$$K_0 \exp\left(\frac{-P\Delta V}{RT}\right) \equiv u.$$

Because the necessary temperature-dependent data are not available, it is not possible to evaluate the terms in equation (10). It is, however, apparent that the theory as outlined above predicts just such a maximum as was experimentally observed and correctly attributed by Bridgman to nucleation but which are here characterized as catalysts or bridges. The region of indifference would seem to arise from a second term in the sum of equation (4). The rate and equilibrium constants in the term are about the same as those in the first term, but the pressure region of stability of the new bridges is different. The second type of bridge is stable at elevated pressures, at which the first type have been reduced to a negligible concentration. Further, the pressure effect on the term $(1 + 1/K)$ has changed the sign of the velocity, and the reaction now proceeds in the opposite direction.

It would seem that a large number of phase transitions can be treated by the method outlined above. An accompanying paper uses the theory to explain an observed optimum of crystal growth of supercooled water on silver iodide crystals.

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[1] F. H. Johnson, H. Eyring, and M. J. Polissar, *The Kinetic Basis of Molecular Biology* (New York: John Wiley & Sons, Inc., 1954), chaps. 7, 8, and 9.

[2] P. W. Bridgman, *Proc. Am. Acad.*, **52**, 57, 1916.

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*THE VISUAL SYSTEM OF THE HONEYBEE**

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The insects, though a major group of animals possessing prominent eyes, have heretofore resisted attempts to learn something of the chemistry of their visual systems. They present unusual difficulties in this regard. Attempts by Wald to extract vitamin A from the heads of *Drosophila*, the grasshopper *Melanoplus*, and the dragonfly *Sympetrum* failed to yield a clear test for this substance, though relatively large amounts of tissue (6–14 gm.) were employed (unpublished experiments cited by Wald and Burg[1]). Similarly, Goodwin and Srisukh[2] have reported the absence of vitamin A in acetone extracts of whole *Locusta* and *Schistocerca*. Wolken[3] and Wolken *et al.*[4] have recently reported an unsuccessful attempt to extract a visual pigment from the heads of *Drosophila*.[5]

Recent experiments on the heads of the honeybee, *Apis mellifera*, however, have revealed the presence of retinene$_1$. Bodies and heads of bees were ground separately with anhydrous sodium sulfate or in some experiments were lyophilized, to dry the tissues. They were extracted first with petroleum ether and then with acetone, which is known to dissociate carotenoids from proteins, and retinene from all known visual pigments. The acetone extracts were evaporated to dryness under reduced pressure and taken up in petroleum ether. Four types of petroleum ether solution were prepared: (A) petroleum ether extract of bodies, (B) acetone

extract of bodies, (C) petroleum ether extract of heads, and (D) acetone extract of heads. These were chromatographed on columns of aluminum oxide (Merck Reagent, "Suitable for Chromatographic Adsorption") "weakened" by the addition of 5 per cent water. Yellow adsorption bands formed, which were eluted with 4, 10, and, finally, 40 per cent (*v/v*) acetone in petroleum ether. The various fractions were evaporated under reduced pressure, and each was taken up in 0.3 ml. chloroform. For the antimony chloride reaction, 0.25 ml. of the test solution was placed in an absorption cell in a Cary recording spectrophotometer. To this was added 1 drop of acetic anhydride and 0.5 ml. of a saturated solution of $SbCl_3$ in chloroform. The spectrum was recorded immediately.

The acetone extract of the heads (extract D) yielded the bright-blue color and absorption peak at 664 mμ, which identifies retinene$_1$ (Fig. 1). The retinene was

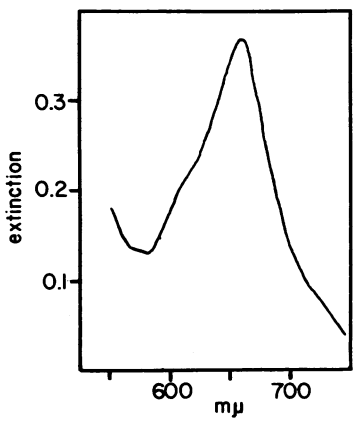

FIG. 1.—Antimony chloride test with a partly purified acetone extract of bee heads. The peak of absorption at about 660 mμ (which moves later to 666 mμ) is specific for retinene$_1$. (This spectrum and those of Fig. 2 were traced from records drawn by the Cary recording spectrophotometer.)

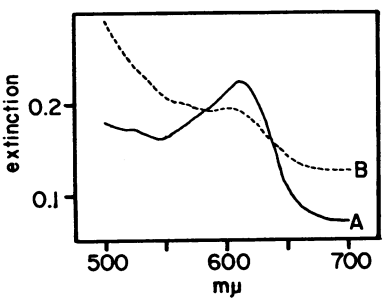

FIG. 2.—(*A*) Antimony chloride reaction of vitamin A, prepared by reducing with potassium borohydride the retinene extracted from bee heads. (*B*) The same, approximately 2 minutes later. Note the characteristic fading of the 618 mμ peak, evident despite the over-all rise in the base line.

found principally in the 10 per cent eluate. Since retinene has been found previously only in eye tissues, it is likely that in these experiments its source is the compound eyes and ocelli. No retinene was detected in the petroleum ether extract of heads (extract C, maximum of 14 gm. tissue extracted); apparently it is present only in bound form, presumably linked to protein, from which it is freed by acetone. A maximum of 0.22 μg. of retinene was obtained per gram fresh weight of heads. This corresponds to slightly more than 3×10^{-6} micromoles per eye, which, per area of retina, is roughly 5 per cent the amount of visual pigment found in cattle. As expected, no retinene could be extracted from the bodies with either petroleum ether or acetone (extracts A and B, maximum of 45 gm. of tissue extracted).

The retinene was further identified by reducing its terminal aldehyde group with potassium borohydride, forming vitamin A.[6] On mixing with antimony chloride,

this yielded the characteristic blue product with an absorption peak at 618 mμ (Fig. 2).

Other carotenoids are present in these extracts, which will be described more fully later.

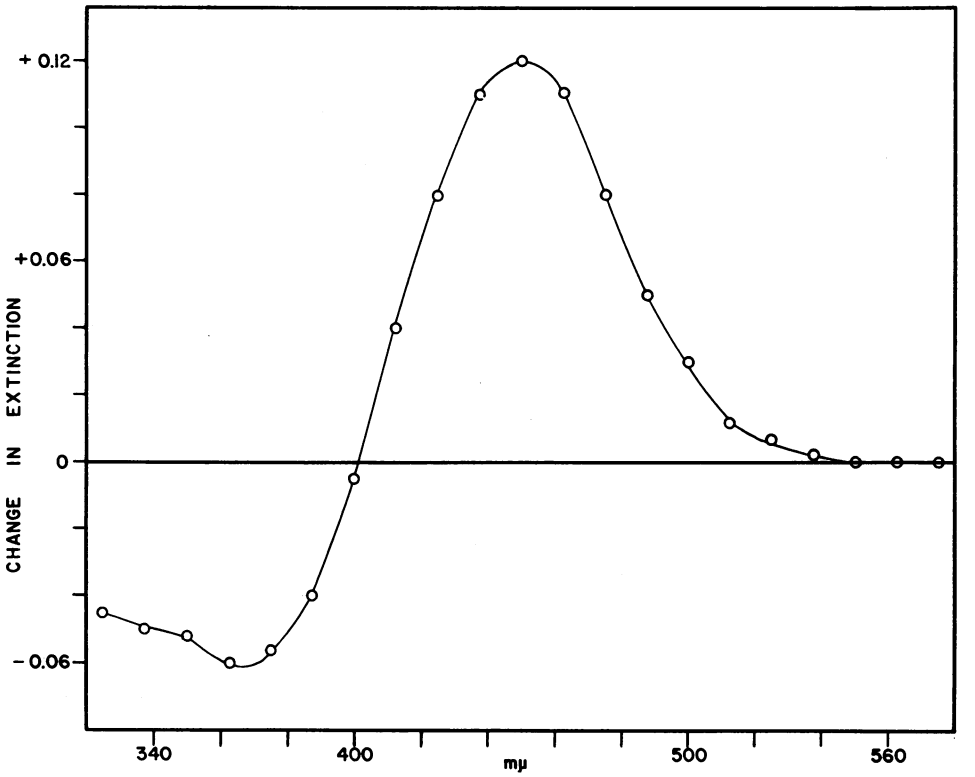

FIG. 3.—Difference spectrum of a photosensitive pigment from the honeybee. An aqueous extract of dark-adapted bees was prepared as indicated in the text. A sample (0.3 ml., pH 6.8) was irradiated for 7 minutes with yellow light (Corning filter No. 3384), and the absorption spectrum was immediately recorded. This was subtracted from the absorption spectrum of the extract before irradiation, to yield the difference spectrum shown. This possesses λ_{max} about 450 mμ. The true absorption spectrum of the photopigment has λ_{max} somewhat shorter, probably close to 440 mμ.

The identification of retinene proved to be of critical importance in the search for photosensitive pigments in the bee. Because retinene has so far been found only as the chromophore of such pigments, it seemed reasonable to assume that it plays a similar role in the bee. However, the extraction of a photosensitive pigment from bees presents special problems. It is possible to estimate the amount of visual pigment present, using as a basis the amount of retinene which can be extracted. Unfortunately, however, the heads of bees contain light-stable pigments in amounts which are vast by comparison. By using the retinene as a marker, it has been possible to devise a fractionation procedure to separate a photosensitive pigment from the bulk of the light-stable contaminants.

Unlike all other visual pigments so far examined, that of the bee is soluble in water without the aid of such solubilizing agents as digitonin. On grinding the

heads of dark-adapted bees in about 10 times their weight of neutral phosphate buffer, about 80 per cent of the total retinene-protein complex is brought into solution. This and all succeeding operations are carried out under dim red light or in complete darkness and at 4° C. The solution is cleared, first, by straining through cheesecloth, then by centrifuging. On bringing the buffer extract to 45 per cent saturation with ammonium sulfate, large amounts of colored contaminants are precipitated, leaving the retinene-protein in solution. The latter is itself precipitated by 60 per cent saturation with ammonium sulfate. The precipitate is redissolved in fresh buffer, and small molecular-weight impurities are removed by dialysis. Further colored contaminants are precipitated with 25 volumes per cent ethanol at −12° C., again leaving the retinene-protein in solution.

Using these techniques, the extract from 20 gm. of heads is concentrated in about 1 ml. of a partially purified solution, which, in favorable extracts, contains nearly 50 per cent of the retinene originally obtainable from the heads. On exposure to light, this solution exhibits a partial bleaching, owing to the presence of a photosensitive pigment with λ_{max} about 440 mμ (Fig. 3). The change in extinction in the region 440–450 mμ is roughly equivalent to the retinene formed by bleaching, if it is assumed that the photopigment possesses an extinction per retinene residue like that of rhodopsin, about 40,000.

It is not yet clear whether this photopigment comes from the compound eyes, the ocelli, or both. Possibly it is only one of several photosensitive pigments in the bee, an insect alleged to have color vision (see, for example, von Frisch[7] and Daumer[8]). Preliminary observations suggest that this pigment is formed from the neo-b isomer of retinene, and further work is now in progress to elaborate this and other points. The spectral sensitivity functions of bee compound eyes and ocelli are also being measured. Evidence has been obtained of one such sensitivity function in drones, maximal at about 440 mμ, which may be based upon the photopigment here described.

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[5] These authors purport to estimate the molecular weight of what they imply to be the visual pigment complex of the rhabdomeres of both pigmented and white-eyed mutants. However, they report that they "have been unable to isolate the visual pigment and determine its concentration . . ." and "no absorption in the visible range was found for the white-eye extracts." The significance of their ultracentrifuge data therefore remains obscure.

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