revert to the onset of breeding or to proceed to the next phase, usually the moult. Ornithology offers no precedent for temporary cessation of parental care; so novel a suggestion requires undeniable proof.

Throughout 1956 Keith (biologist, Australian National Antarctic Research Expeditions) kept records of the development of wandering albatross nestlings at Macquarie Island. The five growth-curves (Fig. 1) show the increase in weight during winter; and Fig. 2 shows a young albatross being fed on July 26.

Tickell[13] also discussed whether adults feed their own or random chicks, a point on which there can be no doubt if the birds are marked. In view of the mounting evidence on this subject, of which only Sladen's[14] study of the Adelie penguin (*Pygoscelis adeliae*), the young of which form crèches, need be mentioned in addition to Richdale's[9] proof in the case of the royal albatross, it would be surprising indeed if the original parent-nestling bond were not maintained. Many observations of banded birds at Macquarie Island confirm that wandering albatross parents feed only their own young, but nests there are much more widely spaced than at Bird Island. Tickell's[13] final conclusion, ". . . it must be held that the wandering albatross, like the royal albatross, breeds only once every two years", also requires birds, the identity, and history during several years, of which are known. Matthews's[5,8] conclusion (quoted above) on similar evidence is more carefully correct. Since 1954, 48 adults and 37 young have been banded on Macquarie Island; frequency of breeding follows the pattern established by Richdale[9] in the royal albatross, namely that successful pairs breed in alternate years and early nest failures enable the next attempt to be made the following summer. Constancy of site and mate is the rule. The full results of the Macquarie Island studies are included in an Australian National Antarctic Research Expeditions report on the birds of this Island to be completed during 1961.

The wandering albatross is a noble bird which has long excited the imagination of poet, voyager and scientist, but canards ill become it. In 1929, after spending a full year at South Georgia and studying large wandering albatross colonies at the end of August, September, November to January, and March, Matthews[5] produced the evidence and correct conclusions on both winter feeding of the young and non-annual breeding of successful parents. Subsequent writers up to 1960, after at most a summer contact with the bird, have done his work less than justice. The time has come to consign much past writing on the wandering albatross to the limbo of ornithological mythology, and to replace speculation with the direct evidence, based on individual identification whenever possible, which modern ornithology demands.

1 Hutton, F. W., *Ibis*, **1**, 276 (1865).
2 Andersson, C. J., *Ibis*, **2**, 324 (1866).
3 Chapman, F. R., *Trans. N.Z. Inst.*, **23**, 491 (1891).
4 Buller, W. L., "Supplement to the Birds of New Zealand" (London, 1905).
5 Matthews, L. H., "Birds of South Georgia", *Discovery* Reports, **1**, 563 (1929).
6 Murphy, R. C., "Oceanic Birds of South America", 561 (Amer. Mus. Nat. Hist., New York, 1936).
7 Rankin, N., "Antarctic Isle" (Collins, London, 1951).
8 Matthews, L. H., "Wandering Albatross" (Macgibbon and Kee, London, 1951).
9 Richdale, L. E., "Post-Egg Period in Albatrosses" (Dunedin, New Zealand, 1952).
10 Paulian, P., "Pinnipèdes, Cétaces, Oiseaux des Iles Kerguelen et Amsterdam", *Mem. de l'Inst. Sci. de Madagascar*, A, **8** (1953).
11 Fisher, J., and Lockley, R. M., "Sea-Birds" (Collins, London, 1954).
12 Jameson, W., "Wandering Albatross" (Hart-Davis, London, 1958).
13 Tickell, W. L. N., *Nature*, **185**, 116 (1960).
14 Sladen, W. J. L., "The Pygoscelid Penguins", F.I.D. Scientific Report No. 17 (1958).

CHEMISTRY OF VISUAL ADAPTATION IN THE RAT

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Rhodopsin Concentration and Visual Sensitivity

THE loss of visual sensitivity after light adaptation, and the recovery of sensitivity during dark adaptation, are common phenomena. Hecht was the first to emphasize the idea that these processes represent the bleaching and synthesis of visual pigment. However, until very recently, experimental attempts to formulate the relationship between visual pigment concentration and visual sensitivity or threshold have not been fruitful, principally because in man, in whom visual thresholds are most commonly determined, pigment concentrations cannot easily be measured. In experimental animals, on the other hand, pigment concentrations can readily be measured, but special means must be employed to estimate visual thresholds.

Recently, in a study of vitamin A deficiency in rats[1], I used the electroretinogram to assay retinal function and sensitivity. I found that the electroretinogram threshold of the eye rose regularly and linearly with time on the deficient diet, similar to the rise of psychophysical thresholds in studies of human vitamin A deficiency[2]. When the measurements of electroretinogram threshold in the deficient rats were compared with the rhodopsin concentrations in the retinas of the same animals, it was found that the logarithm of the electroretinogram threshold rose linearly with fall in rhodopsin concentration.

I have now extended this analysis to light and dark adaptation. That one can use the electroretinogram to reflect the sensitivity of the eye has been demonstrated by several workers[3,4]. The most familiar way to do this is to evaluate the change of the electroretinogram during dark adaptation on the basis of stimulus response curves, so estimating the light intensities needed to elicit constant electroretinogram responses. I achieved essentially the same result by measuring, at various times during dark adaptation, the brightness of stimulus needed to evoke a just-perceptible electroretinogram. The electroretinogram threshold is perhaps preferable to measuring larger, super-threshold responses, which may be complicated by complex and changing waveforms.

The experiments were performed in the following way. Groups of animals were light-adapted for 30 min. in a white porcelain pan, brightly illuminated with three 100-watt flood lamps. At selected times after the lights were turned out, an animal was selected at random and the electroretinogram threshold recorded. Each measurement was made with a fresh animal, anæsthetized with nembutal (10–15 mgm.) 5–10 min. beforehand. To prepare for the first measurement (at 3 min.), anæsthetic was given to one animal a few minutes before the end of light adaptation. After an animal was anæsthetized, it could be prepared for the experiment and its threshold determined in about 3 min. Then the electroretinogram was recorded over a luminance-range of 5 log units.

The stimulus was a 1/50 sec. flash of white light, the luminance of which could be varied continuously with circular photographic wedges. The electroretinogram was recorded from the cornea with cotton wick electrodes connected with a Grass *P*-6 amplifier and Dumont oscilloscope fitted with a camera attachment. Capacity-coupled amplification was used to yield a more stable base line.

To measure rhodopsin, the same or other groups of animals were light-adapted as before on the following day. At appropriate times, an animal was anæsthetized, its eyes removed, and the retinas dissected free and dropped into cold 4 per cent alum solution. The retinas were collected pair by pair in this manner throughout the entire experiment. Each pair of retinas was finally washed twice with water, once again in neutral phosphate buffer, and the rhodopsin was extracted overnight with 0·2 ml. of 2 per cent digitonin. The next day, the extract was centrifuged clear, 0·01 ml. of 1 *M* hydroxylamine was added, and the absorption spectrum was run in a Cary recording spectrophotometer. The solution was bleached with white light for 5 min., and the absorption spectrum re-run. The difference in extinction at 500 mμ before and after bleaching indicated the concentration of rhodopsin. Measurements of rhodopsin in rats during dark adaptation have previously been made by Tansley[5] and Lewis[6]. In all, six complete experiments were performed measuring electroretinogram thresholds, and three complete experiments measuring rhodopsin concentration.

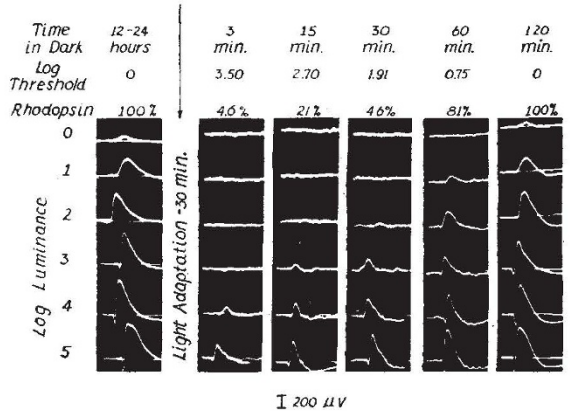

Fig. 1. Electroretinogram responses during dark adaptation in the rat. The top three lines show the time in the dark; the logarithm of the electroretinogram threshold, the lowest luminance necessary to evoke a perceptible electroretinogram; and the rhodopsin content expressed as percentage of the dark-adapted level. The electroretinograms are evoked with 1/50 sec. flashes of white light over a luminance range of 5 log units. The first row of electroretinograms shows the responses of a control animal, dark-adapted overnight. After light adaptation, the electroretinogram threshold is raised about 3·5 log units, and the responses at high luminances are diminished in size. With time in the dark, the threshold returns to normal, and the responses at higher luminances assume the dark-adapted form

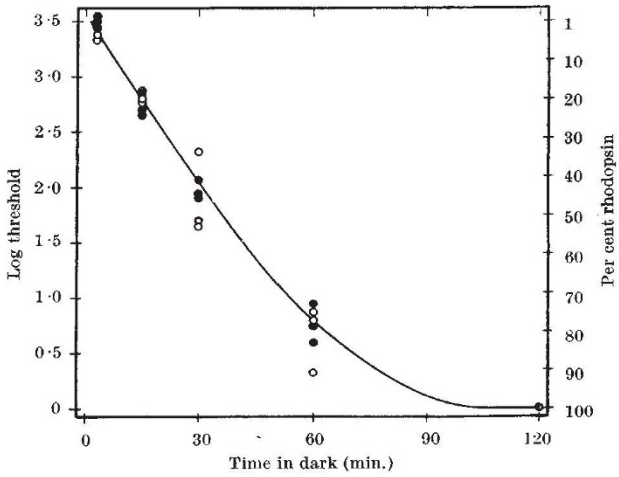

Fig. 2. Dark adaptation in the rat. Following light adaptation, the fall of the logarithm of the electroretinogram threshold parallels the rise in concentration of rhodopsin. Both the threshold and rhodopsin concentration are back to the dark-adapted level in about 100 min. ●, log threshold; ○, rhodopsin

Fig. 1 shows the electroretinogram responses recorded in such an experiment over a range of luminances of 5 log units. At the top of Fig. 1 the time in the dark following light adaptation is shown, and on the next line the logarithm of the electroretinogram threshold, the lowest luminance of light which evoked a perceptible electroretinogram. The normal threshold is set arbitrarily at 1, so that log threshold is 0. The third line shows the rhodopsin content, expressed as percentage of the completely dark adapted level.

The first row of electroretinograms shows the responses of a control animal which had been dark adapted overnight. After 30 min. of light adaptation, the threshold recorded after 3 min. in the dark was raised 3·50 log units (about 3,000 times). The rhodopsin concentration was reduced to 4–5 per cent of the dark-adapted level. With time in the dark, the sensitivity of the eye returned gradually to the dark-adapted state, in parallel with the rhodopsin concentration. Dark adaptation was completed in about 100 min.

Fig. 2 compares the rise in rhodopsin concentration and fall of electroretinogram threshold during dark adaptation. It is seen that the log threshold falls in strict parallelism with the rise in rhodopsin concentration.

Fig. 3 compares the relationship between rhodopsin concentration and log threshold both during dark adaptation and night blindness owing to vitamin A deficiency. It is clear that the logarithm of the electroretinogram threshold, even under these different conditions, rises and falls linearly with the concentration of rhodopsin. This relationship is expressed by the equation:

$$\log I_t/I_0 = 3{\cdot}6\ (R_0 - R_t)/R_0$$

in which I_0 and R_0 are, respectively, the threshold and rhodopsin concentration in dark-adapted control

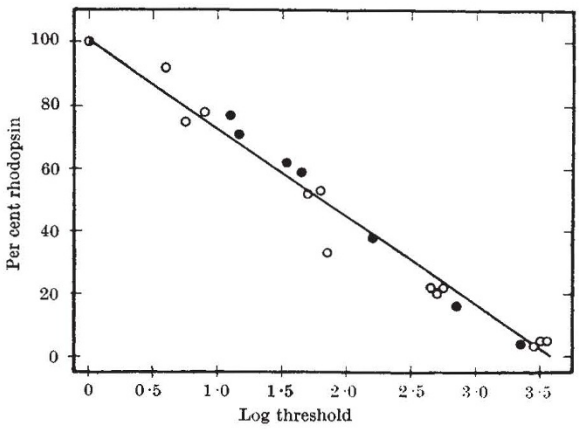

Fig. 3. The relation between rhodopsin content of the retina and the visual threshold in animals dark-adapted after exposure to bright light, and in animals night-blind due to vitamin A deficiency. In both instances, the same relationship is observed: the log threshold rises linearly with fall in rhodopsin concentration ●, Night blindness; ○, dark adaptation

animals, and I_t and R_t are, respectively, the threshold and rhodopsin concentration in vitamin A-deficient or partly dark-adapted animals.

This result is not wholly unexpected. Wald has pointed out the striking qualitative similarity between the synthesis of chicken iodopsin and rhodopsin in solution, and the course of human cone and rod dark adaptation[7]. This comparison is valid only provided that one compares the logarithm of the visual threshold with the concentration of visual pigment. Wald has also offered a theory which may explain such a relation[8].

Recently, Rushton *et al.* have made direct measurements of the rise and fall of human visual pigments in the living human eye during light and dark adaptation[9,10], and these measurements show a striking parallelism between the course of human light and dark adaptation, and the course of bleaching and re-synthesis of the visual pigments. Again, this relationship is evident only when the logarithm of the visual threshold is compared with the concentration of visual pigment[11].

Very recently, Rushton has compared the regeneration of rhodopsin and the course of dark adaptation under the same conditions in a colour-blind subject, and has also found that the logarithm of the threshold falls in parallel with the increase of rhodopsin content in the eye[12].

There have been several previous attempts to correlate the electroretinogram with visual pigment concentration[13–15], all of which have attempted to correlate the increase of visual pigment concentration with the increase in size of the *b*-wave potential elicited with a constant test-flash. As Riggs has pointed out, no simple proportionality exists between magnitude of electroretinogram response and the logarithm of stimulus intensity in the vertebrate eye[3]. Furthermore, the shape of curve obtained by plotting magnitude of response against time in the dark for intensity of a single flash may vary with the luminance of the test-flash selected[16]. As can be seen from Fig. 1, if the test light is too dim, one does not begin to record an electroretinogram until considerable quantities of rhodopsin have accumulated in the retina. This may explain why Granit, Munsterhjelm and Zewi failed to record an electroretinogram in the cat until 40 per cent of the visual pigment had been regenerated[14]. In the rat, as we have seen (Fig. 1), one can record an electroretinogram at any concentration of rhodopsin provided that the test stimulus is bright enough.

On the other hand, if the test stimulus were very bright, large fluctuations in rhodopsin might occur with little effect on the size of the electroretinogram potential. A preferable procedure on all counts is to evaluate the sensitivity of the eye by measuring the light intensities required to evoke a constant potential, perhaps most simply as here the just perceptible electroretinogram.

In summary, following 30 min. of intense light adaptation, the rhodopsin concentration of the eye of the rat is lowered to less than 5 per cent of the dark-adapted level, and the electroretinogram threshold has risen about 3·50 log units (about 3,000 times). With time in the dark, the logarithm of the electroretinogram threshold falls in strict parallelism with the rise of rhodopsin concentration. Both log threshold and rhodopsin content of the eye return to the dark-adapted level in about 100 min.

Distribution of Retinene and Vitamin A in the Eye during Light and Dark Adaptation

On exposure to light, rhodopsin bleaches to a mixture of retinene (vitamin A aldehyde) and the protein, opsin. The retinene may then recombine with opsin, forming rhodopsin again, or it may be reduced to vitamin A. After an intense and prolonged light adaptation, almost all the retinene in the eye is transformed to vitamin A[17].

Several years ago, Wald observed in frogs that the bulk of the vitamin A formed after light adaptation disappears from the retina[17]. More recently it has been shown that the total amount of retinene and vitamin A in the frog's eye does not vary during light and dark adaptation[18]; thus it seemed probable that exchanges of vitamin A occur between the visual cells and the pigmented layers (pigment epithelium and choroid) that lie in contact with them. Probably most, if not all, of these exchanges involve the pigment epithelium, the long processes of which envelop the outer segments of the visual cells. In many animals the pigment epithelium also contains considerable stores of vitamin A, even in the dark-adapted state[17].

Histological evidence for the exchange of vitamin A between retina and pigment epithelium has been obtained by Jancsó and Jancsó[19] and Greenberg and Popper[20], who observed typical vitamin A fluorescence in the pigment epithelium of the rat after light adaptation, which disappeared on dark adaptation. In agreement with the latter observations, I have found that the pigment epithelium of the albino rat contains no detectable stores of vitamin A in the dark-adapted state.

The distribution of retinene and vitamin A during light and dark adaptation has not hitherto been studied quantitatively. The albino rat seemed ideally suited for this purpose, both because in this animal the pigment epithelium contains no vitamin A when dark adapted, and because (unlike the frog) the retina readily separates from the back of the eye in either the light- or dark-adapted condition.

For these experiments, groups of animals (weighing 150–200 gm.) were dark adapted overnight, and then brightly light adapted in a white porcelain pan illuminated with three 100-watt flood lamps. For

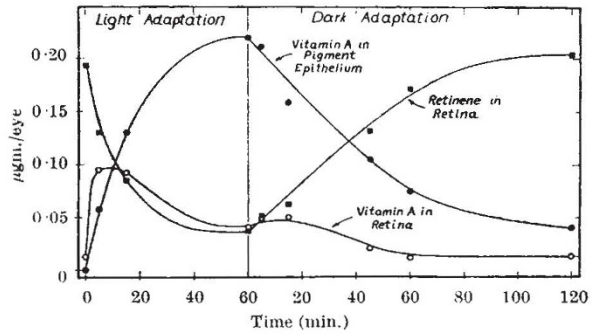

Fig. 4. Distribution of retinene and vitamin A in the eye during light- and dark-adaptation. During light adaptation, the retinene content of the eye falls, as the retinene, liberated on bleaching of rhodopsin, is reduced to vitamin A. Vitamin A in the retina rises for a time, and then declines, while the bulk of the vitamin A moves into the pigment layers. During dark adaptation, these processes are reversed. The retinene content of the eye increases, as rhodopsin is re-formed; and reciprocally the vitamin A in the pigment layers and retina declines. Dark adaptation is completed in about 100–120 min.

dark adaptation, the animals were placed in a small darkroom. At selected times during light- or dark-adaptation, the animals were anæsthetized with nembutal (15 mgm.), their eyes enucleated, and the retinas and pigment layers dissected apart, and placed separately in small mortars. (Throughout this article I shall refer to the back of the eye (pigment epithelium, choroid and sclera) as the pigment layers, even though there is no pigment in these tissues in the albino rat.) For each measurement, three animals (six eyes) were used.

The tissues were dried by grinding with a small amount of sodium sulphate, and the resulting powder transferred to a test-tube and extracted by vigorous stirring in 3 ml. of chloroform. After centrifugation, the clear chloroform extract (2·2–2·4 ml.) was drawn off, evaporated to dryness, and 0·3 ml. of fresh chloroform added. The amount of vitamin A and retinene in the extracts was determined by the Carr–Price reaction. For this, 0·25 ml. of the chloroform extract was mixed with 0·5 ml. of saturated antimony trichloride solution and the absorption spectrum from 700 to 500 mμ run immediately in the Cary recording spectrophotometer. Retinene absorbs maximally at 664 mμ, and vitamin A absorbs maximally at 620 mμ. When mixed, the amounts of vitamin A and retinene can be calculated by means of simultaneous equations[21].

Fig. 4 shows the results. It is convenient to express these in terms of the total potential vitamin A content of the eye; that is, vitamin A plus retinene, free and bound.

In completely dark-adapted animals, about 95 per cent of this potential vitamin A is bound as retinene in rhodopsin. About 5 per cent is stored as vitamin A in the retina. No vitamin A is found in the pigment layers.

On exposure to bright light, rhodopsin is bleached, liberating retinene. The retinene content of the retina, however, rapidly falls, as the released retinene is reduced to vitamin A. Reciprocally, the retinal vitamin A increases, and almost immediately vitamin A appears in the pigment layers.

With further time in the light the retinene content of the retina continues to fall, and after 30 min. reaches a level 10–15 per cent of the total potential vitamin A content of the eye. The retinal vitamin A, on the other hand, increases during the first 10 min. of light adaptation to a maximal level representing about 30 per cent of the total, and then gradually declines. After 45–60 min., it forms plateaux at a level of 10–12 per cent of the total.

The vitamin A in the pigment layers increases throughout the period of light adaptation. After 60 min., 75–80 per cent of the ocular vitamin A is found here. Retinene is never found in the pigment layers; the reduction of retinene to vitamin A takes place solely in the retina, and it is only vitamin A that migrates from the retina into the pigment layers.

It appears from Fig. 4 that there is more vitamin A present in the pigment epithelium after 1 hr. of light adaptation than there is total vitamin A (including retinene) in the eye to begin with. This is probably not the case; this artefact seems caused by my method of extraction, which removes vitamin A more efficiently than retinene. With equal amounts of vitamin A and retinene in a tissue, I extract only about 85 per cent as much retinene as vitamin A. This discrepancy could readily be corrected in the figure, but the raw data as they stand seem explicit enough.

During dark adaptation, these processes are reversed. The retina recaptures vitamin A by binding it as retinene in rhodopsin. The increase in retinene concentration during dark adaptation closely parallels the measured increase of rhodopsin concentration (compare Figs. 2 and 4).

With this increase of retinene, there is a reciprocal fall of vitamin A concentration in the pigment layers. The retinal vitamin A, on the other hand, stays relatively constant during the early stages of dark adaptation, and then gradually declines to its dark-adapted level of 4–5 per cent of the total vitamin A.

Dark adaptation is completed in about 100–120 min. I find at this time, however, a small amount of vitamin A in the pigment layers, which gradually disappears over the next few hours. During this time there is no increase of retinene or retinal vitamin A, so presumably this bit of vitamin A diffuses away into the blood stream. The origin and significance of this superfluous vitamin A is obscure.

Vitamin A in both the eye and body may exist in two forms: it circulates in the plasma as the free alcohol, but it is stored in the tissues primarily as the ester. It has been suggested that the vitamin A alcohol of the tissues is in equilibrium with that of the blood, and that through esterification a tissue can concentrate vitamin A independently of this equilibrium[22]. Krinsky has demonstrated the esterification of vitamin A in the eye tissues of cattle, and has found that much of the vitamin A, both in the retina and in the pigment epithelium, is esterified[23].

It is of interest to inquire as to the state of vitamin A in the pigment layers of the rat after 60 min. of light adaptation. For this, six animals were light adapted for 1 hr., their eyes enucleated, dissected, and the pigment layers extracted for vitamin A, as described above. The ester and alcohol fractions were separated on a column of alumina 'weakened' with 5 per cent water. Esterified vitamin A is eluted from such a column with 4 per cent acetone in petroleum ether, while free vitamin A is eluted with 20 per cent acetone in petroleum ether[24]. The fractions were assayed for vitamin A by the Carr–Price reaction. In two experiments, it was found that 92 per cent of the vitamin A in the pigment epithelium after 60 min. of light adaptation was esterified. Krinsky found that in cattle 87 per cent of the vitamin A in the pigment epithelium was esterified[23].

Vitamin A exists in the tissues in several geometric configurations, *cis-trans* isomers of one another[25]. The bleaching and synthesis of rhodopsin involves a cycle of stereo-isomerization: rhodopsin is synthesized from 11-*cis* (neo-*b*) retinene, and on bleaching releases all-*trans* retinene[25].

11-*cis* Vitamin A has not been identified in any tissue of the body, except in the eye[26]. There Krinsky has found that up to 65 per cent of the vitamin A in the pigment epithelium may be 11-*cis*[23]. It is of interest to inquire what isomer of vitamin A is found in the pigment epithelium after prolonged light adaptation. With this in view, the vitamin A was extracted from the pigment layers of six light-adapted animals as described above. The content of 11-*cis* vitamin A was determined by isomerizing with light in the presence of iodine, according to the method of Wald and Brown[27]. In two such experiments, no 11-*cis* vitamin A was detected.

There is considerable evidence, however, that in some animals the pigment epithelium does isomerize vitamin A[18,23,28], and it seems reasonable that vitamin A while in the pigment epithelium of the rat might undergo isomerization. Recently, Hubbard and Colman have shown that the amount of 11-*cis* vitamin A in the eye of the frog remains relatively low during the course of dark adaptation. Then, when the retina has ceased to remove it to form rhodopsin, the amount of 11-*cis* vitamin A in the eye increases 2–3 fold[18]. It seemed, therefore, that my failure to detect 11-*cis* vitamin A might be due to insufficient time for detectable quantities of 11-*cis* vitamin A to accumulate. I have tested this thesis in preliminary experiments by incubating the backs of light-adapted rat eyes in phosphate buffer for periods up to 4 hr., but have not as yet been successful in demonstrating the production of 11-*cis* vitamin A.

In summary, most of the vitamin A formed from retinene during light adaptation rapidly moves into the pigment epithelium. After 60 min. of light adaptation, 75–80 per cent of the total ocular vitamin A is found in the pigment epithelium, mainly as vitamin A ester. During dark adaptation, the vitamin A returns from the pigment epithelium to the retina as rhodopsin is regenerated. In the fully dark-adapted state, the pigment epithelium contains no detectable amount of vitamin A.

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[1] Dowling, J. E., and Wald, G., *Proc. U.S. Nat. Acad. Sci.*, **44**, 648 (1958).
[2] Wald, G., Jeghers, H., and Arminio, J., *Amer. J. Physiol.*, **123**, 732 (1938).
[3] Riggs, L. A., *J. Cell. Comp. Physiol.*, **9**, 491 (1937).
[4] Best, W., and Bohnen, K., *Documenta Ophthal.*, Sep. x, 351 (1956).
[5] Tansley, K., *J. Physiol.*, **71**, 442 (1931).
[6] Lewis, D. M., *J. Physiol.*, **136**, 624 (1956).
[7] Wald, G., Brown, P. K., and Smith, P. H., *J. Gen. Physiol.*, **38**, 623 (1955).
[8] Wald, G., *Science*, **119**, 887 (1954).
[9] Campbell, F. W., and Rushton, W. A. H., *J. Physiol.*, **130**, 131 (1955).
[10] Rushton, W. A. H., *Nature*, **179**, 571 (1957).
[11] Wald, G., Proc. Nat. Physical Lab. Symp., No. 8, Visual Problems of Colour (H.M.S.O., 1959).
[12] Rushton, W. A. H., Symposium on Life and Light, McCollum-Pratt Institute, Johns Hopkins University (in the press).
[13] Charpentier, G., *Acta Ophthal.*, Kbh. Supp. 9 (1936).
[14] Granit, R., Munsterhjelm, A., and Zewi, M., *J. Physiol.*, **96**, 31 (1939).
[15] Elemus, V., and Heck, J., *Nature*, **180**, 810 (1957).
[16] Johnson, E. P., and Riggs, L. A., *J. Exp. Psychol.*, **41**, 139 (1951).
[17] Wald, G., *J. Gen. Physiol.*, **19**, 351 (1935–36).
[18] Hubbard, R., and Colman, A. D., *Science*, **130**, 977 (1959).
[19] Jancsó, N. von, and Jancsó, H. von, *Biochem. Z.*, **287**, 289 (1936).
[20] Greenberg, R., and Popper, H., *Amer. J. Physiol.*, **134**, 114 (1941).
[21] Krinsky, N. I. (unpublished observations).
[22] Glover, J., Goodwin, T. W., and Morton, R. A., *Biochem. J.*, **41**, 97 (1947).
[23] Krinsky, N. I., *J. Biol. Chem.*, **232**, 881 (1958).
[24] Ganguly, J., Krinsky, N. I., Mehl, J. W., and Deuel, H. J., jun., *Arch. Biochem. Biophys.*, **38**, 275 (1952).
[25] Hubbard, R., and Wald, G., *J. Gen. Physiol.*, **36**, 269 (1952–53).
[26] Brown, P. S. (unpublished observations).
[27] Brown, P. K., and Wald, G., *J. Biol. Chem.*, **222**, 865 (1956).
[28] Hubbard, R., *J. Gen. Physiol.*, **39**, 935 (1956).

EFFECT OF POLYVINYL ALCOHOL ON CHLOROPHYLL AND HÆM IN *CHLORELLA* AND *TENDIPES*

The Level of Chlorophyll in *Chlorella pyridinosa*

IN experiments on *Pisum sativum* L., it was found that polyvinyl alcohol when applied to soil increased the chlorophyll content of the green parts of the plants (ref. 1, and unpublished work by H.F.N. and L.R.). In order to determine what additional factors contribute to this increase we decided to study the alga *Chlorella pyridinosa* which had been bred directly in an aqueous solution of polyvinyl alcohol. Polyvinyl alcohol produced in Poland (known as 'Salvar', 'Polyviol', 'Alvyl', and by other names) was used for these experiments. It was intended to carry out the experiments in aqueous cultures with Pruess medium, on which a rapid growth of *Chlorella* has been observed[2]. However, preliminary experiments showed that one of the elements of that medium, casein, led to errors in measurement. In view of this, cultures from ponds in Białystok were set up in aquaria of 500-ml. capacity. In order to avoid contamination and to remove zooplankton organisms, the culture was filtered by means of a triply folded gauze, after which 400 ml. of the filtrate from the *Chlorella* culture were poured into each aquarium.

The polyvinyl alcohol was prepared as a 10 per cent aqueous solution and different quantities added to the different cultures to give various concentrations of polyvinyl alcohol in them. Concentrations which gave an increase in chlorophyll content were used; they were: 0·00001, 0·0001, 0·001, 0·01, 0·1, 0·5, and 1 per cent. Two aquaria with cultures but no polyvinyl alcohol were used as controls. The experiment lasted 14 days. As early as the tenth day of the experiment, a difference in the intensity of the green colour could be detected with the naked eye. On the fourteenth day of the experiment, 50-ml. specimens were taken from each aquarium and filtered through No. 5 gauzes. The filtered specimens were then dried for three days in a dark room at a temperature of +18° C. Chlorophyll was extracted with ethanol and the content