crease in the serum lipids (7). In the case of heparin, the electrophoretic changes and the reduction in the serum lipids are produced by the action of a "clearing factor" that is a heparin-activated lipoprotein lipase (8). It remains to be determined whether the infusion of a fat emulsion also activates this lipoprotein lipase or has a different mode of action.

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Pyridine Nucleotide Analogs and the Sulfhydryl Nature of Some FAD Enzymes

It has recently been demonstrated by Kaplan and Ciotti (1) that pig brain DPNase will catalyze an exchange between the nicotinamide moiety of DPN (2) or TPN and 3-AP, resulting in the formation of APDPN or APTPN. These analogs have been shown to be active in a number of dehydrogenase systems (3). The average potential of APDPN/APDPNH has been found to be approximately 0.08 v more positive than the DPN/DPNH system. It was therefore of interest to determine whether flavin enzymes could catalyze a transfer of hydrogen or electrons from DPNH or TPNH to the respective 3-AP analogs (4).

All measurements were performed using a Beckman DU spectrophotometer with 3.0-ml cuvettes having a 1.0-cm light path, and all reactions were run at room temperature. APDPNH and APTPNH formation was determined by an increase in optical density at their maximum extinction at 365 mμ and at 400 mμ, where they have significant absorption in contrast to DPNH and TPNH.

Reaction mixtures contained 50 μmoles of phosphate buffer, *p*H 7.5, and 50 μmoles of trisodium citrate dihydrate. In addition, the diaphorase reaction mixtures contained 0.25 μmoles of DPNH, 0.6 μmoles of APDPN, and 0.09 mg of enzyme protein in a total volume of 3.0 ml. The cytochrome-*c* reductase reaction mixtures contained 0.22 μmoles of TPNH, 0.53 μmoles of APTPN, and 0.2 mg of enzyme protein in a total volume of 3.0 ml. Additions of *p*CMB and GSH are noted in Table 1 as final molar concentrations. When *p*CMB was used it was incubated with the enzyme in the phosphate buffer for 5 minutes at 0°C. In measuring GSH reversal of *p*CMB inhibition, GSH was incubated for another 5-minute period before testing. All reactions were started with APDPN for the diaphorase and APTPN for the cytochrome-*c* reductase.

It was found that Straub's DPNH diaphorase (5), a FAD enzyme from pig heart that catalyzes the reduction of dye and inorganic iron (6) also catalyzes a transfer of hydrogen or electrons from DPNH to APDPN, as is indicated by Eq. 1.

$$\text{DPNH} + \text{APDPN}^+ \rightarrow \text{DPN}^+ + \text{APDPNH} \quad (1)$$

This transfer occurred only in the presence of DPNH and APDPN. TPNH and APTPN would not serve as electron donor or acceptor, respectively, with the diaphorase. Investigation of the involvement of sulfhydryl groups in this reaction, as shown in Table 1, revealed that *p*CMB inhibited the transfer reaction. This inhibition could be reversed with GSH. The catalysis of dye reduction by this enzyme was also inhibited by *p*CMB. GSH reversal was not attempted, since the dye is reduced nonenzymatically by GSH.

TPNH cytochrome-*c* reductase (7), a FAD enzyme from pig liver, also catalyzes this transfer reaction, as is given by Eq. 2.

$$\text{TPNH} + \text{APTPN}^+ \rightarrow \text{TPN}^+ + \text{APTPNH} \quad (2)$$

However, with this enzyme, DPNH and APDPN would not serve as electron donor and acceptor, respectively, but required TPNH and the corresponding analog APTPN for transfer. As can be seen in Table 1, *p*CMB inhibited the transfer reaction, and the inhibition was reversed by GSH. Since this enzyme catalyzes the transfer reaction at a much slower rate than the diaphorase, a 60-minute period was used to determine the formation of APTPNH rather than the 3 minutes employed for the diaphorase. The reduction of cytochrome *c* was also inhibited by *p*CMB.

Table 1. Inhibition by *p*CMB of diaphorase and TPNH cytochrome-*c* reductase catalyzing the reduction of APDPN by DPNH and APTPN by TPNH, respectively, and the reversal by GSH.

Reaction mixtures	Diaphorase (μmoles APDPNH formed in 3 min)	TPNH cytochrome-*c* reductase (μmoles APTPNH formed in 60 min)
Control	0.229	0.172
Plus *p*CMB ($2 \times 10^{-4} M$)	0	0
Plus *p*CMB ($2 \times 10^{-4} M$) plus GSH ($10^{-3} M$)	0.234	0.172
Plus GSH ($10^{-3} M$)	0.233	0.172
Minus enzyme plus GSH ($10^{-3} M$)	0	0
Minus DPNH or TPNH plus GSH ($10^{-3} M$)	0	0

These results demonstrate that, in all probability, sulfhydryl groups are involved in the catalytic properties of DPNH diaphorase and TPNH cytochrome-*c* reductase.

Other FAD enzymes such as pig heart DPNH cytochrome-*c* reductase (8), *Neurospora* TPNH nitrate reductase (9), milk xanthine oxidase, and the DPNH oxidase from *Clostridium kluyveri* (10) catalyze these transfer reactions, and demonstrate specificity toward both the reduced pyridine nucleotide and acceptor analog. Nonflavin dehydrogenases and FMN enzymes tested did not appear to catalyze this reaction.

Preliminary experiments indicate that, although only the FAD enzymes tested were active, FAD does not appear necessary for this transfer reaction. In view of this, it is of interest to speculate that FAD enzymes are in such configuration that the reduced pyridine nucleotides can transfer hydrogen or electrons directly to the protein. This reduced protein can then transfer hydrogen or electrons to the specific pyridine nucleotide analog without the involvement of flavin as an intermediate. In reactions involving reduction of dye or cytochrome *c*, the reduced protein would transfer hydrogen or electrons to FAD and then on to dye or cytochrome.

It is of interest to note that, although all the FAD enzymes studied appear to be sulfhydryl enzymes, as demonstrated by *p*CMB inhibition of reactions involving the reduction of dye and cytochrome *c*, they all were not inhibited by *p*CMB in catalyzing the transfer reaction. The transfer reactions from TPNH to APTPN catalyzed by nitrate reductase and from DPNH to APDPN catalyzed by the DPNH oxidase from *Cl. kluyveri*, respectively, were not inhibited by *p*CMB. Milk xanthine oxidase catalysis of hypoxanthine oxidation by oxygen and dye is inhibited by *p*CMB (*11*). When hypoxanthine and APDPN are used as electron donor and acceptor, respectively, inhibition of the reduction of APDPN is accomplished with *p*CMB and reversed with GSH. If, however, DPNH is used as electron donor, dye reduction and the transfer reaction are not inhibited by a concentration of *p*CMB, which would inhibit when hypoxanthine was used as electron donor. This is interesting in view of the belief of Mackler *et al.* (*12*) that all reactions catalyzed by their xanthine oxidase preparations are attributable to one protein.

The involvement of a reduced protein in flavoprotein reactions is now under investigation.

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International Cooperation in Radiobiology through an Agency Sponsored by the United Nations

The following reasons for international cooperation in radiobiology may be considered.

1) The running of numerous power reactors in many countries is going to increase the radioactivity of the atmosphere, the soil, and the waters. It is difficult to establish which level of constant radioactivity is dangerous for man and animals; concentrations of 1/500,000 of phosphorus-32 in fishes (as compared with water) were reported at the Geneva Conference on the Peaceful Uses of Atomic Energy. Possible damages are not limited to the country where the reactor is located; this fact may be the origin of bitter international discussions if measures are not taken beforehand.

The carcinogenic and genetic effects of ionizing radiations on nonhomogeneous populations are unknown and theoretically unpredictable. Different types of research are already contemplated to solve this question; these projects should be discussed on an international basis because they are extremely costly and time consuming.

On the genetic problem of irradiated human populations, T. C. Carter said (A/Conf. 8/P/449), "We now need a research program with three main parts: fundamental studies of mutation; studies of animal populations; and studies of human populations. Such a program would have to be on a very lavish scale and parts of it would almost certainly require international cooperation."

2) Biologists have a great responsibility in the development of peaceful uses of atomic energy. Physicists and industrialists must not disregard the warnings of the biologists despite the fact that these warnings may tend to put limits to their activity.

Some people have interest in emphasizing the biological dangers of radioactivity; others have interest in neglecting them. Margins of safety must be established and constantly revised not only by scientists meeting around a table once a year, but also by their actually working together.

3) Basic discoveries in radiobiology may have important consequences for the generalized use of atomic energy. For instance, the possibility exists of increasing, by chemical substances, the resistance of man to ionizing radiations. The phenomenon of chemical protection against these radiations has been repeatedly demonstrated in animals. Efficient treatment (actually lacking) of accidentally irradiated human beings depends entirely on active pursuit of promising researches in animals. International agreement should be reached before the use of a protector or a treatment is widely advocated. Controls of the experiments and of the substances themselves should be put on an international basis.

4) Countries that do not like to depend on big atomic powers would find in an International Laboratory of Radiobiology a suitable place for obtaining information and training for their scientists.

5) The spirit of collaboration that was prevalent during the Geneva Conference on the Peaceful Uses of Atomic Energy should be perpetuated by the presence, in the same International Laboratory, of biologists from many countries. One may hope that international cooperation would speed up biological research, thus enabling us to keep up with the industrial development of atomic energy and prevent irreparable damage to the human race.

As shown by the Geneva Conference, the whole human race is involved in the widespread use of atomic energy. Many basic biological data are not yet available that would enable us to appreciate the dangers and the possibilities of overcoming them either by protection or by therapeutics. It seems to be the duty of an International Atomic Agency to have at its disposal a body of biologists who are organized in some kind of international institution where facilities for laboratory work would be available.

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Since the foregoing note was received, the United Nations has established a Scientific Committee on the Effects of Atomic Radiation, which met in New York 14–23 March 1956. The recommendations of the committee, which include many of the suggestions made by Z. M. Bacq, were released 9 April 1956 and will be summarized in the 25 May issue of Science.

Myo-Inositol as an Essential Growth Factor for Normal and Malignant Human Cells in Tissue Culture

It has been shown (*1*) that two mammalian cells, a human carcinoma of the cervix (strain HeLa) and a mouse fibroblast (strain L) can be propagated in a medium embodying 13 amino acids, seven vitamins, five salts, glucose, and a varying amount of serum protein, the latter supplied either as whole or dialyzed serum. Each of these components was demonstrably essential for survival and growth. It was subsequently found that a number of other human cell lines, both normal and malignant, could be