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.

MORTON M. WEBER*
NATHAN O. KAPLAN
McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Maryland

References and Notes

1. N. O. Kaplan and M. M. Ciotti, *J. Am. Chem. Soc.* 76, 1713 (1954); N. O. Kaplan, and M. M. Ciotti, *J. Biol. Chem.*, in press.
2. The following abbreviations are used: DPN and DPNH, oxidized and reduced diphosphopyridine nucleotide, respectively; TPN and TPNH, oxidized and reduced triphosphopyridine nucleotide, respectively; 3-AP, 3-acetyl pyridine; APDPN and APDPNH, oxidized and reduced 3-acetyl pyridine analog of DPN, respectively; APTPN and APTPNH, oxidized and reduced 3-acetyl pyridine analog of TPN, respectively; FMN and FAD, flavin mononucleotide and flavin adenine dinucleotide, respectively; *p*CMB, *p*-chloromercuribenzoate; GSH, glutathione.
3. N. O. Kaplan, M. M. Ciotti, F. E. Stolzenbach, *J. Biol. Chem.*, in press.
4. Contribution No. 136 of the McCollum-Pratt Institute. This investigation was aided by grants from the American Cancer Society, as recommended by the Committee on Growth of the National Research Council, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service, grant No. C-2374(C).
5. F. B. Straub, *Biochem. J. London* 33, 787 (1939).
6. M. M. Weber, H. M. Lenhoff, N. O. Kaplan, *Biochim. et Biophys. Acta* 14, 298 (1954).
7. B. L. Horecker, *J. Biol. Chem.* 183, 593 (1950).
8. H. R. Mahler *et al.*, *ibid.* 199, 585 (1952).
9. A. Nason and H. J. Evans, *ibid.* 202, 655 (1953).
10. M. M. Weber and N. O. Kaplan, *Bact. Proc.* (1954), p. 96.
11. J. Harris and L. Hellerman, *Federation Proc.* 12, 215 (1953).
12. B. Mackler, H. R. Mahler, D. E. Green, *J. Biol. Chem.* 210, 149 (1954).

* Postdoctoral fellow in cancer research of the American Cancer Society.

27 February 1956

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.

Z. M. BACQ
University of Liège, Liège, Belgium

25 October 1955

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

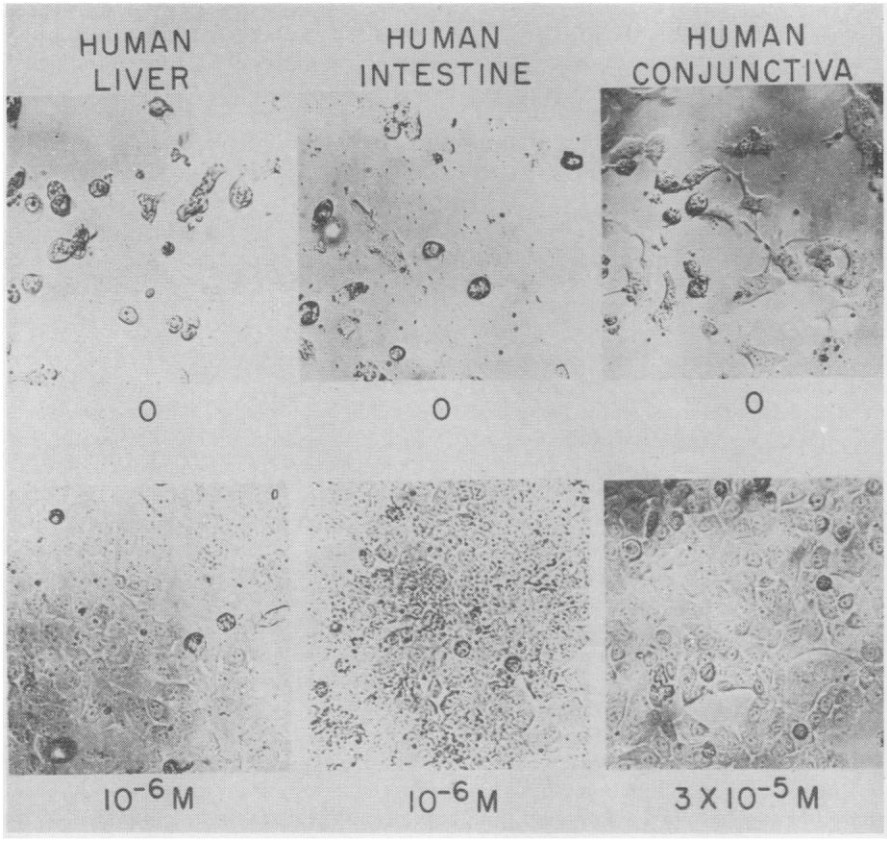

Fig 1. The early death of three normal human cells in the absence of inositol, and their normal growth on its addition to the medium.

Table 1. The inositol requirement of 19 cell lines in tissue culture.

Tissue of origin	Strain identification	Inositol requirement	ED_{50},* M	Maximally effective concn., M
Human carcinoma of cervix	HeLa (Gey)	0 (?)		
Human monocytic leukemia	J-111 (Osgood)	0 (?)		
Mouse fibroblast	L-929 (Earle)	0		
Human epidermoid carcinoma of throat	KB (Eagle)	+	2×10^{-6}	10^{-5}
Human bone marrow (w/metastatic cancer)	Detroit 6 (Stulberg)	+	3×10^{-7}	$1\text{–}3 \times 10^{-6}$
Human carcinomatus ascites	Detroit 30a (Stulberg)	+	3×10^{-7}	$1\text{–}3 \times 10^{-6}$
Human bone marrow (w/metastatic cancer)	Detroit 32 (Stulberg)	+	$3 \times 10^{-7} \pm$	10^{-6}
Human bone marrow (normal)	Detroit 32 (Stulberg)	+	3×10^{-7}	10^{-6}
Human carcinomatus ascites	Detroit 56a (Stulberg)	+	$3 \times 10^{-7} \pm$	10^{-6}
Human malignant lymphoma (cells from pleural fluid)	Detroit 116P (Stulberg)	+	$3 \times 10^{-7} \pm$	$1\text{–}3 \times 10^{-6}$
Human bone marrow (w/metastatic cancer)	Detroit 34 (Stulberg)	+	$3 \times 10^{-7} \pm$	10^{-6}
Human liver	Chang	+	3×10^{-7}	10^{-6}
Human liver	Henle	+	2×10^{-7}	10^{-6}
Human embryonic intestinal epithelium	Henle	+	2×10^{-7}	10^{-6}
Human conjunctiva	Chang	+	3×10^{-7}	10^{-6}
Human foreskin	Leighton	+	3×10^{-7}	10^{-6}
Human embryonic fibroblast	MAF 66 (Microbiological Assoc.)	+	$10^{-6} \pm$	3×10^{-6}
Mouse adenocarcinoma ("Sarcoma")	S-180	+	2×10^{-7}	10^{-6}
Human bone marrow (normal)	Detroit 98 (Stulberg)	+	$3 \times 10^{-7} \pm$	10^{-6}

* Concentration permitting 50 percent of maximum growth.

propagated in the same basal medium, but only when it was supplemented with 10 percent whole human serum; when the latter was replaced by dialyzed serum, most of these cell strains died, either in the very first passage or on subdivision. When the dialyzed serum was supplemented with an equivalent amount of serum ultrafiltrate, normal growth was obtained. The dialyzable factor of serum required by these cells, over and above the 26 essential components previously identified, could be wholly replaced by myo-(meso-)inositol and presumably was either the compound or a dialyzable derivative of it.

A total of 19 cell lines (17 human and two mouse) have now been studied with respect to their inositol requirement (see Table 1). Seven cultures derived from cancer tissue, nine derived from normal tissue, and three cultures were obtained from the bone marrow of patients with metastatic carcinoma. Of these 19 cell lines, the nine that are listed in the bottom section of Table 1 failed to grow even on the first subculture in an inositol-free medium (see Fig. 1). The seven lines listed in the middle section of the table grew for a period of 5 to 7 days but regularly died on subculture. With the addition of inositol, however, all 16 lines grew at a normal rate, and all continued to grow at a normal rate on serial subculture. The contrast between the death of these cells in an inositol-deficient medium and their rapid growth in the same medium supplemented with inositol is illustrated for three normal cells in Fig. 1. The amounts of inositol required by these 16 cell lines were remarkably consistent, the concentrations necessary for maximum growth varying between 10^{-5} and $10^{-6} M$ (Table 1).

Of the three remaining cell lines, the mouse fibroblast has to date given no indication of an inositol requirement and has been carried for months in an inositol-free medium, supplemented with 1 percent dialyzed serum. Inconsistent results have, however, been obtained with the remaining two cell lines (HeLa strain, human leukemia J-111). In several experiments these two strains were propagated for 6 to 8 weeks in an inositol-free medium, maintaining an average generation time of approximately 48 hours. Occasionally, however, the HeLa cell has failed to give sustained growth in a medium supplemented with dialyzed serum unless inositol was added; and similarly inconsistent results have been obtained with the J-111 leukemia cell.

Inositol has been shown to be essential for the survival and growth of a number of microorganisms (*2*). However, as Schopfer (*3*) has pointed out, although myo-inositol was perhaps the first of the microbial growth factors to be discovered, it is one of the last to be identified as a vitamin in animal metabolism.

Its metabolic function remains obscure, although it is suspected to have a lipotropic effect independent of that of choline (*4*).

Inconsistent results have been obtained in attempts to produce symptoms of inositol deficiency in animals. In mice, alopecia was produced in approximately half the animals (*5*); and in rats also inositol caused a positive growth response and prevented and cured a generalized alopecia (*6*). More recently, however, inositol was not found to have a demonstrable effect in the growth of rats on an amino acid-sucrose-corn oil-salt-vitamin diet (*7*). Similarly conflicting results have been reported with respect to its possible relationship to the "spectacle eye" syndrome in rats (*8*). A growth-promoting effect has been noted in cotton rats (*9*); and the omission of both inositol and PABA from the diet of golden hamsters led to the death of some of the animals (*10*). In man there has been no evidence of an inositol requirement.

The demonstration of its vitamin function in animals or man is complicated by its possible production by the intestinal flora (*11*) and by its partial biosynthesis (*12*) and is further complicated by the reported dependence of its effects on the presence or absence of other B vitamins (*13*).

Under these circumstances, the present demonstration that inositol is an essential growth factor for a wide variety of human cell lines in tissue culture is of particular significance. The effective concentrations, higher than those of most of the other essential vitamins, are of the same order of magnitude as the choline requirement of the mouse fibroblast. Whether inositol is used as a metabolite —for instance, for incorporation into phospholipides—or whether it functions as a vitamin remains to be determined, as does the degree to which it can be replaced by its isomers or by related compounds. To date, no compound has been found capable of substituting for myo-inositol.

Contrary to some of the results obtained in animals, the presence of pantothenic acid in the medium did not obviate the need for inositol, and the addition of PABA similarly had no qualitative or quantitative effect. It is of interest also that, although inositol has been reported to have a carcinolytic action in experimental animals (*14*), in the present experiments it was essential for the growth of five of the seven tumor lines tested.

When inositol was added to the 26 essential factors previously identified, every cell line here described could be grown for long periods in a medium supplemented only with serum protein. It thus becomes possible to determine the specific amino acid and vitamin requirements of all these human cell lines, deriving from a variety of normal and malignant tissues, with a view to ascertaining possible quantitative or qualitative differences.

HARRY EAGLE
VANCE I. OYAMA
MINA LEVY
AARON FREEMAN

Section on Experimental Therapeutics, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda 14, Maryland

References

1. H. Eagle, *J. Biol. Chem.* **214**, 839 (1955); H. Eagle, *J. Exptl. Med.* **102**, 37, 595 (1955); H. Eagle *et al.*, *J. Biol. Chem.*, in press; H. Eagle, *Arch. Biochem. and Biophys.*, in press; *Proc. Soc. Exptl. Biol. Med.*, in press.
2. E. V. Eastcott, *J. Phys. Chem.* **32**, 1094 (1928); R. J. Williams, R. E. Eakin, E. E. Snell, *J. Am. Chem. Soc.* **62**, 1204 (1940); G. W. Beadle, J. Biol. Chem. **156**, 683 (1944).
3. W. H. Schopfer, *Bull. soc. chim. biol.* **33**, 1113 (1951).
4. G. Gavin, E. W. McHenry, *J. Biol. Chem.* **139**, 485 (1941); J. C. Abels *et al.*, *Proc. Soc. Exptl. Biol. Med.* **54**, 157 (1943); G. Gavin, J. M. Patterson, E. W. McHenry, *J. Biol. Chem.* **148**, 275 (1943).
5. D. W. Woolley, *J. Nutrition* **28**, 305 (1944).
6. T. J. Cunba *et al.*, *Proc. Soc. Exptl. Biol. Med.* **54**, 236 (1943).
7. M. H. McCormick, P. N. Harris, C. A. Anderson, *J. Nutrition* **52**, 337 (1954).
8. P. L. Pavcek and H. M. Braun, *Science* **93**, 502 (1941); E. Nielson and C. A. Elvejhem, *Proc. Soc. Exptl. Biol. Med.* **48**, 349 (1941).
9. J. M. McIntire, B. S. Schweigert, C. A. Elvejhem, *J. Nutrition* **27**, 1 (1944).
10. J. M. Cooperman, H. A. Waisman, C. A. Elvejhem, *Proc. Soc. Exptl. Biol. Med.* **52**, 250 (1943).
11. D. W. Woolley, *J. Exptl. Med.* **75**, 277 (1942).
12. J. W. Halliday and L. Anderson, *J. Biol. Chem.* **217**, 797 (1955); W. H. Daughaday, J. Larner, C. Hartnett, *ibid.* **212**, 869 (1955).
13. Literature recently summarized by E. R. Weidlein, *The Biochemistry of Inositol*, Bibliographic Ser., Bull. No. 6 of the Mellon Institute, Pittsburgh, Pa.
14. M. L. Hesselbach and D. Burk, *Record Chem. Progr. Kresge-Hooker Sci. Lib.* **5**, 37 (1944); D. Laszlo and C. Leuchtenberger, *Science* **97**, 515 (1943).

6 March 1956

Biochemical Action of CDAA, a New Herbicide

One of the more active herbicides that has been discovered since the introduction of 2,4-dichlorophenoxyacetic acid is α-chloro-*N,N*-diallylacetamide (CDAA) (*1*). This herbicide, which has only recently been announced, is grass-specific and preemergent. It is also extremely selective, affecting only certain members of the monocotyledons and dicotyledons. For example, when it is applied at the appropriate dosage, it will destroy giant foxtail and pigweed in either corn or soybean fields without injuring the crop.

The mode of action of such a selective herbicide is of sufficient interest to warrant studies of the respiratory processes in moderately susceptible and susceptible plants (wheat and ryegrass, respectively). Germinating seeds of wheat and ryegrass were treated with CDAA at 10 ppm in the absence and presence of sulfhydryl-containing compounds. The data in Table 1 on oxygen uptake, changes in respiratory quotients, and growth show that ryegrass seeds are more susceptible to CDAA than wheat seeds. This finding is in agreement with results obtained from greenhouse studies. The possibility exists that the site and/or mode of action of CDAA in wheat is different than it is in ryegrass since the respiratory quotient of wheat was increased in the presence of this herbicide, whereas the respiratory quotient of ryegrass was markedly decreased.

The reversal studies suggest that sensitive sulfhydryl enzymes in both wheat and ryegrass are inhibited by CDAA, and this reaction could be related to the classical alkylation of sulfhydryl groups by iodoacetate and iodoacetamide (*2–3*). The reversal produced by calcium pantothenate was attributed to its conversion to coenzyme A. Reversal studies with coenzyme A per se were not significant; however, this may have been caused by an inability of the nucleotide to penetrate the seed at the proper site.

Since the growth of wheat and ryegrass seedlings was almost completely inhibited despite the observed effects on the respiratory quotients and respiration rates, a simple detoxification of CDAA by inter-

Table 1. Influence of α-chloro-*N,N*-diallylacetamide (CDAA) on the respiration, respiratory quotients, and growth of germinating wheat and ryegrass seeds. The respiratory quotients were determined according to the "direct method" of Warburg (*3*).

Compound* and concn. (ppm)	O_2 uptake [μlit/hr 100 mg of tissue (wet wt.)]	Respiratory quotient	Cotyledonary growth after 120 hr (mm)
Wheat			
Control	49.0	0.96	16.9
CDAA (10)	38.0	1.12	0.8
GSH (15)	42.0		15.5
CDAA (10) + GSH (15)	45.0		1.0
Ryegrass			
Control	16.0	1.00	21.3
CDAA (10)	3.4	0.62	0
GSH (15)	17.4	0.96	20.6
CDAA (10) + GSH (15)	18.8	0.94	0
Ca-pan (15)	16.8	1.02	19.3
CDAA (10) + Ca-pan (15)	11.0	1.05	0
α-lipoic acid (4)	16.0	1.02	21.5
CDAA (10) + α-lipoic acid (4)	11.5	1.07	0

* The abbreviations represent the following: GSH, reduced glutathione; Ca-pan, calcium pantothenate.