gels[5,6], no change in frequency and little change in intensity of the carbonyl vibration at 1652 $cm^{-1}$. We conclude that the carbonyl group does not undergo inter-base hydrogen bonding in the ordered structure but that the purine ring is probably hydrogen-bonded by the amino group of another molecule. Comparison of the spectra with those of the isoguanosine cation (bands at 1697, 1652, and 1613 $cm^{-1}$) and anion (broad band with $\nu_{max}$ at 1631 $cm^{-1}$) demonstrates that the nucleoside is uncharged in the ordered structure. The temperature dependence of infrared intensity (Fig. 5) reflects the cooperative melting of an ordered structure. The transition is much broader than those observed with the homopolynucleotide helices, but similar to those of the guanylic acid gels[5,6].

Rotatory dispersion curves of the aggregate and of the unassociated monomer are given in Fig. 6. The gel has a positive specific rotation about six times that of unassociated monomer, suggesting that the structure is highly asymmetric and probably helical.

We shall later report demonstrations of the tautomeric forms of the neutral and charged molecules and propose a structure for the helix based upon these studies. Syntheses of the 5'-phosphate and pyrophosphate of the nucleoside are in progress.

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## Fluorescence decline in purple bacteria resulting from carotenoid absorption

An initial decline in bacteriochlorophyll fluorescence as a result of excitation in the region of carotenoid absorption was measured with chromatophores of purple bacteria. This decline occurs only at high intensities of exciting light, exceeding $1.5 \cdot 10^4$ erg $\cdot$ $cm^{-2} \cdot sec^{-1}$ with *Rhodospirillum molischianum* and $4.5 \cdot 10^4$ erg $\cdot$ $cm^{-2} \cdot sec^{-1}$ with *Rhodospirillum rubrum*.

In Table I the intensity of fluorescence of chromatophore suspensions of *R. molischianum* and *R. rubrum* measured after 15 sec of illumination, is given. The intensity of exciting light at different wavelengths is adjusted such that the fluorescence intensity amounts to 100 units at the onset of illumination. The chromatophores were adsorbed on filter paper. Fluorescence was measured at 900 nm. Exciting light was obtained from a 100-W, 12-V incandescent lamp (Philips P35S, type 13116C/C4). Monochromatic light of indicated wavelengths was isolated with interference filters. Fluorescence was analysed with a Bausch and Lomb grating monochromator (trans-

TABLE I

INTENSITY OF BACTERIOCHLOROPHYLL FLUORESCENCE FROM CHROMATOPHORES OF *R. molischianum* AND *R. rubrum* AFTER 15 sec OF ILLUMINATION

Fluorescence intensity at time $t = 0$ is taken 100. Intensity of exciting light at 511 nm is $2 \cdot 10^4$ erg·cm$^{-2}$·sec$^{-1}$ with *R. molischianum* and $5.5 \cdot 10^4$ erg·cm$^{-2}$·sec$^{-1}$ with *R. rubrum*. Intensity of exciting light at other wavelengths is adjusted to yield a same deflection of 100 at time $t = 0$. Dark time between readings is 2 min. The values are an average of 6 experiments.

| | *Wavelength of excitation in nm* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *645* | *588* | *566* | *545* | *525* | *511* | *497* | *471* | *458* | *424* |
| *R. molischianum* | 100 ± 1 | 100 ± 1 | 95 ± 5 | 89 ± 6 | 66 ± 9 | 61 ± 6 | 75 ± 4 | 73 ± 6 | 80 ± 7 | 87 ± 5 |
| *R. rubrum* | 100 ± 1 | 98 ± 1 | 94 ± 1 | 83 ± 4 | 85 ± 6 | 84 ± 7 | 85 ± 7 | — | 90 ± 4 | — |

mitted band width 7 nm). Table I shows that light absorbed in the yellow bacteriochlorophyll band at about 590 nm does not result in a marked decrease in fluorescence, while light absorbed in the region of the carotenoid bands (from 530 to 450 for *R. molischianum* and from 550 to 450 for *R. rubrum*, *cf.* ref. 1) effects an appreciable "quenching". In the far red a slight "quenching" effect was observed by light absorbed in the weak bacteriochlorophyll band of *R. rubrum* at 800 nm (corresponding to 99 on the scale of Table I), while absorption in the main band around 870 nm proved to be ineffective.

The rate of fluorescence decline depends strongly on light intensity. At incident intensities of about $4 \cdot 10^5$ erg·cm$^{-2}$·sec$^{-1}$ white light (650 to 400 nm), fluorescence is "quenched" in 10 sec to about 50 % of its initial value, while the final level amounts to only 10–15 %.

If illumination is stopped the fluorescence capacity is restored in the dark. The amount of restoration, usually between 50 and 90 %, depends on time and intensity of pre-illumination. "Half time" of dark restoration is about 8 sec.

Cooling the chromatophores results in a slight increase in the rate of fluorescence decline during illumination. The percentage reversibility in the dark often is increased at temperatures between −15° and −35°, but reversibility vanishes at temperatures below −60°. At liquid nitrogen temperature (−196°) the quenching rate is still similar to that at −20° (and thus somewhat higher than at room temperature), but no reversibility in the dark can be measured. Warming up the chromatophores to temperatures above −30° results in a reappearance of the reversibility.

Light quanta absorbed by carotenoids in *R. rubrum* and *R. molischianum* are only 25–40 % effective in producing bacteriochlorophyll fluorescence[2]. In *Rhodopseudomonas spheroides* a higher percentage of energy transfer, about 80–90 %, was measured[3]. It seems likely that the "quenching effect" is produced by that fraction of the quanta which is not transferred to bacteriochlorophyll. In agreement with this suggestion it was found that the "quenching effect" in chromatophores of the latter species is much less pronounced than it is in the former ones.

With intact cells of *R. rubrum* and *R. molischianum* no fluorescence "quenching" was detected at room temperature. After cooling to about −10° a "quenching effect" of only 5–10 % was detected, which is reversed in the dark. Between −20° and −40°, fluorescence "quenching" was found to be similar to that of chromatophores while

the effect is nearly totally reversible in the dark. Below —60° to liquid nitrogen temperature "quenching" is similar to that of chromatophores and not reversed in the dark.

With the purple sulfur bacterium *Chromatium* a fluorescence "quenching effect" was also noticed. Below —20° "quenching" and dark restoration was similar to that of athiorhodoceae, though at —80° still 40 % of "quenching" is reversible and even at liquid nitrogen temperature a slight reversibility was seen. At room temperature, and during cooling to about —10°, a fluorescence "quenching" was measured with *Chromatium*, but at a rate at least 8 times lower than that at about —30°.

Fluorescence of chromatophores is also strongly quenched by the addition of oxidants, such as potassium ferricyanide or hydrogen peroxide. Suspension of chromatophores of *R. rubrum* in ferricyanide/ferrocyanide mixtures with a potential of 540 mV (with respect to normal hydrogen electrode) results in a quenching of fluorescence as compared with suspensions made in pure buffer (Tris, pH 7.2). The light-induced decline of fluorescence, however, was not lost in this sample, though it was slightly smaller as compared to the sample in buffer. Suspensions of chromatophores in ferricyanide/ferrocyanide mixture of 400 mV showed a fluorescence of the same order of magnitude as the pure buffer samples, but the light-induced fluorescence "quenching effect" was only very weak. At high concentrations of ferrocyanide, or after addition of ascorbate or sodium dithionite, fluorescence intensity was high, but the fluorescence "quenching effect" was completely lost.

These results suggest that the light-induced fluorescence "quenching effect" is due to some photo-oxidation reaction resulting from carotenoid absorption. Whether or not this reaction is mediated by the weak 800-nm bacteriochlorophyll band cannot yet be decided. The reaction appears to be maximal in a certain region of redox potentials. Preliminary experiments indicated that for *R. rubrum* this is in the neighbourhood of 500 mV. This photo-oxidation in chromatophores should then be nearly independent of temperature, while the dark reduction is stopped below —40°.

The carotenoid-induced "quenching effect" seems to be more general in photosynthetic systems. A similar decline of fluorescence during illumination at liquid nitrogen temperature was measured with the 695-nm fluorescence band (*cf.* ref. 4) of chlorophyll *a*-containing organisms. In Chlorella and spinach leaves this decline was found to occur only as a result of illumination with blue or violet light.

MURTY AND RABINOWITCH[5] measured chlorophyll *a* fluorescence of mixtures of chlorophyll *a* and β-carotene dissolved in 3-methoxybutanol-1. They found a quenching of chlorophyll fluorescence in light absorbed partly by carotenoids (at 415 nm), while no quenching occurred when exciting light was absorbed by chlorophyll only (at 660 nm). They suggested that this phenomenon could be ascribed to a quenching of the second excited state of chlorophyll *a* by β-carotene. Such a mechanism might be assumed to be temperature independent. The "quenching effect" in purple bacteria and also that in chlorophyll *a*-containing cells, needs some time to develop, while the lifetime of the second excited state of chlorophyll is of the order of $10^{-11}$ sec. Hence the suggestion of MURTY AND RABINOWITCH does not seem to be valid for the carotenoid-induced fluorescence decline in the structure *in vivo*.

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## Action de l'ocytocine sur la composante active du transport de sodium par la peau de grenouille

A la suite des travaux de USSING *et al.*[1–3] sur la peau de grenouille et de LEAF *et al.*[4] sur la vessie de crapaud, il est généralement admis que l'hormone neurohypophysaire stimule le transport de sodium en augmentant la perméabilité passive pour le sodium de la face muqueuse des cellules épithéliales. Si cette perméabilité agit comme facteur limitant, la quantité de sodium pénétrant dans les cellules dans le sens du gradient électrochimique augmente en présence d'hormone, et en conséquence la quantité transportée activement à travers la face séreuse. Dans cette interprétation, l'hormone n'agit pas directement sur le mécanisme de transport actif de sodium lui-même.

Nous avons recherché les effets de l'ocytocine sur les flux de sodium à travers la peau de grenouille (*Rana esculenta*), *in vitro*, dans des conditions expérimentales où le flux net de sodium est inversé. Ceci est réalisé en abaissant la concentration du sodium dans la solution externe, et en remplaçant le $Cl^-$ de la solution de Ringer par du $SO_4^{2-}$ auquel la peau est peu perméable[5]; les autres ions sont en concentrations égales dans les milieux baignant les deux faces; le courant de court-circuit est enregistré simultanément sur plusieurs éléments de surface d'une même préparation selon une technique antérieurement décrite[6]; on compare toujours deux à deux les fragments symétriques par rapport à la ligne médiane de l'abdomen. Dans un certain nombre d'expériences, les flux unidirectionnels (entrant ou sortant) de sulfate et de sodium ont été mesurés à l'aide de $^{35}SO_4^{2-}$ et de $^{24}Na^+$.

Le courant de court-circuit change de sens pour des concentrations du sodium externe ($[Na^+]$ ext.) inférieures à 1 mequiv/l.

I. Dans ce domaine de concentrations, on observe une relation linéaire entre courant de court-circuit et log de $[Na^+]$ ext.; la pente de la droite est augmentée par l'ocytocine (10 mU/ml). Il existe des valeurs de $[Na^+]$ ext. pour lesquelles le sens du courant est négatif pour le fragment témoin et positif pour le fragment traité (la Fig. 1 en donne deux exemples). Les mesures simultanées de flux montrent que le flux entrant de sodium augmente sous l'influence de l'ocytocine dans tout le domaine de $[Na^+]$ ext. étudié et rend compte à lui seul des variations observées du courant de court-circuit (*cf.* Fig. 1); par contre le flux entrant de $SO_4^{2-}$ ne montre pas de variation systématique dans les mêmes conditions.

II. Lorsque pour une concentration donnée $[Na^+]$ ext. correspondant à des courants de court-circuit négatifs, on ajoute de l'ocytocine (10 mU/ml) sur l'un des fragments, on observe que le courant de court-circuit de ce fragment devient moins