are also available for the swift transportation of sick and wounded.

If I were to name another and one of the most admirable characteristics of American culture, it would be the gradual union of the physical and the human sciences, and more especially the union of the natural sciences with the social sciences and the humanities. In these troubled days the scientists can take little satisfaction in the social consequence of their discoveries. The material contributions of science alone do not create a rich and satisfying life. Nor do the intellectual values of science alone provide the spiritual satisfaction which men crave. Scientists are merely partners of many others in mankind's great endeavor. Science liberates men from the fear of unknown natural forces, frees men from grinding toil for mere survival, subdues pain, and cures sickness. Thus, science frees men to enjoy art and music and literature and the beauties of nature and religious faith. Science makes possible the enjoyment of much that science alone cannot give. Scientists are partners of those in other walks of life who seek to improve man's estate.

I should be blind to the status of modern American science if I did not recognize its critics and opponents. Many are torn between *fear* of new horrors science may add and *hope* that science will build a better world. Without science, which created the atomic bomb, we would still be defenseless against natural forces and disease. Would we rather be the *certain* victims of natural forces or *possible* victims of atomic energy misused by man? The question is: Do we have courage to understand the facts of nature and educate our fellowmen to use them for human welfare?

Science provides the building stones of a better world—but the world will be as we choose to make it.

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The Enzymatic Reduction of the Retinenes to the Vitamins A

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THE RETINENE$_1$, formed by the bleaching of rhodopsin, is converted to vitamin A_1 by a reaction for which reduced cozymase (DPN—H_2) serves as coenzyme (*10*). Retinene$_1$ is vitamin A_1 aldehyde (*2*); and the essential process is the transfer of two hydrogen atoms from DPN—H_2 to this molecule, reducing its carbonyl group to the primary alcohol group of vitamin A_1:

$$\underset{\text{retinene}_1}{C_{19}H_{27}CHO} + \text{DPN—}H_2 \xrightarrow{\text{retinene reductase}} \underset{\text{vitamin } A_1}{C_{19}H_{27}CH_2OH} + \text{DPN}$$

In the outer segments of the retinal rods this system is coupled with a second one which reduces DPN (*10*).

The reduction of retinene$_1$ has been followed in cell-free bries of whole retinas, in suspensions of isolated outer segments of rods (*10*), and in freshly prepared solutions of rhodopsin in aqueous digitonin (*3, 10*). Such fresh rhodopsin solutions lose the capacity to reduce retinene$_1$ within 3–4 hrs after preparation. This is because they lose their DPN—H_2 by the action of an enzyme widespread in animal tissues and particularly active in brain, to which retina is closely related (*4*). Rhodopsin solutions left at room temperature for 18 hrs, which have entirely lost the ability to reduce retinene$_1$, are reactivated by addition of new DPN—H_2. The apoenzyme, retinene reductase, is therefore relatively stable; the inactivation of fresh rhodopsin solutions is due to the loss of the coenzyme.

The retinene reductase system has now been fractionated into its components, all in true solution. Two components are in a satisfactory state of purity and chemical definition: the coenzyme, DPN—H_2, prepared by Ohlmeyer's method (*5*); and the substrate, synthetic retinene$_1$, prepared by the chromatographic oxidation of crystalline vitamin A_1 on manganese dioxide (*2, 8*).

The apoenzyme has not yet been isolated as a pure substance, but it has been prepared free of the other components. It is extracted with dilute salt solutions from homogenized frog or cattle retinas, forming a

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clear, almost colorless solution. From this it is precipitated by half-saturation with ammonium sulfate and may be redissolved without loss of activity. It can also be dialyzed in a Visking membrane for as long as 18 hrs against M/15 phosphate buffer (pH 6.8; 5° C) without harm. It is destroyed by heating at 100° C within 30 sec. Its pH optimum lies at about 6.5.

Retinene$_1$ is a typically fat-soluble substance and was originally introduced into our enzyme system with the aid of digitonin, with which it forms a water-soluble complex. This step proved to be unnecessary, however, since the retinal extracts which contain our apoenzyme take up retinene$_1$ directly. This in itself is evidence that retinene$_1$ couples with water-soluble substances from the retina. Primarily it attaches to protein, for it is precipitated from such solutions with the protein fraction.

It has been known for some time that in the product of bleaching rhodopsin *in solution,* retinene$_1$ remains loosely coupled with protein (*7*). In this condition it displays the properties of a pH indicator. Synthetic retinene$_1$ does not exhibit this behavior; nor does natural retinene$_1$ after adsorption and elution (*8*). Ball, *et al.* (*1*) have now shown that the pH indicator property is characteristic of retinene$_1$ in the coupled condition; synthetic retinene$_1$ acquires this property on condensing spontaneously with certain proteins, amino acids, and aromatic amines. Indeed, further evidence that the synthetic retinene$_1$ taken up by our apoenzyme solutions is coupled in this manner is that it has become a pH indicator. It has in fact taken on the properties generally associated with the product of bleaching rhodopsin in solution.

It is of some importance to note that retinene$_1$ is not restricted to a single complex in its retinal associations. Rhodopsin and retinene reductase are two distinct proteins. Retinene$_1$ normally originates on rhodopsin protein, but it must migrate onto the apoenzyme preparatory to its reduction. Such migrations of retinene$_1$ and changes of the molecules with which it is coupled must play an important part in retinal metabolism.

In the rods of fresh-water fishes, lampreys, and some amphibia, rhodopsin is replaced by porphyropsin (*9*). The bleaching of porphyropsin forms retinene$_2$. This is reduced to vitamin A$_2$ by an enzyme system entirely comparable with that which reduces retinene$_1$ in cattle and frogs.

This system may be assembled from the following components: the apoenzyme, contained in a saline extract of homogenized fresh-water fish retinas (sunfish, yellow perch); as coenzyme, DPN—H$_2$; and as substrate, synthetic retinene$_2$, prepared from vitamin A$_2$ by chromatographic oxidation on manganese dioxide (*8*).

The apoenzyme from fresh-water fishes, however, reduces retinene$_1$ as effectively as it does retinene$_2$. Conversely, the frog retinal apoenzyme reduces retinene$_2$ as well as retinene$_1$. There is no reason at this time to designate the apoenzyme differently in the rhodopsin and porphyropsin systems, since it is capable, in cooperation with DPN—H$_2$, of reducing either retinene to the corresponding vitamin A. It can be referred to simply as retinene reductase.

It was remarked above that retinal homogenates and extracts contain an enzyme which destroys DPN. In our fresh-water fish extracts this destructive action was so intense as to block the reduction of retinenes even when DPN—H$_2$ had been added. It is essential to protect the coenzyme in such preparations and it is advantageous to do so also in extracts of frog and cattle retinas.

Cozymase can be protected by adding nicotinamide to the enzyme system, in a final concentration of about 0.03 molar (*4*). It has been reported that α-tocopheryl phosphate (vitamin E phosphate; 0.0015 molar) similarly protects DPN (*6*). This last reagent has another beneficial effect on our system—as an antioxidant it inhibits the oxidation of the vitamins A formed as products of the reaction.

The retinene reductase system as most effectively assembled therefore reveals an extraordinary degree of vitamin interaction *in vitro.* The main process presents the novel phenomenon of one vitamin regenerating another, in that the DPN—H$_2$ which reduces the retinenes to the vitamins A, contains as its central component the antipellagra factor, nicotinamide, a member of the vitamin B complex. While so engaged, DPN—H$_2$ is protected from cleavage by the presence of free nicotinamide. The latter is aided in this action by vitamin E phosphate, which simultaneously protects the vitamins A formed in the main reaction from oxidative destruction.

A complete account of these experiments will appear in the *Journal of General Physiology.*

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