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Reactions of Vitamin A with Acceptors of Electrons

INTERACTIONS WITH IODINE AND THE FORMATION OF IODIDE

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1. The reactions of retinol and retinoic acid with iodine were investigated since knowledge of the chemical reactions of vitamin A with acceptors of electrons may shed light on its biochemical mode of action. 2. Colloidal retinol, but not retinoic acid, reacts with iodine to yield a blue–green complex that rapidly decomposes, giving iodide and an unknown species with $\lambda_{max.}$ at 870 mμ. 3. In addition, both retinol and retinoic acid reduce iodine to iodide by a reaction that does not involve an intermediate coloured complex; this reaction appears to yield unstable carbonium ion derivatives of the vitamin. 4. The presence of water greatly facilitates the production of iodide from vitamin A and iodine. 5. Possible chemical pathways involved in these reactions are discussed. 6. It is suggested that the chemical properties of retinol and retinoic acid that underlie their biochemical behaviour might be apparent only when the molecules are at a lipid–water interface, and that vitamin A might be expected to react with a number of different electron acceptors *in vivo*.

It is reported in the preceding paper (Lichti & Lucy, 1969) that electron transfer can occur when retinol, or retinoic acid, interacts with 7,7,8,8-tetracyanoquinodimethane or with chloranil (tetrachloro-1,4-benzoquinone), and that the radical anions of 7,7,8,8-tetracyanoquinodimethane and chloranil are formed under appropriate conditions. The present paper reports some reactions of vitamin A with iodine, which include reduction of iodine to iodide. Molecular iodine is known to form charge-transfer complexes with many different donors of electrons (Andrews & Keefer, 1964) and, on complete electron transfer, the I^- ion is produced. In our investigations particular attention has been paid to the reactions of vitamin A with iodine that occur when water comprises the bulk environment, since the amphipathic nature of retinol that is responsible for its surface-active (Bangham, Dingle & Lucy, 1964) and membrane-active (Lucy, 1964) properties indicates that the vitamin may be located at a lipid–water interface *in vivo*.

The present findings on the chemical behaviour of vitamin A are thought to be consistent with the possibility that the vitamin may be involved in electron-transfer reactions *in vivo* (Dingle & Lucy, 1965). If vitamin A is involved in electron-transfer reactions *in vivo*, the widespread biological actions of the vitamin (Moore, 1957) may arise from its interactions with one or more acceptor molecules in biochemical processes occurring on, or in, different membranes.

A preliminary communication has been published (Lucy & Lichti, 1967).

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MATERIALS AND METHODS

Reagents. Synthetic crystalline retinol (Roche Products Ltd., Welwyn Garden City, Herts.), retinoic acid (Distillation Products Industries, Rochester, N.Y., U.S.A.) and I_2 (AnalaR; British Drug Houses Ltd., Poole, Dorset) were used without further purification. All aqueous solutions of I_2 were freshly prepared. The concentrations of I_2 in the aqueous solutions used were determined by completely extracting the I_2 into light petroleum followed by spectrophotometric measurements of the I_2 in the organic solvent. Retinol was handled as described by Dingle & Lucy (1962).

Spectrophotometric measurements. Most of the spectra were obtained by means of a Unicam SP.700 recording spectrophotometer. All spectral measurements were made at room temperature.

Uptake of OH^- *ions.* Consumption of OH^- ions was measured by means of a Radiometer automatic titrator, used as a pH-stat at pH 7·0, with standardized 0·01 M-NaOH as the titrant. In procedure A, retinol and iodine in ethanol were added separately under N_2 to previously boiled phosphate buffer (0·5 mM). Since a small uptake of base occurred with I_2 alone, the I_2 was added first and the pH brought to 7·0 before addition of retinol. The rate of addition of base levelled off about 3–4 min. after addition of

the retinol; consumption of OH^- ions/mole of retinol was calculated for the plateau region. In procedure B, solutions of retinol and I_2 in ethanol were mixed, and a sample of the mixture was added to the phosphate buffer. To correct for the base uptake due to I_2 alone, a separate titration was made of I_2 added to the buffer from ethanol. All titrations were made at room temperature.

RESULTS

Interactions of retinol and iodine in water. Addition of a fresh solution (6 ml.) of iodine in water (80 μg./ml.) to ethanol (0·02 ml.) containing retinol (20 mg./ml.) immediately yields a dark precipitate: the heterogeneous reaction mixture appears blue by reflected light and dark green by transmitted light. A comparable result is obtained if the iodine solution is added to a fresh colloidal suspension of retinol in water, prepared by rapidly adding water (5 ml.) to ethanol (0·05 ml.) containing retinol (20 mg./ml.). It appears that, whichever of these two techniques is employed, the iodine reacts with colloidal aggregates of dispersed retinol to form the coloured complex, since the complex is not formed when retinol and iodine are mixed in ethanol. Neither is the coloured substance formed when retinol and iodine are allowed to interact in ethanol and then subsequently added to water (see below). Some degree of micellar organization of retinol molecules may therefore be a prerequisite for the formation of the coloured complex.

No coloured precipitate is produced with retinol when sufficient potassium iodide is added to the aqueous iodine solution to destroy the absorption band at 460 mμ of iodine in water. In the presence of excess of iodide, the iodine-containing solution exhibits only the intense absorption bands at 286 mμ and 354 mμ due to the I_3^- ion (cf. Robin, 1964). It would thus seem that free iodine is necessary for the formation of the coloured precipitate with retinol.

The coloured heterogeneous reaction mixture produced on the interaction of iodine with colloidal retinol initially has a pronounced absorption maximum at about 610 mμ (16·4 kcyc./cm.) (Fig. 1). This absorption band is presumably due to the presence of a new molecular species, since the absorption maxima of iodine and of retinol in water are at 460 mμ and 325 mμ respectively and neither substance has an absorption band in the 600–900 mμ region (Fig. 1).

Investigation of the ultraviolet absorption of the reaction mixture in the 320–330 mμ region, where colloidal retinol has its maximum, reveals that the absorption due to retinol is considerably decreased within 1 min. of addition of iodine; the position of maximum absorption also shifts to about 295 mμ (34 kcyc./cm.). The spectrum of the reaction mixture then remains relatively unchanged during the succeeding 30 min. (Fig. 2). A decrease in extinction and changes in the shape of the spectrum of colloidal retinol occur during the autoxidation of retinol by molecular oxygen in an aqueous environment (Fig. 2). The changes occurring with molecular oxygen involve the development of fine structure in the spectrum of the dispersed retinol (Lucy, 1965), however, and they differ from those observed in the reaction of retinol with iodine in water (Fig. 2). Nevertheless a shift of the absorption maximum to about 295 mμ also occurs in the autoxidation reaction after a relatively long time (1–2 hr.) (J. A. Lucy, unpublished work).

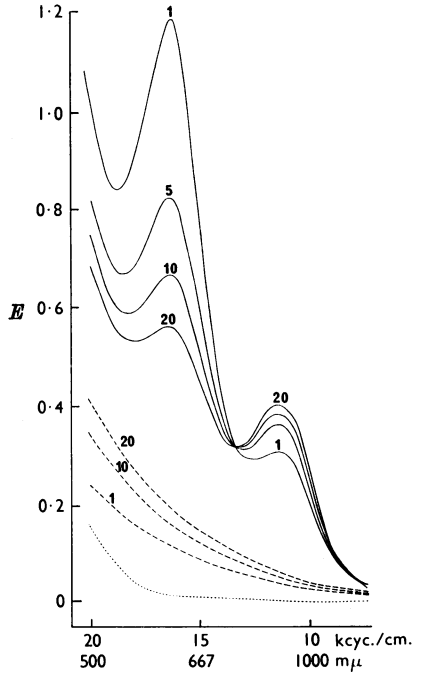


Fig. 1. Spectral changes with time (in minutes) of a mixture of I_2 (0·3 mM) and retinol (0·24 mM) in water (——). The aqueous I_2–retinol reaction mixture was prepared by adding 50 μl. of a solution of I_2 (60 mM) in ethanol to 10 ml. of N_2-saturated water; this was then added to 50 μl. of a solution of retinol (48 mM) in ethanol. Corresponding spectra of retinol (0·24 mM) (– – – –) and of I_2 (0·3 mM) (. . . . .) in N_2-saturated water are also shown. The spectrum of the I_2 solution is stable for at least 30 min.

Decomposition of the retinol–iodine complex. The visible colour of the retinol–iodine complex fades quite quickly, and spectral studies reveal that the decomposition of the complex is accompanied by the development of new absorption bands in the ultraviolet and near-infrared regions of the spectrum.

Iodine in water has an absorption maximum in the ultraviolet at about 205 mμ (48·8 kcyc./cm.); its

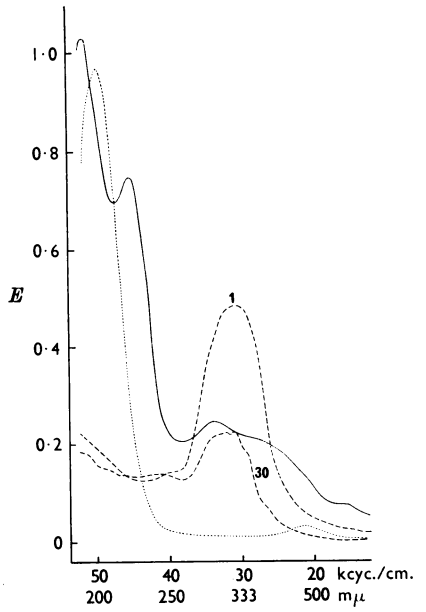


Fig. 2. Spectrum of the reaction mixture (——) containing I_2 (0·044 mM) and retinol (0·026 mM) 30 min. after mixing in air-equilibrated water. Spectra of retinol (0·026 mM) 1 min. and 30 min. after suspending in air-equilibrated water (----) and of I_2 (0·044 mM) in air-equilibrated water (.....) are also shown. The spectrum of the I_2 solution is stable for at least 30 min.

extinction at 226 mμ (44·3 kcyc./cm.) is 25% of that at 205 mμ. Within 1 min. of preparing the reaction mixture of iodine and colloidal retinol, the iodine peak at 205 mμ is no longer apparent. During the first minute a shoulder develops at about 226 mμ (44·3 kcyc./cm.) and a distinct new maximum is present at this wavelength after 30 min. (Fig. 2). A second new maximum is also observable at about 195 mμ (51 kcyc./cm.). Control experiments show that none of these changes occurs either with a solution of iodine in water or with colloidal retinol (which has a minimum at 226 mμ) over a 30 min. period (Fig. 2). Since the I^- ion in water is known to have maxima at 194 mμ and 226 mμ (Franck & Scheibe, 1928), these observations indicate that iodine is reduced to iodide as the retinol–iodine complex decomposes.

Experiments were carried out to compare the rate of decay of the extinction of the coloured complex at 610 mμ and the formation of the peak attributed to iodide at 226 mμ. The reaction mixture was placed in a 1 cm. cuvette, and the decrease at 610 mμ was followed continuously for 15 min. The increase at 226 mμ was determined similarly in an experiment in which a 1 mm. cuvette was employed. The rates of change at both wavelengths were most rapid during the initial 5 min. (Fig. 3). Although the shapes of the two curves of Fig. 3 would seem to be consistent with the interpretation that iodide is derived from the coloured complex of retinol with iodine, experiments described below indicate that iodide can also be formed by a pathway that is independent of the coloured complex.

If iodide is formed from the retinol–iodine complex, equivalent cationic material is presumably produced simultaneously. It is therefore noteworthy that a new species, which absorbs at 870 mμ (11·5 kcyc./cm.), is formed in addition to iodide as the coloured complex decays (Fig. 1). In a control experiment the absorption due to retinol dispersed in water was observed to increase generally in the 800–900 mμ region, apparently as a result of a change in the state of aggregation of the dispersed retinol. No maximum developed at 870 mμ, however, in the absence of iodine. An isosbestic point is formed at about 740 mμ (13·5 kcyc./cm.) as the retinol–iodine complex decomposes (Fig. 1) and this may indicate that the material absorbing at 870 mμ is being produced directly from that absorbing at 610 mμ, but no firm conclusions can be drawn in view of the heterogeneous nature of the reaction mixture. Nevertheless, the formation of iodide and the production of the species absorbing at 870 mμ both occur most rapidly during the 5 min. period immediately after the interaction of iodine with colloidal retinol (Fig. 1). It is suggested therefore that the band at 870 mμ may be due to the positive counterion that is formed from the retinol–iodine complex at the same time as the iodide.

Recovery of iodine. Since the above spectral studies indicated that the iodine of the complex is rapidly reduced to iodide, experiments were made to recover iodine after reoxidation of the iodide. Samples of the reaction mixture containing the retinol–iodine complex were extracted once with light petroleum at different times after formation of the complex, and the recovered iodine was estimated by means of its characteristic absorption at 525 mμ in light petroleum. Repetition of the extraction process yielded no further iodine from individual samples and, after one extraction, the aqueous samples gave no colour on acidification and treatment with starch. A blue colour ($\lambda_{max.}$ 615 mμ) formed in the presence of acid and starch, however, when the iodide present in these solutions was oxidized by sodium nitrite. A comparison of the progressively decreasing quantities of free iodine recovered with the increasing densities of colour observed in the starch test with sodium nitrite is shown in Fig. 4 for periods up to 10 min. after the initial formation of the retinol–iodine complex.

The blue starch–iodine reaction can be used,

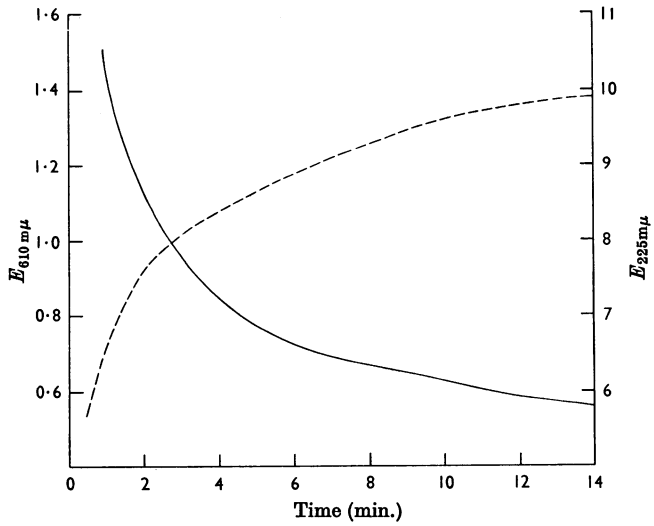


Fig. 3. Changes in extinction with time (in minutes) of the reaction mixture of retinol (0·025 mM) and I_2 (0·66 mM) in air-equilibrated water. The extinction at 610 mμ (——) was measured in a 1 cm. cuvette, and that at 225 mμ (----) in a 1 mm. cuvette.

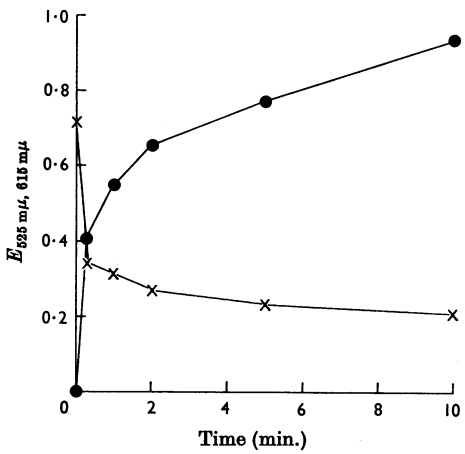


Fig. 4. Comparison of the rate of consumption of I_2 (extinction at 525 mμ; ×) with the rate of formation of I^- ions (extinction at 615 mμ; ●) as indicated by the starch–iodide reaction, when retinol reacts with iodine. The aqueous I_2–retinol reaction mixture was prepared by adding 15 ml. of a freshly prepared solution of I_2 (0·48 mM) in air-equilibrated water to 60 μl. of retinol (70 mM) in ethanol.

within appropriate concentration limits, as a measure of the iodide produced from the interaction of retinol with iodine. In two separate determinations, 1·0 and 1·5 moles of iodine were consumed/mole of retinol (iodine being present initially in a slight molar excess). After 10 min. 2·0 and 1·3 moles of iodide/mole were found to have been produced. A detailed quantitative analysis of this system was not made, however, in view of the likelihood (considered below) that iodide is produced by two simultaneous but independent reaction pathways that yield respectively either 1 or 2 moles of iodide/mole of iodine consumed.

Formation of iodide in the absence of the coloured retinol–iodine complex. Water greatly facilitates the production of iodide from iodine and retinol. Thus the I^- ion is apparently not formed, or at least is formed much more slowly, when retinol and iodine are mixed in ethanol rather than in water, as indicated by the slow increase in the ultraviolet absorption spectrum at 222–227 mμ (44–45 kcyc./cm.) (Fig. 5). Nevertheless, the absorption of the reaction mixture in ethanol (0·02 mM-retinol and 0·04 mM-iodine) undergoes a marked decrease with time in the region of the spectrum where retinol absorbs maximally (325 mμ or 30·8 kcyc./cm.). After 40 min. the absorption maximum has shifted to about 295 mμ (34 kcyc./cm.) and this may indicate that an addition reaction of iodine with a double bond of retinol has occurred. These spectral changes occur much more rapidly if the reaction is carried out at higher reactant concentrations (10 mM).

When retinol and iodine are allowed to interact for a short time in ethanol at concentrations of 10 mM, and the reaction mixture is then diluted 1000-fold with water, the spectrum due to iodide

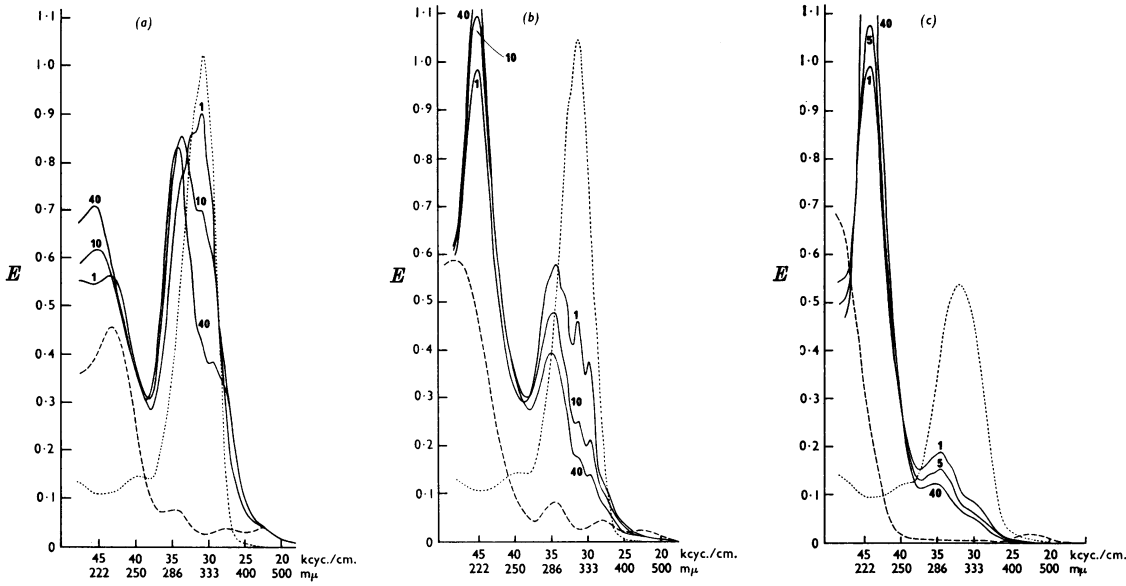


Fig. 5. Spectral changes with time (in minutes) during the reaction of retinol (0·021 mM) with I_2 (0·040 mM) in (*a*) ethanol, (*b*) aq. 50% ethanol and (*c*) water. ——, Reaction mixture; ----, solution of I_2 (0·040 mM) 1 min. after preparation; ....., solution or dispersion of retinol (0·021 mM) 1 min. after preparation. The spectra of the I_2 and retinol controls changed only slightly during 40 min., since all experiments were done in N_2-saturated solvents.

appears immediately but no coloured complex is observed. This experiment indicates that the production of a coloured complex is not essential for the process of electron transfer from retinol to iodine.

It is noteworthy that, after 1 min. in aq. 50% (v/v) ethanol, absorption maxima are apparent at 295, 323 and 339 mμ (34·0, 31·0 and 29·5 kcyc./cm.) and a shoulder is observable at 308 mμ (32·5 kcyc./cm.) in addition to the iodide peak at 226 mμ (44·3 kcyc./cm.). After 40 min. the iodide peak has slightly increased. The multiple absorption bands in the 300–345 mμ region have virtually disappeared by this time, leaving a prominent absorption maximum at about 295 mμ (34 kcyc./cm.). Since the latter band and that due to iodide, but not the multiple bands between 300 mμ and 345 mμ, are formed within 1 min. when retinol reacts with iodine in water (Fig. 5), it seems possible that the multiple bands noted in aq. 50% ethanol may be due to an intermediate that is produced in the electron-transfer process and that is more stable in ethanol–water than in water alone.

Retinoic acid and iodine. Retinoic acid dispersed in water has a broad absorption maximum at about 370 mμ (27 kcyc./cm.) and, apart from a decrease in extinction and a further increase in the width of the band, the spectrum of the dispersed material remains unchanged for at least 20 min. at room temperature. Dispersed retinoic acid does not give a coloured complex with iodine comparable with the retinol–iodine complex. Failure to form such a complex may be due to the difficulty of preparing sufficiently concentrated dispersions of retinoic acid, the acid being more difficult to disperse than retinol.

Iodine nevertheless reacts readily with retinoic acid in water as is shown by the marked decrease within 1 min. of the absorption due to retinoic acid at about 370 mμ and the appearance of a band at about 226 mμ corresponding to the I^- ion (Fig. 6). As with retinol and iodine, new material with a $\lambda_{max.}$ at about 295 mμ is also formed. The development of the band at 295 mμ can be accelerated by pre-mixing retinoic acid and iodine (at concentrations of 10 mM) in ethanol and then diluting the mixture 1000-fold with water. When retinoic acid and iodine interact in aq. 50% ethanol, the I^- ion is again rapidly produced, although its appearance is slightly slower than in water alone. With aq. 50% ethanol new peaks also develop between 294 and 345 mμ (34 and 29 kcyc./cm.) (Fig. 6); the behaviour of retinoic acid and iodine therefore resembles that of retinol and iodine at this concentration of water. Further, as with retinol, iodide does not seem to be produced when retinoic acid and iodine react in ethanol, although interactions nevertheless occur that lead to the formation from retinoic acid of material absorbing at shorter wavelengths (295 mμ).

Consumption of OH^- *ions.* As in the reactions of

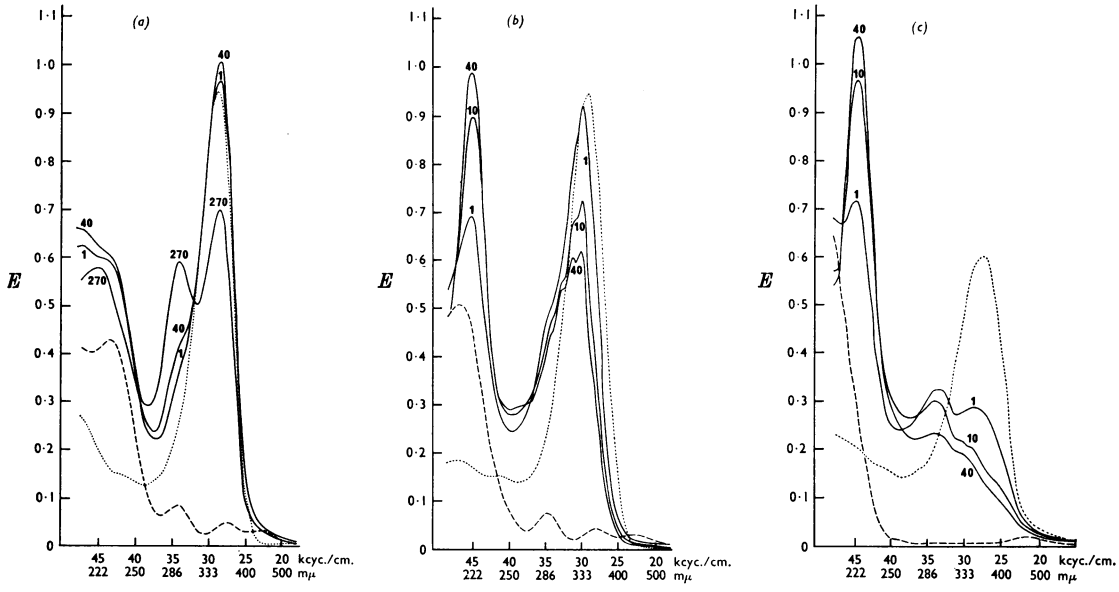


Fig. 6. Spectral changes with time (in minutes) during the reaction of retinoic acid (0·020 mM) with I_2 (0·040 mM) in (*a*) ethanol, (*b*) aq. 50% ethanol and (*c*) water. ——, Reaction mixture; – – – –, solution of I_2 (0·040 mM) 1 min. after preparation; . . . . ., solution or dispersion of retinoic acid (0·020 mM) 1 min. after preparation. The spectra of the I_2 and retinoic acid controls changes only slightly during 40 min., since all experiments were done in N_2-saturated solvents.

vitamin A with 7,7,8,8-tetracyanoquinodimethane and chloranil reported in the preceding paper (Lichti & Lucy, 1969), consumption of OH^- ions is a feature of the interactions of both retinol and retinoic acid with iodine. Uptake of OH^- ions was measured in two different ways. In method A, vitamin A in ethanol was added to iodine dissolved in dilute buffer of the desired pH; in method B, vitamin A and iodine were mixed in ethanol and the reaction mixture was then added to the buffer. Method A allows colloidal aggregates of retinol to form when the retinol is added to the buffered iodine solution, and in this procedure the coloured retinol–iodine complex is formed. In method B an interaction between retinol and iodine occurs in the ethanol, as indicated by the observed spectral changes that are reported above. Apparently as a result of this interaction the coloured retinol–iodine complex is not produced when the ethanolic reaction mixture is subsequently added to water. Whichever procedure is used, the pH of the buffer falls to a low value (approx. pH 3) by the time the sample addition is complete, and about 1 min. elapses before the pH is fully restored to its initial value by the titrator. The rapid fall in pH indicates that either an acid is being formed or some other reaction is occurring that liberates protons, such as the reaction of a carbonium ion with water. From a consideration of the known reaction of electron donors (see the Discussion section) it is thought that a carbonium ion may be involved.

In a series of experiments in which the ratio of

Table 1. *Uptake of* OH^- *ions associated with the reaction of retinol and iodine at* pH 7·0

Uptake of OH^- ions was measured with a Radiometer automatic titrator, used as a pH-stat at pH 7·0 as described in the Materials and Methods section. In series A, vitamin A and I_2 were added separately to the dilute buffer; in series B vitamin A and I_2 were premixed in ethanol and the mixture was added to the buffer. The values in parentheses in the fifth column represent the numbers of determinations.

Procedure	Concn. of I_2 (mM)	Concn. of retinol (mM)	I_2/retinol molar ratio	OH^- ions consumed (moles/mole of retinol)
A	0·52	0·52	1·0	1·3±0·1 (3)
A	1·04	0·52	2·0	2·0±0·1 (3)
A	1·56	0·52	3·0	2·6±0·3 (3)
A	1·72	0·151	11·8	4·8±0·2 (3)
A	1·815	0·120	15·1	6·8 (1)
A	1·815	0·060	30·3	6·7 (1)
B	0·52	0·52	1·0	1·6±0·1 (3)
B	1·04	0·52	2·0	3·4±0·1 (3)
B	1·77	0·15	11·8	6·7±0·6 (3)

iodine to retinol was varied, the quantity of OH^- ions consumed/mole of retinol was observed to depend on the molar ratio of the reactants (Table 1). The finding that at least six OH^- ions can be consumed/molecule of retinol appears to imply that the electrons of at least three double bonds of retinol are available for transfer to iodine when the ratio of acceptor to donor molecules is very high.

The decreased consumption of OH^- ions in method A as compared with method B (Table 1) is considered to result from the formation of the coloured complex between retinol and iodine in method A. It is suggested that the colloidal aggregates of retinol produced in method A may react with iodine by two competing pathways. In one reaction retinol forms an unstable coloured complex, whereas in the other it may form a carbonium ion derivative that reacts with water to consume OH^- ions. Formation of the coloured complex would thus decrease the quantity of retinol available for carbonium ion formation. In procedure B apparently only the carbonium ion pathway is followed. With retinoic acid consumption of OH^- ions was more comparable in methods A and B. Thus in experiments with iodine (0·59mM) and retinoic acid (0·15mM), the mean values of $3·0 \pm 0·2$ and $3·4 \pm 0·1$ moles of OH^- ion consumed/mole of retinoic acid were obtained from three determinations each by methods A and B respectively. This is consistent with the fact that retinoic acid does not form a coloured complex with iodine.

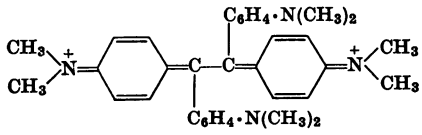
After the initial readjustment of the pH, uptake of OH^- ions continued very slowly for 10–20min. in method A but not in method B. This behaviour was more pronounced with retinoic acid than with retinol, and it is therefore unlikely to be associated with the decay of the coloured retinol–iodine complex. The slow consumption of OH^- ions may occur because method A allows the formation of aggregates of retinol molecules that, if they are not involved in the coloured retinol–iodine complex, may react relatively slowly with iodine to yield carbonium ions. This interpretation would appear to be supported by the noticeably slower consumption of OH^- ions in experiments with retinoic acid and iodine, since retinoic acid gives much larger aggregates than retinol in water.

DISCUSSION

A number of substances resemble retinol in forming blue complexes with iodine (Cramer, 1955). In their early studies on the interactions of saponarin and cholalic acid with iodine Barger & Field (1912) concluded that no substance that is dissolved as separate molecules will give this reaction; the 'typically colloidal reaction' of benzacridines and acridines with iodine to give blue or brown colours (Kermack, Slater & Spragg, 1930) has also been referred to by Albert (1966) as evidence that solutions of the ions of many acridines may contain dimers or highly aggregated micelles. The formation of the coloured complex of iodine with retinol may thus depend partly on the ability of retinol to form colloidal aggregates in aqueous dispersions (cf. Straus, 1939; Lucy, 1965). By contrast retinoic acid, which does not form a blue complex with iodine, cannot be dispersed in water at concentrations equal to those used with retinol since gross aggregation occurs. Cramer (1955) suggested that the most important factor contributing to the formation of blue inclusion compounds containing iodine, such as the starch–iodine complex, may be electronic interactions of the donor–acceptor type between the host molecule and iodine, and Szent-Györgi (1960) has discussed the formation of a blue complex of iodine with cortisone ($\lambda_{max.}$ 740mμ) in terms of charge transfer. Thus a second factor contributing to the formation of a coloured complex of retinol with iodine is probably the electron-donor character of retinol.

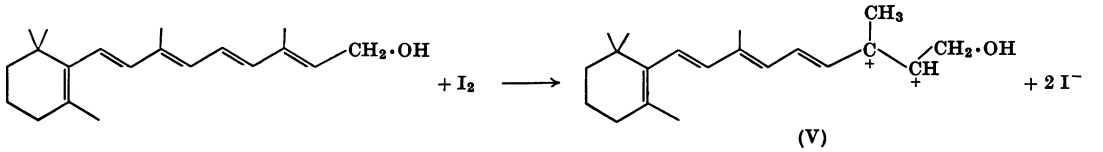
The instability of the blue retinol–iodine complex apparently stems from the strength of the donor properties of retinol, since our observations indicate that iodine is reduced to iodide as the coloured complex decays. If the coloured material is equivalent to a charge-transfer complex, of the type [retinol.I_2], dissociation may subsequently yield an ionic complex of the type [retinol.I]$^+I^-$, in which the cation [retinol.I]$^+$ absorbs at 870mμ. This dissociation would resemble that of the complex between pyridine and iodine, which gives rise to the 'inner complex' of *N*-iodopyridinium iodide, containing the cation $(C_6H_5.NI)^+$ (Reid & Mulliken, 1954). It is relevant that infrared-absorption bands due to (γ-picoline$_2$.I)$^+$ and I_3^- ions have been observed with polar solvents in addition to the band associated with the charge-transfer complex between γ-picoline and iodine (Haque & Wood, 1967). The production of iodide from the charge-transfer complex formed between triphenylarsine and iodine has also been interpreted in terms of the formation of an inner complex containing I^- ion (Bhat & Rao, 1966).

The interactions between retinol and iodine that we have observed have some resemblance to those between β-carotene and iodine reported by Lupinski & Huggins (1962) and Lupinski (1963). In polar organic solvents β-carotene reacted with iodine to give material having a new absorption band at 1000mμ; new bands also appeared at 290 and 360mμ, which were attributed to the I_3^- ion. It has been proposed by Lupinski (1963) that the solid β-carotene–iodine complex, $C_{40}H_{56},2I_2$, is best represented by the structure $(C_{40}H_{56}.....I^+)I_3^-$, in



(I)

(III) (IV) (II)



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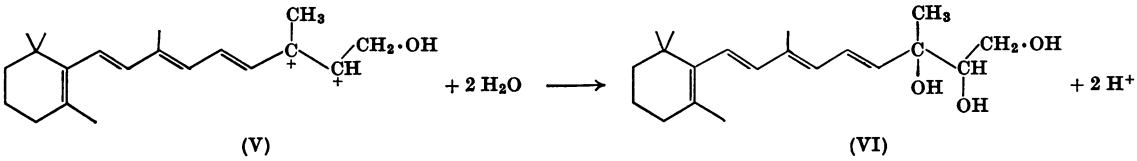


(V) (VI)

which the cation contains an iodonium ion rather than a carbonium ion. Ebrey (1967) has suggested that the band at 1000mμ observed by Lupinski (1963) is due to β-carotene that has had its absorption band shifted by the interaction with iodine (cf. Platt, 1959), and he has proposed that the complex may have the structure $I_3^-...C^+.C:[C-C=]_n.-C.C^-...I^+$. Since both the band at 1000mμ observed by Lupinski (1963) with β-carotene–iodine, and that at 870mμ found in the retinol–iodine system in the present experiments, are about 540mμ from the normal absorption bands of β-carotene and retinol respectively, a similar interpretation may apply to the decomposition of the initial coloured retinol–iodine complex. On this basis retinol may have mediated a dissociation of the neutral iodine molecule into the iodonium ion, I^+, and the iodide ion, I^-, by virtue of forming a relatively stable 'trimolecular charge-transfer complex' with the two ions (Platt, 1959). Since vitamin A would not be consumed in such a process, a reaction of this type, which may be regarded as a catalytic reaction, might be of significance in relation to the mechanism of the systemic actions of the vitamin *in vivo*.

Our observations on the production of iodide in the absence of a coloured intermediate complex indicate the existence of a second mechanism for the production of iodide from vitamin A and iodine. This would appear to involve a direct electron transfer from vitamin A to iodine, and may be similar in principle to the reaction of tetrakis-(*p*-dimethylaminophenyl)ethylene with iodine (Anderson, Elofson, Gutowsky, Levine & Sandin, 1961) in which two I^- ions are formed and a dication (I) is produced that contains two positively charged nitrogen atoms. Since a double bond in retinol (or retinoic acid) is presumably the source of the electrons that are transferred from the vitamin to iodine, the electron-deficient product will probably contain one or two carbonium ions. The reaction product may be comparable in this respect with the dicarbonium ion (II) formed in the reaction of tetra(dimethylamino)ethylene (III) with tetracyanoethylene (IV) (Wiberg & Buchler, 1963).

If the dication (V) were produced on the reduction of iodine by retinol, it would be expected to react with water or added OH^- ions (cf. Sorensen, 1965) to yield the polar product 13,14-dihydroxyretinol (VI), which has one double bond less than the original molecule. Since compound (VI) has four conjugated double bonds, it would be expected to have an ultraviolet-absorption maximum at about 290mμ. It is therefore noteworthy that at least two OH^- ions are consumed/molecule of retinol, and that material absorbing at about 295mμ is produced when retinol and iodine, or retinoic acid and iodine, react in the presence of water. It seems, however, that more than one double bond of retinol is apparently available for electron transfer since

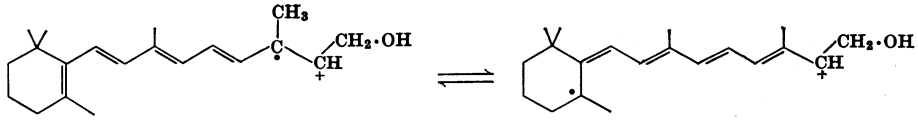
the observed consumption of OH^- ions indicates that more than two carbonium ions are formed/molecule of retinol in the presence of excess of iodine.

Although a mechanism for the formation of iodide involving direct electron transfer from vitamin A to iodine would be consistent with the behaviour of vitamin A towards TCNQ that is reported in the preceding paper (Lichti & Lucy, 1969), it must not be overlooked that loss of one double bond from retinol by any means will yield a product having an absorption maximum at about 295mμ. Thus the product of an addition reaction of iodine with a double bond would be expected to absorb in this region of the spectrum. Iodine does not react readily with unsaturated olefinic systems, however, since unlike the addition of chlorine and bromine the addition of iodine is normally reversible and the position of the equilibrium lies well to the side of free olefins (De La Mare & Bolton, 1966). It remains possible, nevertheless, that iodine may interact with retinol by an electrophilic addition reaction to yield a carbonium ion intermediate $>C(I)-C^+<$ that may react rapidly with the aqueous solvent to give the grouping $>C(I)-C(OH)<$; this behaviour would be comparable with that observed when the more reactive bromine molecule interacts with olefinic acids in water (Read & Read, 1928). In this instance, the number of moles of OH^- ions consumed/mole of retinol will equal the number of moles of iodine reacting. By contrast, the number of moles of OH^- ions consumed/mole of retinol will be twice the number of moles of iodine reacting in an electron-transfer reaction such as that yielding the compounds (V) and (VI). Although the reaction of some molecules of vitamin A with iodine by an electrophilic addition reaction cannot be excluded in our studies, particularly with retinoic acid, the consumption of 1·6 and 3·4 moles of OH^- ions/mole of retinol in experiments in which the molar ratios of iodine/retinol were 1·0 and 2·0 respectively (Table 1, procedure B) would not seem to be explicable solely in terms of electrophilic addition. Furthermore, the electrophilic addition of iodine, followed by a reaction of carbonium ion intermediates with the solvent, cannot account for values that are greater than 5·0 for the number of moles of OH^- ions consumed/mole of retinol.

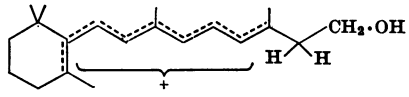
The development of spectral fine structure in the 305–340mμ region that we observed when retinol and iodine interacted in aqueous 50% ethanol, and the subsequent appearance of a single band at about 295mμ, parallel the spectral changes noted in studies on the autoxidation of colloidal retinol in sodium chloride solution (Lucy, 1965). Since the rapid autoxidation of retinol and the development of fine structure within 1min. does not occur in the presence of aqueous 50% ethanol (Lucy, 1966) similar products may be formed when retinol reacts with iodine in aq. 50% ethanol and when colloidal retinol is autoxidized by molecular oxygen in sodium chloride solution, especially as a rapid fall in pH occurs both in the autoxidation reaction (J. A. Lucy & F. U. Lichti, unpublished work) and when retinol reacts with iodine.

Derivatives of retinol having the *retro* configuration exhibit spectral fine structure at a relatively long wavelength as compared with the parent compound (e.g. retinyl acetate has one absorption band at 325mμ; *retro*-retinyl acetate has three bands at 333, 348 and 367mμ; Beutel, Hinkley & Pollak, 1955). The development of multiple bands at about 310, 325 and 340mμ in experiments with retinol may therefore indicate that a rearrangement of double bonds has occurred, and the position of the multiple bands could indicate that one double bond has been destroyed. As discussed in the preceding paper (Lichti & Lucy, 1969), loss of a single electron from retinol may give a radical ion grouping of the type $>C^+-C^{\bullet}<$. By analogy with the work of Blatz & Pippert (1968) on the carbonium ion formed by protonation of retinyl acetate, the loss of a single electron from retinol might thus yield resonance forms such as (VII*a*) and (VII*b*); the reaction of structure (VII*b*) with water would then give a hydroxylated radical (IX) containing a double bond adjacent to the ring as in the *retro* series of compounds. It is relevant that Beutel *et al.* (1955) have postulated that the conversion of vitamin A into *retro*-vitamin A under acid conditions may proceed via the intermediate 'conjugated acid' (VIII). If structure (VII*b*) were a more stable resonance form than structure (VII*a*), the major intermediate reaction product would be the radical (IX) and this might exhibit spectral fine structure. Since it is known that organic radicals can behave as electron donors (Buley & Norman, 1964), a further electron-transfer reaction in which the radical (IX) is the donor may then occur and give rise to the structures (X*a*) and (X*b*). If structure (X*a*) were more stable than structure (X*b*), the final product, after reaction with water, would be 13,14-dihydroxyretinol (compound VI, see above), which would absorb at a shorter wavelength than retinol and, like retinol, it would have no fine structure.

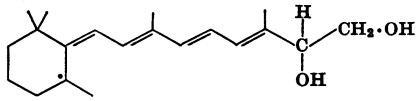
Isenberg & Baird (1962) have remarked that the enhancement of charge transfer and free-radical formation by polar solvents may have biochemical implications. They also suggested that, since the formation of free radicals in biochemical systems takes place in an aqueous environment, interactions with the medium may be expected to play a significant role. Such considerations are particularly apposite in relation to vitamin A, which has surface-active and membrane-active properties.



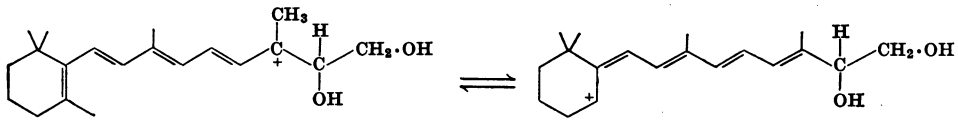
(VII*a*) (VII*b*)



(VIII)



(IX)



(X*a*) (X*b*)

In the present experiments the characteristic spectrum of the I^- ion did not develop at all rapidly when retinol interacted with iodine in ethanol containing less than 5% of water. The observations reported in the preceding paper (Lichti & Lucy, 1969), that an aqueous sodium chloride medium is apparently necessary for successful electron transfer from retinol to chloranil, provide another example of the effect of environment on retinol. This is also seen in the autoxidation of retinol by molecular oxygen, which occurs most readily in aqueous sodium chloride, less readily in water and much more slowly in organic solvents (Lucy, 1965, 1966). The influence of the environment in these reactions leads us to suggest that the chemical properties of retinol and retinoic acid that are responsible for their biochemical behaviour might become apparent only when the molecules are at an interface between lipid and water. In such a situation molecules of vitamin A make contact with the aqueous phase but simultaneously retain the properties that they exhibit in a non-polar environment. It is noteworthy that retinal in aqueous 2% digitonin behaves as if it were in a somewhat non-polar environment with regard to the generation of free radicals by incident light (Grady & Borg, 1968). It may also be relevant to the biochemistry of retinol that the rates of bimolecular chemical reactions occurring in micellar systems can be increased by including both reactants in or on the micelle (cf. Bruice, Katzhendler & Fedor, 1968).

Our findings on the effect of the environment on retinol contrast with those published by Bhowmik, Jendrasiak & Rosenberg (1967) on charge-transfer complexes of lecithin with iodine. These workers observed the formation of I_3^- ions when iodine interacted with lecithin in water or in carbon tetrachloride, and when lecithin was in the solid state. Wobschall & Norton (1967) have concluded, however, that water is necessary for the formation of the blue complexes of certain steroids with iodine.

The experiments reported in the present paper indicate that the donor reactions of vitamin A *in vitro* are not restricted to reduction of substances like 7,7,8,8-tetracyanoquinodimethane and chloranil, which, when they are reduced to their respective radical anions, accept a total of only a single electron. In addition both observations (F. U. Lichti & J. A. Lucy, unpublished work) on the autoxidation of retinol in an aqueous environment (Lucy, 1965, 1966) and the studies of Grady & Borg (1968) on light-induced free radicals of vitamin A indicate that one molecule of the vitamin may serve as a donor of an electron to a second molecule under certain circumstances. It is suggested that this lack of specificity may possibly be of biological

significance, since it might enable the vitamin to react with a number of different acceptors in different environmental situations *in vivo*.

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