A LIGHT-SENSITIVE YELLOW PIGMENT FROM THE HOUSE-FLY*

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ABSTRACT

Extraction of house-fly heads with neutral phosphate buffer yielded a dark brown solution from which a number of pigments were separated, either wholly or partially, by chromatography on a column of calcium phosphate mixed with celite. One of the pigments was light-sensitive, and had a yellow color, with a spectral absorption maximum at 437 mμ in phosphate buffer at pH 6.5. Several consecutively eluted fractions from each chromatogram of the house-fly head extract showed the characteristic absorption curve of this pigment with no trace, spectroscopically, of the other pigments of the extract. The products of bleaching the pigment at pH 6.5 had an absorption curve showing plateaus at 440 to 460 mμ and 350 to 360 mμ and an inflection at about 250 mμ. Above pH 8.0 the pigment bleached in the dark giving an absorption maximum at about 380 mμ, and inflections at 290 mμ and at about 250 mμ. With 2.5 to 5 N HCl or H_2SO_4 an absorption maximum at 470 to 475 mμ was obtained with either the unbleached or the bleached pigment. With sulfosalicylic acid, ethanol, or heating at 100°C., a part of the pigment was precipitated, leaving a light-stable yellow supernatant.

This light-sensitive house-fly pigment cannot as yet be identified with any of the previously known insect pigments or with the photosensitive pigments of other animals, though these latter compounds exhibit some similarity in their spectroscopic properties.

INTRODUCTION

The fine structure of the rhabdomeres of the insect eyes[1] is comparable in many respects with that of the retinal rods and cones (1). The chemistry of the

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[1] For electron micrographs and structure of the retinula cells in the compound eye, see three consecutive articles, by W. H. Miller, by J. J. Wolken *et al.*, and by T. H. Goldsmith and D. E. Philpott, in *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 421–448.

photoreceptors in the insect is, however, still largely unknown. One approach to this problem is a search for light-sensitive pigments in the eyes of insects. Though such pigments are not necessarily involved in the processes leading to stimulation of the photoreceptors, their possible significance in this respect and in the over-all reaction of the eyes to light has to be considered.

Forrest and Mitchell (2) have elucidated the chemical structure of a photolabile pteridine from a mutant of *Drosophila*. A connection between this compound and vision has not been established, though the necessity of considering it has been mentioned (3, 4). Goldsmith (5) has demonstrated the presence of a photosensitive pigment in phosphate buffer extracts of honey-bee heads. There is evidence that this pigment is related chemically to the visual pigments of other animals.

In the present work, a light-sensitive yellow pigment has been extracted from house-fly heads with phosphate buffer, and has been separated from a number of other pigments by chromatography on calcium phosphate mixed with celite. This procedure, based on a method of separating proteins developed by Tiselius (6), is an adaptation of one developed for the purification of cattle rhodopsin (7).

Experimental Methods

Materials.—House-flies (*Musca domestica*) were obtained from the Gulf Research and Development Co., Harmarville, Pennsylvania, and were packed in dry ice during transit. They were kept frozen in the freezer compartment of a refrigerator until they were used. The heads were detached and separated from the rest of the bodies by the freezing and sieving techniques of Moorefield (8).

Extraction.—The separated heads were ground in a mortar with 0.05 M Na_2HPO_4: KH_2PO_4 buffer, pH 7.0, until no intact heads could be seen. The mixture was then frozen overnight to break up the tissues further, allowed to thaw, and centrifuged at 12,000 R.P.M. for 20 minutes.

Chromatography.—Columns were prepared by mixing 3.17 gm. of celite (Johns-Manville Products Corporation, New York) with 50 ml. of 0.2 M $CaCl_2$ using a magnetic stirrer. Stirring was continued while 60 ml. of 0.2 M Na_2HPO_4 was poured into the mixture. The resulting suspension of calcium phosphate and celite was stirred for an additional 5 minutes and poured into glass columns (1.0 cm. diameter) with sintered glass filters at the lower end. The suspended material was allowed to settle; during this time part of the fluid drained through the column. The sedimented material was stirred carefully with a long glass rod to eliminate air bubbles. A pressure equal to about 40 cm. of water was then applied to the top of the column to remove the excess fluid and to pack the solid particles. At no time during this or any of the other operations was the fluid level allowed to fall below that of the sedimented particles. 20 ml. of distilled water was then allowed to drain through the column. The average length of packed, sedimented, material used for chromatography was 8 cm. The rate of addition of the 0.2 M Na_2HPO_4 in the initial operation affects the properties of the adsorption column to a certain extent. A rapid addition and mixing gave columns with good adsorbing properties, though fluid flowed through them rather slowly.

The supernatant from centrifuging the phosphate buffer extract of about 2000 heads was poured onto a prepared column and the fluid allowed to drain through. Adsorption proceeded best when the solution was diluted to give a 0.025 M buffer concentration. The column was then developed and eluted with 0.2 M phosphate buffer (Na_2HPO_4:KH_2PO_4); first 15 ml. at pH 7.0 and then 50 ml. at pH 6.5. Slight positive pressure from an air line was used in the second stage of the elution. The effluent was collected in fractions of 3 ml. each. All operations involved in preparing and developing the chromatogram were carried out in a dark room under dim yellow or red illumination.

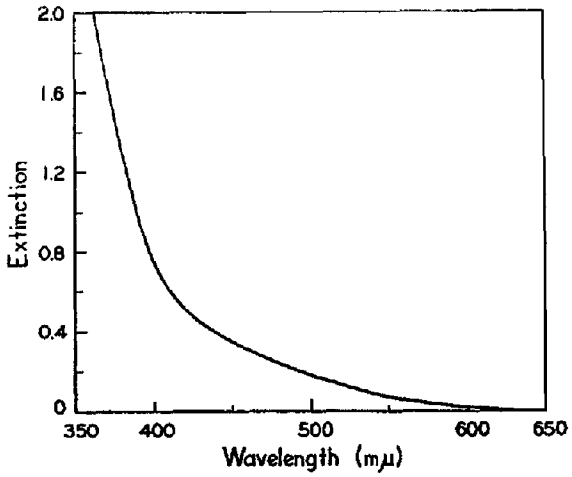

FIG. 1. Absorption spectrum of the fluid which drained through the calcium phosphate and celite column on applying the house-fly head extract in 0.025 M phosphate buffer, pH 7.0.

Spectrophotometry.—The absorption spectra of effluent fractions were obtained with the Beckman DK.1 recording spectrophotometer. Buffer solutions of the same strength were used as compensating blanks. The positions of absorption maxima were checked using the Beckman DU spectrophotometer.

Nitrogen Estimations.—The micro-Kjeldahl-nesslerization technique, as previously described by Bowness (7), was used.

RESULTS

1. Fractions from the Chromatogram.—After extracting the heads and centrifuging, the supernatant was a dark red-brown color. When the supernatant was placed on a prepared column, a light yellow fluid drained through. The absorption spectrum of this material is shown in Fig. 1. The presence of three other pigments became apparent on eluting with 0.2 M phosphate buffers. A pigment with a peak of absorption at about 545 mμ, and an inflection at about 515 mμ, and another with a peak at about 408 mμ were evident in the effluent fractions

(see Fig. 2), though they were only obtained mixed with the yellow material of Fig. 1 or with a fourth pigment (Fig. 3). The fourth pigment, the last to come off the column with 0.2 M phosphate buffer, was colored yellow, was light-sensitive, and, at pH 6.5, had an absorption peak at 437 mμ. The spectra of the later effluent fractions containing the 437 mμ pigment showed no trace of the absorption maxima of the other three pigments eluted from the column with phosphate buffer. These later fractions showed a slight inflection in the ultra-

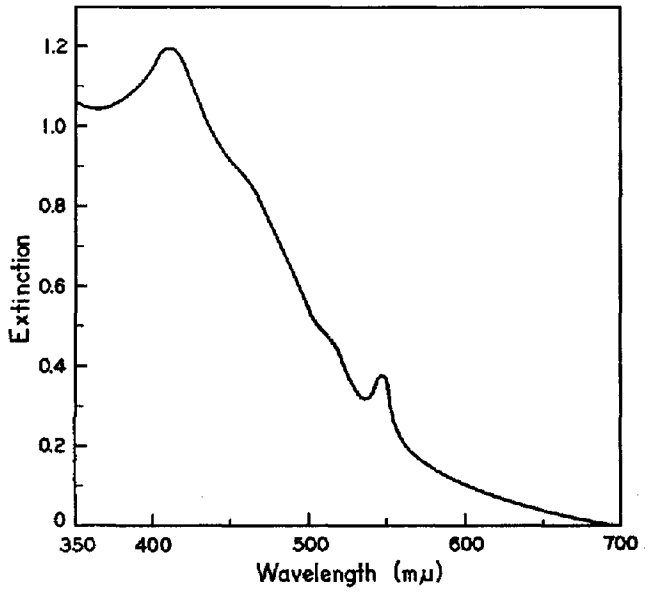

FIG. 2. Absorption spectrum of the first fraction eluted with 0.2 M phosphate buffer, pH 7.0, from the calcium phosphate and celite column, loaded with the materials adsorbed from the house-fly head extract.

violet at 290 mμ and, in dilute solution, a slight inflection at about 250 mμ (Fig. 4). After all the light-sensitive pigment had been eluted, a dark red-brown material remained at the top of the column which could be eluted from the extruded column with 2 N KOH. If, on the other hand, this pigment was allowed to stay on the column for a day or two, it turned into a red material, which, immediately after eluting from the extruded column with 1 M acetate–acetic acid buffer, pH 4.8, showed a peak of absorption at 490 mμ, changing to 440 mμ on standing (Fig. 5).

2. Quantity of the Light-Sensitive Pigment.—From a single extraction of 3 gm. of house-fly heads (about 1800 heads) approximately 43 ml. of effluent containing the light-sensitive pigment (about 10 ml. of this also contained a little of

the other pigments), with an average $E_{1\,cm.}$ of 0.295, was obtained. A second extraction of the same material yielded, on chromatography, about 20 ml. more of effluent containing the pigment, with an average $E_{1\,cm.}$ of 0.281.

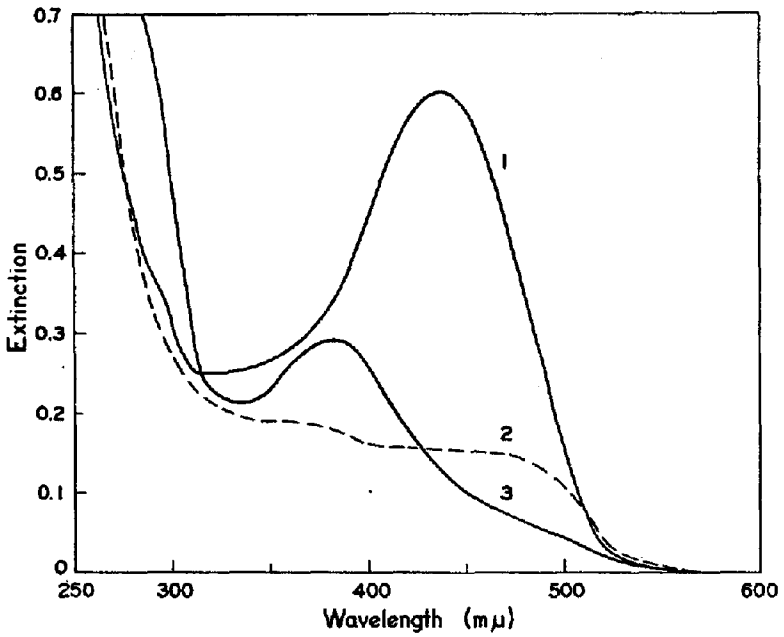

FIG. 3. Absorption spectra of the photosensitive pigment and the products o bleaching this pigment.

Curve 1. Eluate (10 ml.; henceforth called "eluate P") with 0.2 M phosphate buffer at pH 6.5, the loaded column previously having been eluted with 15 ml. of 0.2 M phosphate buffer at pH 7.0, and 10 ml. at pH 6.5.

Curve 2. Eluate P, after bleaching with white light at about 600 foot-candles for 2 hours at 12°C.

Curve 3. 2 ml. of eluate P mixed with 0.3 ml. 2 N KOH and allowed to stand 30 minutes in the dark.

3. Bleaching of the Light-Sensitive Pigment.—

Change in spectra: In both the bleaching by light at pH 6.5 and bleaching in the dark at pH 8.0 there appeared to be an initial shift of the 437 mμ absorption maximum to a longer wavelength, producing a broader peak with a somewhat lower extinction than the original. There was also an increased absorption of light in the 500 to 540 mμ region. At pH ~12 the main peak was soon replaced by a different one at 380 to 385 mμ (Fig. 3). At pH 6.5 in the light the main absorption peak was gradually replaced by a plateau of absorption or an inflection at 440 to 460 mμ, together with a second plateau at 350 to 360 mμ (Fig. 3).

The ultraviolet absorption spectrum of the pigment also showed changes during bleaching. When the pigment was bleached by light in solutions at pH 6.5 the inflection at 290 mμ gradually disappeared, while an inflection at about 250 mμ became clearly evident (Fig. 4). In solutions at a pH above 8.0 inflections at about 250 mμ and at 290 mμ were very pronounced. If the solutions bleached in alkali were then made acid, the 290 mμ inflection disappeared.

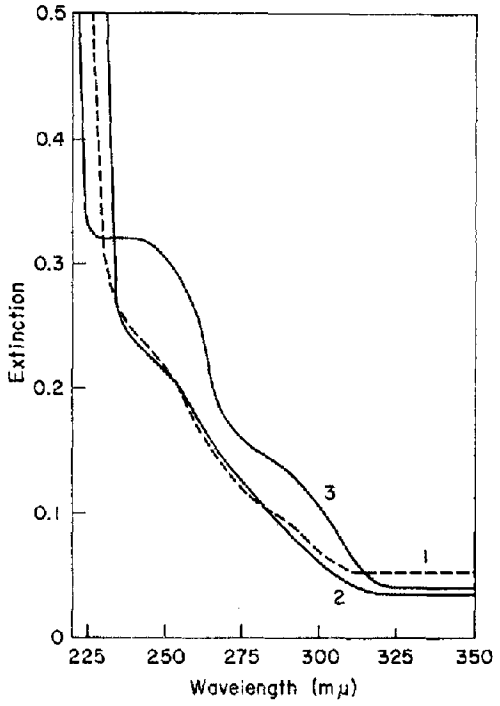

FIG. 4. Ultraviolet absorption spectra of the solutions described under Fig. 3., each diluted 1 in 5 with distilled water. Numbering is the same as in Fig. 3.

Speed of bleaching: The changes in extinction at 437 mμ and 520 mμ on exposure to fluorescent light for various times in enclosures at 12°, 20°, and 24°C. are shown in Table I. In solutions with a pH higher than 8.0 bleaching by light was slower, and difficult to detect because of a spontaneous bleaching in the dark. At pH 8.0 in the dark, about 23 per cent of the extinction at λ_{max} of a solution of the pigment was lost in 30 minutes at 24°C. A second portion of the same solution exposed to a fluorescent tube (about 200 foot-candles) for 30 minutes at 24°C. lost about 38 per cent of the extinction at λ_{max}. In the dark a 38 per cent loss was observed in 2 hours at 24°C. At pH 12, 86 per cent of the extinction at λ_{max} was lost in 4 minutes in the dark at 24°C. After 15 minutes there was no further trace of a peak or inflection at 437 mμ in this solution.

Difference spectra: Measurement of the absorption spectrum of an unbleached solution of the pigment, kept in the dark at 24°C., using as a compensating blank a second portion of the same solution which had been exposed for 10 minutes in an enclosure at 12°C. to light from a fluorescent tube, gave a curve (Fig. 6) with maxima at 432 mμ and 280 to 290 mμ, a minimum at 520 mμ, and a plateau at about 240 mμ. The changes in the visible spectrum of the pigment on exposure for 1 to 10 minutes at 24°C. to light from a fluorescent tube are shown in Fig. 7.

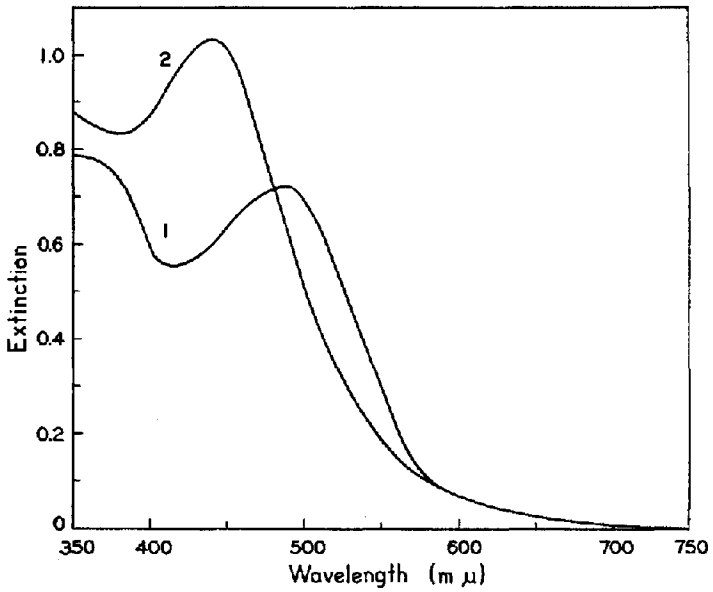

FIG. 5. Absorption spectra of:

1. A centrifuged extract, made with 1 M acetate–acetic acid buffer, pH 4.8, of the top 2 cm. of the extruded calcium phosphate and celite column, the loaded column having been eluted with 15 ml. of phosphate buffer at pH 7.0 and 50 ml. at pH 6.5.
2. Solution (1) allowed to stand 2 hours in the dark.

4. Other Reactions and Properties of the Light-Sensitive Pigment.—

Strong HCl or H_2SO_4: The absorption peak of the unbleached or the bleached pigment shifted to 470 mμ in 2.5 N HCl and to 475 mμ in 5 N H_2SO_4.

Sulfosalicylic acid: An orange-brown precipitate was obtained on standing when 20 per cent (by weight) of sulfosalicylic acid was dissolved in a solution of the pigment. From 10 ml. of pigment solution (pH 6.5) with an $E_{1\,cm.}$ value of 0.350 at 437 mμ, about 0.2 mg. of orange-brown material was obtained, after centrifuging and washing the precipate with water and ethanol, and drying. This material contained about 10.5 per cent nitrogen, as estimated by the micro-Kjeldahl–Nessler procedure. The supernatant obtained after centrifug-

ing down the orange-brown precipitate had still a yellow color with an absorption maximum at 460 mμ, and at λ_{max} almost two-thirds of the $E_{1\,cm.}$ of the original pigment solution. The 460 mμ peak was unaffected by light.

Ethanol: Addition of 4 volumes of ethanol to 1 volume of the light-sensitive pigment solution in pH 6.5 phosphate buffer precipitated the phosphates together with a brownish material similar to that obtained with sulfosalicylic acid. On centrifuging, a light-stable yellow supernatant with λ_{max} at about 425 mμ was obtained. When this supernatant was allowed to evaporate and the residue redissolved in 3 N HCl, a solution with λ_{max} at 470 mμ was obtained.

TABLE I

Changes in Extinction at 437 and 520 mμ of the House-Fly Light-Sensitive Pigment in 0.2 M Phosphate Buffer, pH 6.5, on Exposure to Light

The data were calculated from absorption curves obtained with the Beckman DK. 1 recording spectrophotometer, before irradiation with light, and after irradiation for the stated times.

Experiment	Conditions	Per cent decrease in extinction at 437 mμ							Increase at 520 mμ as per cent original E_{437}						
		Min.				Hrs.			Min.				Hrs.		
		1	4	10	30	1	5	20	1	4	10	30	1	5	20
1. Original E_{437} = 0.85	12°C.; 400 to 450 foot-candles	5.9	15.9	23.5	37.6	52.1	78	78	2	5.2	7.5	10.2	11.4	3.0	3.0
2. Original E_{437} = 0.62	20°C.; 150 to 200 foot-candles	4.9	13.0	20.2	38.0	53.2	73	78	3.6	5.2	7.8	10.1	10.8	6.0	4.5
3. Original E_{437} = 0.85	20°C.; 150 to 200 foot-candles	7.0	17.1	25.1	40.0	56.0	—	79	4	5.6	7.4	10.4	9.8	—	4.0
4. Original E_{437} = 0.35	24°C.; 150 to 200 foot-candles	7.5	17.5	27.8	43.2	60.2	77	77	4.9	7.1	7.8	8.5	8.0	2.3	2.1
5. Original E_{437} = 0.35	24°C.; 150 to 200 foot-candles	8.0	16.8	27.6	42.9	60.1	76	77	4.5	7.2	8.5	8.6	7.9	3.0	2.8

When the original supernatant was made alkaline, the absorption maximum shifted to about 360 mμ.

The precipitate partly redissolved in the phosphate buffer solution produced by adding water to wash the precipitate. The absorption spectrum of a solution in 1 N KOH of the undissolved material showed a low plateau at 350 to 360 mμ and strong inflections at 290 and 250 mμ.

Thermal bleaching in the dark: The rates of bleaching of the 437 mμ peak at 50°, 60°, and 100°C. in the dark are shown in Fig. 8. Associated with the decrease in absorption at 437 mμ there was a rise at 350 to 360 mμ. When there was no further change in spectrum with continued heating, the 350 to 360 mμ inflection was the only one detectable. During the heat bleaching a deposit was obtained, largely in the form of a white coagular precipitate. After centrifuging down and washing with water this material was dissolved in 0.2 N NaOH and

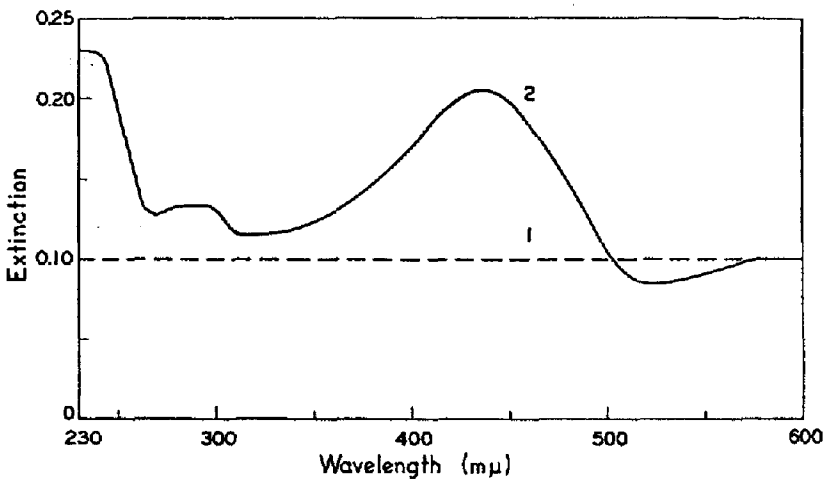

FIG. 6. Difference spectra obtained by measuring the absorption spectrum of one portion of eluate P of Fig. 3, using, as compensating blank, a bleached portion of the same solution.

Curve 1, before, and curve 2, after, bleaching the first portion for 10 minutes at 12°C. with white light at about 200 foot-candles, while the second portion was kept in the dark at 24°C.

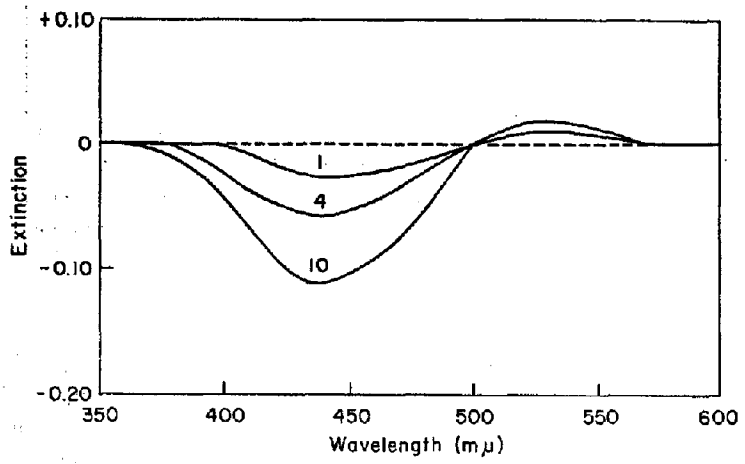

FIG. 7. Difference spectra obtained at 24°C., after three different exposure times of a solution of the light-sensitive pigment (E_{437} = 0.355) in 0.2 M phosphate buffer, pH 6.5, to fluorescent light at about 200 foot-candles. The curves were obtained by subtracting the spectra at 1, 4, and 10 minutes exposure from the spectrum of the unexposed solution. The increase in extinction in the 500 to 540 mμ range was so small between 4 and 10 minutes, that the two curves were drawn as one in this region.

the spectrum obtained. Only general absorption was found in the visible region, but in the ultraviolet there was a peak at 290 mμ and a slight inflection around 250 mμ.

Nitrogen Content of Pigment Solutions.—Estimations of the nitrogen content of three different preparations of the pigment showed a mean value of 0.011 mg. N for $E = 1.0$ at 437 mμ. During the determinations (by the micro-Kjeldahl method) it was noticed that a purple color was obtained after nearly all the water had been distilled off from the digest mixtures containing sulfuric acid.

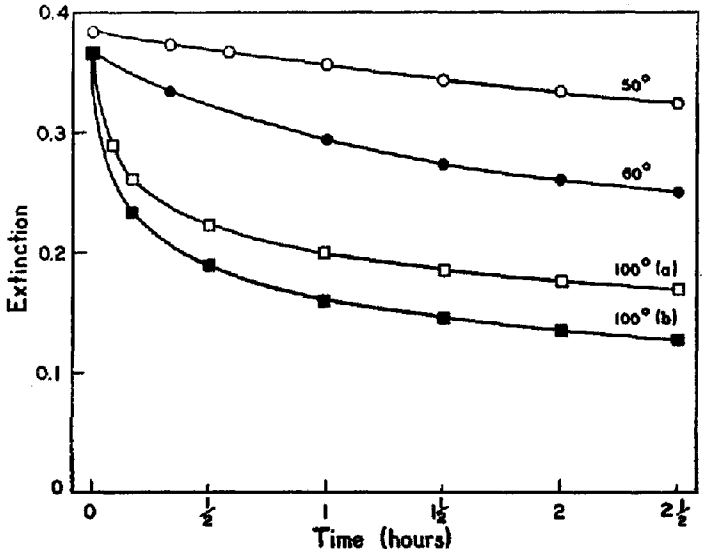

FIG. 8. Thermal bleaching of solutions of the light-sensitive pigment in 0.2 M phosphate buffer at pH 6.5. The curve labelled 100° (*b*) shows results obtained by heating at 100°C., then cooling, and centrifuging for 10 minutes at 12,000 R.P.M.

DISCUSSION

The results described show that a light-sensitive yellow pigment, with a 437 mμ absorption maximum at pH 6.5 in phosphate buffer solution, can be extracted from house-fly heads, and can be separated from a number of light-stable pigments. Evidence of two kinds indicates that the light-sensitive pigment obtained from the chromatograms was a material of approximately constant composition. (*a*) In several separate chromatograms, three or more consecutively eluted 3 ml. fractions showed almost exactly the same absorption spectrum. Fig. 3 (curve 1) shows one such spectrum from one chromatogram. The ratios of minimum extinction (at about 325 mμ) to maximum (at 437 mμ) for four consecutive 3 ml. fractions were all between 0.425 and 0.435 for this chromatogram; extinction values at 437 mμ were between 0.585 and 0.605.

Though the extinction values were different, the corresponding spectra from other chromatograms were of almost the same shape. Ratio of minimum to maximum extinction in the best fractions from these chromatograms ranged from 0.42 to 0.50. (*b*) The manner and the rate of bleaching of the pigment were very similar in a number of tests with light-sensitive fractions from several chromatograms (see Table I, and figures in the text of the results).

That the 437 mμ pigment is bleached by light, rather than heat, on exposure to the light source, is shown by: (*a*) the decreased absorption at 437 mμ found in a solution exposed to light at 12°C., as compared with the same solution kept in the dark at 24°C. (Fig. 6); (*b*) the slow rate of bleaching on heating the solution in the dark at 50° (Fig. 8) as compared with the rate of bleaching in the light at 12°C.

The rate of bleaching of the 437 mμ peak of the house-fly pigment (as illustrated in Table I) is slow compared with that of such photosensitive pigments as cattle or frog rhodopsin. The difference spectra of Fig. 7 and the figures in Table I indicate that the pigment undergoes a different, and somewhat less obvious, change which shows itself during the first half hour of irradiation through an increase in the absorption of light in the region of 520 mμ. The observation that the 437 mμ peak broadens during bleaching, with a shift to a longer wavelength of the center of the peak, is probably associated with the occurrence of the 520 mμ peak in the difference spectra. Unless the light-sensitive pigment is inhomogeneous, which for reasons discussed earlier seems unlikely, the simplest explanation of these changes is that a product (also light-sensitive, see Table I) is formed during bleaching, which has an absorption curve similar to that of the original pigment, but with the whole curve shifted slightly to longer wavelengths. The bleaching of the light-sensitive pigment (Fig. 3) appears to be almost complete in 2 hours at 12°C. and 600 foot-candles. The remaining absorption spectrum after this time consisted mainly of (*a*) inflections or plateaus of absorption at 440 to 460 mμ and 350 to 360 mμ, (*b*) general absorption due to suspended particles; this was reduced by centrifuging.

The animal pigments were comprehensively reviewed by Fox (9) in 1953.[2] The 437 mμ pigment cannot as yet be identified with any of the previously characterized insect pigments. The yellow photolabile pteridine described in 1954 by Forrest and Mitchell (3) has absorption maxima at 440 and 268 mμ in 0.1 N sodium hydroxide and at 409 and 279 mμ in 0.1 N hydrochloric acid. The house-fly light-sensitive pigment has an absorption maximum at about 380 mμ in 0.1 N alkali, and the shift when this solution is made acid is in the opposite

[2] The insect pigments were reviewed by Wigglesworth (23) in 1949. One of the pigments obtained from *Drosophila* by Wald and Allen (24) had an absorption maximum at 436 mμ. No light sensitivity was reported for this pigment; the absorption curve resembles the 440 mμ form of the pigment shown in Fig. 5, rather than the light-sensitive house-fly pigment.

direction to that found generally with the pteridines (10). The photodecomposition of the yellow pteridine from *Drosophila* yields an intensely fluorescent material (3). No fluorescent products have been observed either visually, or with the fluorescence attachment of the Beckman DK spectrophotometer, after bleaching the light-sensitive house-fly pigment.

Goldsmith (5, 11) has shown that a photosensitive pigment is present in phosphate buffer extracts of honey-bee heads. Partial purification of the extracts indicated that a retinene-protein complex was present, which, on the basis of a difference spectrum obtained by irradiating a solution containing this and other pigments with yellow light was stated to have an absorption maximum probably close to 440 mμ. The absorption maximum of the light-sensitive house-fly pigment at pH 6.5 thus probably lies very close to that of the honey-bee pigment. However, no retinene has as yet been obtained from the house-fly pigment, nor is there any evidence of a retinene peak, either in the absorption spectra of the products of bleaching this pigment, or in the difference spectra of Figs. 6 and 7. Solutions with an absorption band at about 328 mμ were obtained after bleaching the house-fly pigment, adding ethanol, evaporating to dryness, and extracting with chloroform. A transient blue color was obtained when antimony trichloride was added to some extracts made this way, but no absorption peak in the visible region was found in the resulting solutions. Vitamin A_1 has a principal absorption maximum at 328 mμ, but it gives a blue color with a peak at 620 mμ. It must be assumed that substances other than vitamin A_1 could have been responsible for the band at about 328 mμ.

Although, without the demonstration of the presence of retinene or vitamin A, it is not possible to consider them indicative of the chemical structure of the house-fly pigment, it is of interest to note a number of similarities in spectroscopic properties between this pigment and the visual pigments which have been found in many animals (see Wald (12), Dartnall (13)). Firstly, there are the pH indicator properties shown by the light-sensitive house-fly pigment and by the products of bleaching this pigment. On bleaching in the light at pH 6.5 a solution with plateaus of absorption at 440 to 460 mμ and 350 to 360 mμ is produced. Addition of a strong acid to this material gave a plateau or a peak at 470 to 475 mμ. In alkaline solution there was a plateau at 360 mμ only. Absorption maxima in these three wavelength regions are given by the retinylideneamines and indicator yellow under similar conditions of pH (14, 15) though the 440 mμ form of retinylideneamines is not stable except at pH 1 (16). An absorption maximum at 380 mμ was produced at pH ~12 with the house-fly pigment. A peak at this wavelength is obtained with squid metarhodopsin at pH 9.9 or from cattle metarhodopsin at pH ~13 (17). Secondly, the bleaching process of the house-fly pigment, as with the visual pigments (12) appears to involve more than one stage.

There are a number of observations which, though not conclusive, indicate

that protein may be a part of the light-sensitive house-fly pigment. Firstly, the ultraviolet absorption spectrum of the pigment, either bleached or unbleached, exhibited an inflection at 290 mμ in alkaline solution. At pH 6.5 the unbleached pigment showed only a slight inflection at 290 mμ, while the bleached pigment showed none. Most proteins show a band at 290 mμ in alkaline solution, though in neutral or acid solution they have a similar band at 275 to 280 mμ (18). However, the peak at about 250 mμ of the pigment in alkali, though given by cystine, is not characteristic of a protein. Secondly, the heat bleaching of the pigment at 100°C. gave a coagular precipitate which, when dissolved in 0.2 N sodium hydroxide, showed a peak at about 290 mμ. Thirdly, a precipitate, containing about 10.5 per cent of nitrogen, was obtained from the light-sensitive pigment solution upon addition of sulfosalicylic acid.

At present no definite relationship is evident of the 437 mμ light-sensitive pigment with vision in the house-fly or with those visual pigments which have been characterized in higher animals. Behavior studies with the house-fly and other insects (19–21) have shown that light exerts a maximum of attractiveness for them at about 365 mμ. There is thus no correspondence between the absorption spectrum of the light-sensitive house-fly pigment at pH 6.5 and the response curve of the insect. However, any comparison of maxima in these response curves with absorption maxima of possible visual pigments is probably invalid unless complicating factors mediating between the light incident on the eye, stimulus of the photoreceptor, and response of the effectors can be discounted. Electrophysiological measurements made by Goldsmith (11) with the compound eyes of drone bees indicated a peak of sensitivity at 440 mμ. As yet, no corresponding measurements have been made with the house-fly, but it seems that the light-sensitive pigment which we have separated should be considered as a possible visual pigment until further evidence is available to indicate more exactly the structure and function of this pigment.

No attempt was made to separate or investigate the four light-stable pigments in the phosphate buffer extract of the house-fly heads. The absorption spectrum (Fig. 1) of the light yellow pigment which drains through the column with 0.025 M phosphate buffer appears to be of the melanin type. The red pigment, which required 2 N KOH or 1 M acetate–acetic acid buffer at pH 4.8 for elution from the column, exhibited a shift in absorption maximum (from about 490 to 440 mμ) in changing from alkaline to acid conditions (Fig. 5). This is similar to the shift shown by rhodommatin, a red pigment obtained from insects by Butenandt, Schiedt, and Biekert (22), which is possibly related to the pteridines (2).

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