growth, and 71.5% as effective in causing the storage of vitamin A in the rat liver.

The experiments we have described introduce a new factor in the biochemistry of vitamin A—that of stereochemical specificity in its interactions with enzymes and other proteins. Certain of its reactions involve this factor acutely, others do not. We have already encountered an example of each kind: specificity in the reaction of retinene with opsin, relative indifference in the reaction of vitamin A or retinene with alcohol dehydrogenase. This type of relation will need to be considered in all future work on vitamin A metabolism.

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## Making Names of Biological Taxa from Greek Stems


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The names applied to genera and higher taxonomic groups (taxa) in biology (including zoology, botany, and bacteriology) are frequently, even preferably, derived from the Greek. Appropriate transliteration is essential if the Latin name resulting is to be in good form. The Latins developed certain rules which they generally followed when adapting a Greek word to Latin usage. Presumably these rules are the ones to be observed in biology. As stated by Linnaeus in his *Critica Botanica* (Hort trans.): "When Greek names are transliterated into Latin, the equivalents used by the Romans from all time must be adopted in representing the Greek letters." And yet there is nowhere to be found a complete and sufficient summary of these principles in any of the three international biological codes of nomenclature, nothing adequate to guide in the formulation of *new* names from Greek stems. The statements appended to botanical and zoological codes are incomplete and in some cases misleading. There is in consequence much unwarranted confusion in the spelling of biological names. The bacteriologist is deeply concerned because he must constantly use the names proposed in all three major fields of biology.

A preliminary statement in the form of a recommendation relative to the transliteration of Greek to