

## THE VISUAL CYCLE AND PROTEIN DENATURATION

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Communicated December 28, 1935

If recent advances in knowledge of visual purple are examined in the light of our present understanding of protein denaturation, it is perhaps possible to perceive what chemical reaction takes place, when light strikes the retina. In this paper it is shown that there is evidence indicating that light denatures a conjugated protein, visual purple, and that denaturation reverses in the dark.

It has been known for over fifty years that visual purple plays a rôle in vision. Light bleaches visual purple, converting it into visual yellow, in both the intact eye and the excised retina. In the dark visual purple reappears. The rate of bleaching varies with the wave-length of the incident light, and it has been observed that the curve relating rate of bleaching of visual purple to wave-length is almost identical with the curve relating luminosity to wave-length in the dark-adapted eye.<sup>1,2</sup>

It has also long been known that visual purple is a colloid. Wald<sup>3</sup> has collected the evidence indicating that it is a conjugated protein. All of this evidence is concerned with one of the most characteristic protein properties, denaturation. The exceedingly varied agents that cause protein denaturation also result in the destruction of visual purple, so that it would appear that visual purple is a protein.

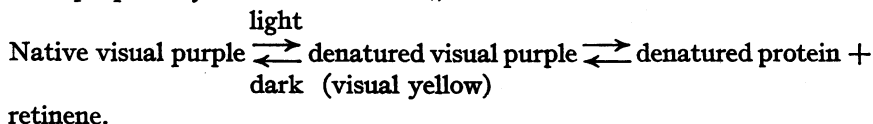
Before proceeding with the discussion of visual purple some of the properties of other conjugated proteins will be considered. Of these hemoglobin is the best understood. The denaturation of hemoglobin is completely and readily reversible.<sup>4,5</sup> The denaturation of globin is also reversible.<sup>6</sup> If heme is added to native globin, hemoglobin is formed; if it is added to denatured globin, hemochromogen or parahematin (the reduced and oxidized forms of denatured hemoglobin) is formed. In denatured hemoglobin it is possible to demonstrate an equilibrium between the conjugated protein on one side and globin and free heme on the other.<sup>7,8</sup> In native hemoglobin if there is such an equilibrium, it is shifted entirely to the side of the conjugated protein. Since the bond between denatured globin and heme is much looser than that between native globin and heme, it is necessary to denature hemoglobin in order to separate heme and globin. Native hemoglobin  $\rightleftharpoons$  denatured hemoglobin  $\rightleftharpoons$  denatured globin + heme  
(hemochromogen or parahematin)

Recently another conjugated protein, the "yellow ferment" of Warburg, has been the subject of a brilliant investigation by Theorell.<sup>9</sup> In this sub-

stance a protein is joined to a yellow pigment, a flavine. Experiments essentially similar to those on hemoglobin have been performed. The bond between denatured protein and flavine appears to be much looser than that between native protein and flavine, so that the method of separating protein and pigment involves the denaturation of the protein. In view of the closely analogous experiments on hemoglobin the conclusion is justified that the ferment is reconstituted only when the flavine is joined to protein the denaturation of which has been reversed.

The experiments on hemoglobin and the "yellow ferment" demonstrate that in these conjugated proteins the bond between protein and prosthetic group is much weaker when the protein is denatured than when it is native. Separation of the two components of the conjugated protein involves denaturation, and reconstitution of the original conjugated protein requires reversal of denaturation. Reversal of denaturation has also been observed in serum albumin<sup>10</sup> and trypsin.<sup>11,12</sup> A careful study of protein denaturation has shown that it is a definite chemical reaction and not a vague disintegration of the protein.<sup>13</sup>

Returning to visual purple, and in particular to the important recent experiments of Wald,<sup>3</sup> it will be apparent that our knowledge of hemoglobin and the "yellow ferment" is applicable to this conjugated protein. Wald has shown that the pigment in visual yellow, retinene, is a carotene-like substance and that it is convertible into the well-known carotene, vitamine A. In visual purple the pigment is very firmly attached to protein, but in visual yellow retinene is loosely held (if, indeed, it is bound at all) and can be readily separated from protein. Retinene can be prepared from visual purple only after the action of light or of a denaturing agent such as chloroform. The behavior of visual purple resembles that of other conjugated proteins in which a carotene is the prosthetic group. In all of these conjugated proteins the color of the native complex varies from purple to green, and that of the denatured complex varies from red to yellow. In each case the prosthetic group is firmly bound by native protein, loosely bound by denatured protein. The carotenoid-proteins in this respect resemble hemoglobin and the "yellow ferment." All these facts have led me to the conclusion that visual yellow is the denatured form of visual purple. Light denatures visual purple, thereby causing its color to change from purple to yellow. In the dark, denaturation reverses.



The function of retinene in visual purple is to provide an increased absorption coefficient in the visible, to sensitize the protein. The protein itself differs from other proteins in having a lower energy of activation, so

that it is denaturizable by a quantum of visible light, whereas other proteins require a quantum of ultra-violet light to be denatured. The absorption band of visual purple has a maximum in the visible spectrum at about 5300 A.U. At this wave-length one quantum contains 53,700 calories. Proteins possess several absorption bands in the ultra-violet, that of longest wave-length being at 2800 A.U., at which wave-length a quantum contains 101,700 calories. The temperature coefficient of denaturation of egg albumin and of many other proteins is about 600 for an interval of ten degrees. This corresponds to an energy of activation of approximately 150,000 calories per mole. A quantum of light at 2800 A.U. is insufficient to activate egg albumin, and experiment shows that some heat, in addition to the light, is needed to coagulate egg albumin after it has been radiated.<sup>14,15</sup> A quantum of light at 5300 A.U. would contain far too little energy to activate egg albumin. This difficulty does not, however, exist in the case of visual purple because when it is denatured by heat the energy of activation is only about 75,000 calories per mole,<sup>3</sup> a value not widely different from the energy content of a light quantum at 5300 A.U.

This paper has been concerned with the primary effect of light on the retina rather than with the way in which an impulse is conveyed to the nerve endings. Since the two processes are intimately related, it may be noted that the difficulty of extracting visual purple (it can be dissolved in bile salts only) suggests that it forms part of the actual structure of the rods of the retina and its denaturation might therefore have a direct effect on the configuration of the nerve ending.

*Conclusion.*—In vision at low illuminations light denatures visual purple in the retina. Denaturation reverses in the dark.

I am much indebted to Professor Linus Pauling for the discussions we have had.

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<sup>3</sup> Wald, G., *Jour. Gen. Physiol.*, 19, 351-373 (1935).

<sup>4</sup> Anson, M. L., and Mirsky, A. E., *Ibid.*, 9, 169-179 (1925).

<sup>5</sup> Anson, M. L., and Mirsky, A. E., *Ibid.*, 17, 399-408 (1934).

<sup>6</sup> Anson, M. L., and Mirsky, A. E., *Ibid.*, 14, 605-609 (1931).

<sup>7</sup> Anson, M. L., and Mirsky, A. E., *Jour. Physiol.*, 60, 50-67 (1925).

<sup>8</sup> Anson, M. L., and Mirsky, A. E., *Jour. Gen. Physiol.*, 12, 273-288 (1928).

<sup>9</sup> Theorell, H., *Biochem. Z.*, 278, 263-289 (1935).

<sup>10</sup> Anson, M. L., and Mirsky, A. E., *Jour. Gen. Physiol.*, 14, 725-732 (1931).

<sup>11</sup> Northrop, J. H., *Ibid.*, 16, 323-337 (1932).

<sup>12</sup> Anson, M. L., and Mirsky, A. E., *Ibid.*, 17, 393-398 (1934).

<sup>13</sup> Mirsky, A. E., and Anson, M. L., *Ibid.* In the press.

<sup>14</sup> Bovie, W. T., *Science*, 37, 373 (1913).

<sup>15</sup> Clark, J. H., *Jour. Gen. Physiol.*, 19, 199-211 (1935).