Reports

Accumulation of Reduced Pyridine Nucleotides by Illuminated Grana

The ability of illuminated chloroplasts to reduce pyridine nucleotides has previously been demonstrated indirectly by coupling the photochemical process with a suitable dehydrogenase and measuring the formation of the product of the dehydrogenase system (*1, 2*). However, up to the present time, no accumulation of reduced pyridine nucleotides by illuminated grana or chloroplasts has been reported. It has heretofore been suggested (*2, 3*) that the inability of pyridine nucleotides to undergo directly measurable photochemical reduction may be a consequence of their low oxidation-reduction potential (E_0' at *p*H 7.0 = − 0.32 v) since most substances that are effective as oxidants in the Hill reaction have high oxidation-reduction potentials (E_0' at *p*H 7.0 = + 0.1 to + 0.4 v). In this paper (*4*), data are presented which show that, under the proper conditions, photochemical reduction of pyridine nucleotides can indeed be demonstrated directly.

The results of a typical experiment are presented in Table 1 (*5*). The data clearly indicate that, when diphosphopyridine nucleotide (DPN) is incubated with grana in the light, either aerobically or anaerobically, reduced DPN (DPNH) accumulates in the reaction mixture. It can be seen that more DPNH is formed under anaerobic conditions than aerobically. Further, no reduction of DPN occurs in the dark under aerobic conditions. In separate experiments it has been established that this reduction is light-dependent even under anaerobic conditions.

Table 1. Reduction of DPN by illuminated grana aerobically or anaerobically.

Conditions	Amount of DPNH formed (μmole)
Aerobic-dark	0
Aerobic-light	6.7
Anaerobic-light	19.8

Table 2. Effect of DPN concentration.

Amount of DPN initially present (μmole)	Amount of DPNH formed (μmole)
13.3	8.1
26.6	16.7
39.9	21.8

The effect of the concentration of DPN initially present in the reaction mixture on the amount of DPNH formed is illustrated by the data presented in Table 2 (*6*). It can be seen that the amount of DPNH formed is almost directly proportional to the amount of DPN present initially. It does not appear that we have reached a saturating level of DPN even at an initial concentration of DPN of about $4 \times 10^{-3} M$.

In one experiment, which was carried out anaerobically in the light for 125 minutes, it was possible to show the accumulation of 34 μmole of DPNH, which corresponds to about 85 percent of the DPN present initially.

Triphosphopyridine nucleotide (TPN) and the acetylpyridine analog of DPN (AP-DPN) are also reduced by illuminated grana, although to a lesser extent than is DPN. When the reduction process is carried out aerobically in the light, the amounts of reduced TPN and reduced AP-DPN formed correspond to approximately 60 percent and 40 percent, respectively, of the amount of DPNH formed (*7*). Under identical conditions, nicotinamide mononucleotide is not reduced to any measurable extent. It is interesting that the amount of reduction observed with AP-DPN is less than that with DPN, even though the oxidation-reduction potential of AP-DPN is about 0.08 v more positive than that of DPN (*8*). The finding that nicotinamide mononucleotide was not reduced indicated that the reduction is an enzyme-catalyzed reaction and that the enzyme is highly specific for the intact dinucleotide structure.

The stereospecificity exhibited by the enzyme that catalyzes the reduction is different from that responsible for the oxidation of the reduced pyridine nucleotides, since deuterium from the medium is incorporated into the oxidized pyridine nucleotides by illuminated grana (*9*). The deuterium has been shown to be present at that position of the pyridine nucleotide which undergoes reversible oxidation-reduction (*10, 11*).

It has been observed that at low grana concentrations—that is, less than 0.06 mg of chlorophyll per milliliter—no measurable reduction of DPN takes place, even when the initial concentration of DPN is about $3.8 \times 10^{-3} M$. This finding might explain why previous investigators have not observed the reduction of DPN.

Recent experiments indicate that it is possible to demonstrate reduction of pyridine nucleotides under these conditions of low grana concentration, provided that a soluble extract from chloroplasts is added. A description of this extract is in preparation.

The accumulation of DPNH by illuminated grana is difficult to interpret in terms of known oxidation-reduction potentials if the reduction is a 1-quantum process. If the reduction of DPN is pictured as proceeding according to Eq. 1, then the energy required for this reaction is approximately + 52 kcal/mole.

$$DPN^+ + H_2O \rightarrow DPNH + H^+ + \tfrac{1}{2} O_2 \quad (1)$$

Since the energy content of 1 einstein (6×10^{23} quanta) of red light is about 44 kcal, it is evident that the reduction of DPN by illuminated grana would proceed only to a very small extent at *p*H 7. Arnon (12) has calculated that the energy of 1 quantum of red light is sufficient to maintain the ratio of DPNH/DPN at 10^{-5} at *p*H 7.0 and an oxygen tension of 0.2 atm. The results presented here are inconsistent with this calculation. It would appear, therefore, that the reduction of DPN by illuminated grana requires more than 1 quantum of red light. Since the reduction of DPN is a 2-electron process, it is possible that the reaction requires 1 quantum per electron transferred to the DPN. This does not necessarily imply that the reaction proceeds through a free radical intermediate, since Barltrop *et al.* (*13*) have presented a mechanism for reduction of DPN via thioctic acid which requires 2 quanta of red light per molecule of DPNH formed.

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4. Contribution No. 142 of the McCollum-Pratt Institute. This investigation was supported by a research grant [RG-4143 (C)] from the National Institutes of Health, U.S. Public Health Service. We wish to express our gratitude to S. P. Colowick for many helpful suggestions.
5. The "grana" were prepared as follows: 25 g

of spinach leaves were deveined and ground for 3 min in a Waring Blendor with 200 ml of cold 0.01*M* Na_2HPO_4-KH_2PO_4 buffer of *p*H 7, containing 0.01*M* KCl. After filtering through cheesecloth, the filtrate was centrifuged for 2 min at 4600*g*. The supernatant was centrifuged for 20 min at 18,000*g*; the residue was suspended in buffer and centrifuged again at 18,000*g*. The final residue was made up in 0.05*M* Na_2HPO_4-KH_2PO_4 buffer of *p*H 7, containing 0.01*M* KCl. No attempt has been made to ascertain that this preparation consists solely of grana. Each reaction mixture contained grana equivalent to 4.92 mg of chlorophyll, 38.1 μmole of DPN, 500 μmole of Na_2HPO_4-KH_2PO_4 buffer of *p*H 7.05, and 100 μmole of KCl. The final volume was 10 ml. The dark control flask was wrapped with tinfoil. The anaerobic flask was evacuated continuously with a water aspirator, and the aerobic flask was open to air. The flasks were incubated for 10 min in the dark and for 60 min in the light (one 100-w bulb per flask at a distance of approximately 6 in.) with shaking at 13 to 14°C. After incubation, the samples were centrifuged to remove the grana, and the DPNH concentration in the supernatant solution was determined by measuring the decrease in optical density at 340 mμ upon the addition of acetaldehyde and yeast alcohol dehydrogenase.

6. Each reaction mixture contained grana equivalent to 4.34 mg of chlorophyll, DPN, 500 μmole of Na_2HPO_4-KH_2PO_4 buffer of *p*H 6.98, and 100 μmole of KCl. The final volume was 10 ml. The flasks were incubated aerobically for 80 min in the light with shaking at 12°C. After incubation, the samples were treated as indicated in Table 1.
7. Reduced TPN and reduced AP-DPN were estimated spectrophotometrically from their absorption at 340 mμ and 365 mμ, respectively. The extinction coefficient of reduced AP-DPN has been determined by N. O. Kaplan and M. M. Ciotti [*J. Biol. Chem.*, in press].
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"Adenoviruses": Group Name Proposed for New Respiratory-Tract Viruses

The discovery of a new group of viruses, affecting primarily the respiratory tract, has led to the need for a meaningful, specific, and acceptable name for these agents, both as viruses and in relation to diseases with which they are associated. In the first published report of the isolation of these viruses, Rowe and his associates (*1*) used the term *adenoid degeneration agent*, abbreviated as *A.D. agent*. The 13 strains reported at that time were recovered from human adenoids removed surgically and cultivated in tissue culture. Independently, Hilleman and Werner (*2*) reported the isolation of five agents, termed *Respiratory illness (RI) agents*, during an epidemic of acute respiratory disease (ARD) and pneumonitis among recruits at Fort Leonard Wood, Mo. One of these agents (strain RI-67) was shown by complement-fixation and neutralization tests to be etiologically associated with the epidemic disease (*2*). Confirmation and expansion of their findings soon followed (*3–5*). Further information was rapidly acquired (*6–22*), indicating that these viruses comprised a family or group and that they were related to several clinical syndromes. Huebner and his associates (*6*) proposed the term *adenoidal-pharyngeal-conjunctival (APC)* agents or viruses as the group name.

The problem of terminology was discussed informally for some months by investigators and others interested in the field at the National Institutes of Health, at Walter Reed Army Institute of Research, at several universities both in this country and abroad, and by members of such groups as the Scientific Advisory Committee of the Common Cold Foundation. These discussions culminated in a meeting in New York City on 25 May 1956 of the undersigned representatives of the early investigators in the field and others interested in seeking a satisfactory solution to the problem.

Agreement was reached on the term *adenovirus group*, which suggests a characteristic involvement of lymphadenoid tissue (*23*), as well as the tissue of the first reported isolation, and is in accordance with the proposals concerning nomenclature of the Subcommittee on Viruses of the International Nomenclature Committee (*24, 25*). For the present, members of the group would be indicated by serotype numbers in accordance with the classification of Huebner *et al.* (*6, 9*). Thus far, 12 types have been reported from human and two from simian sources (*9*). Information is not yet available to permit a completely detailed description of the adenovirus group. The viruses at present included in this group, however, have the following characteristics. (i) They produce acute infection of respiratory and ocular mucous membranes with associated follicular enlargement of submucous lymphadenoid tissue in these areas and also of the regional lymph glands. Virus has frequently been isolated from adenoid or tonsillar tissues from persons without clinical signs of acute illness. (ii) Multiplication in tissue culture of certain types of human and simian cells takes place readily and leads to increased acid formation and distinctive cytopathic changes. As is shown by electron micrographs, the nuclei of virus-infected cells may contain symmetrical arrays of virus-like particles. (iii) An antigen unique to this group, demonstrable in the complement-fixation test, is shared by members of the family. (iv) Antigenic type specificity is demonstrable by the neutralization test. (v) No strain as yet has produced manifest illness in commonly used laboratory animals.

With respect to terminology of the diseases caused by these viruses, it is proposed that the usual practice be followed of employing a clinical diagnostic term followed by etiological identification, such as, for example, acute respiratory disease (ARD) caused by adenovirus type 4; pharyngitis or pharyngoconjunctival fever caused by adenovirus type 3; follicular conjunctivitis caused by adenovirus type 6; keratoconjunctivitis caused by adenovirus type 8; or pneumonitis or atiypical pneumonia caused by adenovirus type 7. The use of such terminology will eliminate confusion that might arise from the facts that a single serotype can produce clinically different diseases and, conversely, that clinically similar illnesses may be produced by different adenovirus serotypes as well as by unrelated agents.

In making the foregoing proposals regarding terminology, the undersigned realize that they have no official status conferred by any national or international body dealing with nomenclature. They have, however, found that the term *adenovirus group* is acceptable among the investigators most concerned. Accordingly, it is suggested that this designation be generally employed in the interest of avoiding further confusion in the literature until ultimately a satisfactory nomenclature can be established for viruses.

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