living portion of *Millepora* colonies (the coenosarc) is restricted to the superficial layers of the skeleton (*6*). Individual polyps are sealed off from the inner part of the skeleton by a series of complete tabulae, and, although a system of organic filaments occupies minute canals in the outer layers of the skeleton, these filaments degenerate below the surface, leaving the canals open (Fig. 1B). Oxygen produced by zooxanthellae would have no direct access to the internal skeleton and therefore would not be trapped inside.

On the other hand, the architecture of the *Millepora* skeleton is such that it could easily trap gas produced internally, which may explain why this is the only coral in which this phenomenon has been found to date. The central region is occupied by large, open pores. This is surrounded by the coenosteum, in which the polyps are located. The coenosteum is highly porous but has little communication with the surrounding seawater. In addition, it has a dense peripheral rim. The *Millepora* skeleton therefore has a very high porosity but relatively low permeability. In contrast, scleractinian skeletons have higher permeabilities, and any gases produced inside them can easily diffuse into the surrounding seawater.

Millepora skeletons collected for detailed morphological analysis showed phenomenally high incidences of infestation by boring algae (Fig. 1C). Infestation was highest near the periphery, and in fresh specimens it is evidenced by an intense green rim just below the surface, but algal borings were present throughout the colonies (Fig. 2). Borehole diameters were variable (between about 6 and 20 μm). It is likely that photosynthesis by these algae produced the O_2, causing N_2 to come out of solution in the internal water to equilibrate with the O_2. The origins of the trace gases are uncertain, but the CO_2 is probably respiratory.

How much, if any, of this O_2 escapes to the surrounding seawater is unknown. Much of it could be used by the endoliths in nighttime respiration. By midmorning, however, significant amounts of gas are usually present in the skeletons. Conservative rough estimates of the amount of gas trapped inside *Millepora* colonies by late afternoon (*7*) suggest that the reservoir of O_2 at -10 m is at least 300 ml per square meter of colony vertical projection area.

This gas has been found in *Millepora* heads on the Great Barrier Reef from Lizard Island in the north at least as far south as the fringing reefs of the central region, and it is likely that the phenomenon is even more widespread. There are wider implications to the findings: if endolithic algae in coral heads can produce large quantities of O_2, their contribution in other substrates may be considerable. Boring algae are ubiquitous in shallow marine carbonates and are especially abundant in tropical regions (*8*). It is possible that they could affect the O_2 and CO_2 concentrations of overlying waters and hence influence measurements of reef productivity. Future investigators of reef dynamics who are engaged in analyses of dissolved gases should make every effort to assess the contribution by endolithic algae.

NIGEL BELLAMY
MICHAEL J. RISK*
Australian Institute of Marine Science, Private Mail Bag No. 3, Townsville Mail Sorting Office, Queensland 4810

References and Notes

1. R. E. Johannes and Project SYMBIOS team, *BioScience* **22**, 541 (1972); B. D. Scott and H. R. Jitts, *Mar. Biol.* **41**, 307 (1977); S. V. Smith, *Limnol. Oceanogr.* **18**, 106 (1973).
2. H. T. Odum and E. P. Odum, *Ecol. Monogr.* **25**, 291 (1955); D. W. Kinsey and B. E. Kinsey, *Aust. J. Mar. Freshwater Res.* **18**, 23 (1967).
3. P. S. Davies, in *Proceedings of the Third International Coral Reef Symposium*, D. L. Taylor, Ed. (Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Fla., 1977), vol. 1, p. 392; E. H. Gladfelter and R. K. Monahan, in *ibid.*, vol. 2, p. 390.
4. We collected the gas underwater by breaking coral branches under an inverted glass funnel connected to a separatory funnel. The gas was transferred underwater to prerinsed (distilled water and then seawater) 5-ml Teflon septum glass vials. Only minute amounts of seawater were included with the samples. Samples were refrigerated immediately after extraction. Analyses were performed at the Queensland Government Chemical Laboratories in Brisbane; in the analyses a Hewlett-Packard 5840 gas chromatograph fitted with a thermal-conductivity detector was used.
5. B. Roffman, *Comp. Biochem. Physiol.* **27**, 405 (1968); A. Svoboda, in *Physiology and Behaviour of Marine Organisms*, D. S. McLusky and A. J. Berry, Eds. (Plenum, New York, 1980), p. 381.
6. H. Boschma, in *Treatise on Invertebrate Paleontology*, vol. F, *Coelenterata*, R. C. Moore, Ed. (University of Kansas, Lawrence, 1956), p. F90.
7. Whole *Millepora* fronds covering a vertical projection area of about 50 cm^2 were broken out of larger colonies and placed inside plastic bags. Working at the same depth as that at which the coral grew, we placed the bagged heads on a plywood board and bashed them with hammers to produce the maximum coral destruction and gas release consistent with a minimum number of holes punctured in the bags. (Gas volumes as produced are therefore underestimates of the total volume present.) Gas was extracted from the bags underwater with a hypodermic syringe. Volumes were read underwater directly from the syringe and were not corrected to sea level.
8. S. Golubic, *Am. Zool.* **9**, 747 (1969); W. S. Rooney and R. D. Perkins, *Geol. Soc. Am. Bull.* **83**, 1139 (1972); J. H. Schroeder, *Neues Jahrb. Geol. Palaeontol. Monatsh.* **1**, 16 (1972); D. R. Kobluk and M. J. Risk, *J. Exp. Mar. Biol. Ecol.* **27**, 107 (1977).
9. We thank R. Nordberg, T. Peters, and S. Tudhope for field assistance and the captains and crews of R.V. *Lady Basten* and R.V. *Sirius*. A. Nott helped to obtain analytical results. The scanning electron microscopy was performed at James Cook University of North Queensland, with the help of J. Darley. L. Brady provided valuable photographic aid. We thank B. Chalker, J. Bunt, and E. Drew who read an earlier version of this report. Supported by the Australian Institute of Marine Science, the Natural Sciences and Engineering Research Council of Canada, and the International Development Research Centre, Ottawa.
* Permanent address: Department of Geology, McMaster University, Hamilton, Ontario, Canada L8S 4M1.

6 October 1981

Proliferative Capacity of Murine Hematopoietic Stem Cells in vitro

Abstract. *Large numbers of granulocytes can be collected repeatedly from the supernatant medium of long-term cultures of mouse bone marrow cells. A constant relationship was found between the number of adherent hematopoietic stem cells and the lifetime cell production per culture. The data indicate that there is a limit to the proliferative capacity of normal and of irradiated stem cells. A similar limitation was found in the production of marked granulocytes from clonal cultures of "beige" C57 (*bg/bg^J*) stem cells placed in limiting dilutions into stromal culture layers.*

Long-term cultures of mouse bone marrow cells maintain stem cell differentiation and undergo extensive self-renewal (*1*). Hematopoiesis is established on the flask bottom, where a stromal network supports the formation of large, confluent, granulopoietic aggregates (*2*). As they mature, the flat, spread granulocytes round up and become suspended in the culture medium. If the system's longevity is tested by repeated harvesting of the nonadherent cells, one observes three phases which resemble those first seen by Hayflick (*3*) in serially subcultured, diploid human fibroblasts. A typical example, shown in Fig. 1a, displays an initial lag phase, a middle plateau phase, and a terminal senescent phase. The plateau is affected by the frequency of feeding. Twice weekly feedings elicit higher cell production early on when compared to the once weekly feeding mode. Yet, the terminal phase invariably occurs and thus appears unrelated to nutritional factors. Senescence in vitro has been interpreted as expression of a general biological limit to the division capacity of somatic cells (*4*). Despite the obvious relevance of this concept for cellular regeneration, and although a large amount of work has been conducted in the area (*5*), very few reports have

confirmed the phenomenon in other than diploid fibroblastic cells (*6*). We now present evidence that mouse hematopoietic stem cells undergo senescence in vitro. The evidence is based on irradiation experiments and on presentation of presumed clonal cultures of unirradiated bone marrow cells.

We chose to irradiate because this would reveal any limit to the absolute number of stem cell divisions if there were only a few hematopoietic stem cells present and if these were undergoing extensive self-renewal.

Thirty-two primary mouse (C3H) bone marrow cultures were set up in 25-cm^2 culture flasks as described (*2*). After 21 days they were divided into four groups which received 50, 300, or 550 rad from a cesium-137 gamma irradiation source (122 rad/min), or no treatment. The cultures were fed with a mixture of fresh (two-thirds) and conditioned (one-third) cell-free medium (*2*) either once or twice weekly. At each feeding, the cells suspended in the medium were harvested, counted, and assayed for colony-forming units by the spleen colony technique (*7*). Figure 1 shows cell yields from one harvest each week. In this system, more than 90 percent of the cells in the supernatant are viable granulocytes (*2*) with an exponential disappearance function of 1.8 days half time (*8*). Since harvesting intervals exceeded the natural disappearance time, the cell removal per se did not impose significant manipulation of the cultures. Figure 1 therefore represents essentially unperturbed hematopoiesis during the postirradiation period.

The cells were irradiated before the cultures had attained plateau phase levels of at least 4 million suspended cells. Recovery was established by a rise near or above this mark 3 or 4 weeks after irradiation. Although this characteristic was acquired by all irradiated cultures, the production levels and the duration of time they were maintained varied inversely with the irradiation dose: the terminal phase commenced after 22, 12, and 10 weeks in cells that received 50, 300, and 550 rad, respectively, and after 22 weeks in control flasks. Could this shift from apparent recovery to failure be caused by stromal degradation, loss of adherent stem cells, or insufficient feeding? The supporting stromal layers showed no early degradation, although rarefaction occurred late in the terminal phase. The concentration of hematopoietic stem cells (that is, the colony-forming units) in the supernatant suspension was decreased in irradiated flasks (see Table 1), thus ruling out increased loss of stem cells from the productive underlayer. More frequent feeding led to high cellularity initially and improved the hematopoietic recovery; however, it did not inhibit or delay the terminal phase. According to these data, hematopoiesis

Table 1. Adherent and nonadherent colony-forming units (spleen) (CFU-S) and cell harvests. The total accumulated number of suspended cells and CFU-S per flask, collected weekly (see Fig. 1), is given in the (b) columns. The (a) column shows the adherent CFU-S which survived the irradiation and which are the ultimate precursors of the offspring shown under (b). Values in (a) are estimated from a replicate experiment with actual CFU-S survival of 470 ± 20 (50 rad), 128 ± 18 (300 rad), 0.9 ± 0.3 (550 rad), and 0.7 ± 0.4 (800 rad). The linear regression of the log-transformed values yielded an intercept of 1.84 (indicating a shoulder curve) and a D_0 of 102 rad (where D_0 is the dose that reduced survival to 37 percent on the exponential part of the curve).

Dose (rad)	Adherent CFU-S 1 day after irradiation	Nonadherent CFU-S (from week 2)		Nonadherent cells (from week 1)	
		1×	2×	1× (10^6)	2× (10^6)
	(a)	(b)	(b)	(b)	(b)
0	488	19,614	17,702	133.8	133.9
50	551	16,484	15,274	143.5	150.2
300	47	2,643	1,738	42.4	65.5
550	4	310	155	18.3	21.2
		*Doublings per surviving CFU-S**			
0		5.3	5.2	18.1	18.1
50		4.9	4.8	18.0	18.1
300		5.8	5.2	19.8	20.4
550		6.3	5.3	22.1	22.3

*ln (b/a)/ln 2.

Table 2. Effect of overlaying stromal cultures with fresh marrow cells in limiting dilutions. Stromal cultures that were free of "spontaneous" hematopoiesis (*13*) were overlaid at 5 weeks with limiting dilutions of fresh marrow cells carrying the "beige" C57B1 (*bg/bg*^{J}) giant lysosome marker, or with mutation-free C57B1 marrow, or with a mixture of both types of cells. The cells with supernatant media from "positive" cultures were harvested, counted, and evaluated for donor type every week. Based on the Poisson expectation of "negative" cultures (*15*), λ is an estimate of the number of stem cells per flask which gave rise to hematopoiesis.

Overlaid per flask			Flasks with hematopoiesis				Hematopoiesis in individual flasks			
Cells		CFU-S*	Week 3		Week 6		Donor type	Time after overlay (week)	Total cell harvest (10^5)	Stem cell doublings‡
C57B1	*bg/bg*^{J}		*x/n*	λ†	*x/n*	λ				
1000		0.1	0/6	0.0	1/6	0.2	C57 (100 percent)	6 to 7	6.1	19.2
	1000	0.1	2/12	0.2	3/12	0.3	Beige (96 percent)	4 to 5	3.7	18.5
							Beige (100 percent)	7 to 9	3.3	18.3
							Beige (91 to 100 percent)	6 to 19	22.4	21.1
2000		0.2	1/10	0.1	0/10	0.0	C57 (99 percent)	4	1.0	16.6
	2000	0.2	0/10	0.0	3/10	0.4	Beige (83 to 96 percent)	5 to 9	3.2	18.3
							Beige (87 to 97 percent)	6 to 9	2.9	18.1
							Beige (90 to 100 percent)	4 to 9	13.9	20.4
1000	1000	0.2	3/10	0.4	2/10	0.2	C57 (97 to 99 percent)	4 to 7	6.8	19.4
2000	2000	0.4	3/10	0.4	0/10	0.0	0			
4000	4000	0.8	8/10	1.6	2/10	0.2	Beige (99 percent)	4 to 7	1.8	17.5
							Beige (91 to 100 percent)	6 to 15	16.4	20.6
							C57 (96 to 100 percent)	4 to 6	6.8	19.4
			0/20	0.0	0/20	0.0	0			

*The content of CFU-S was 10 per 10^5 cells in C57B1 marrow and 12 per 10^5 cells in *bg/bg*^{J} marrow. †Estimate for "stem cells per flask," where $\lambda = -\ln[(n - x)/n]$, n is the number of flasks in a group, and x is the number of positive flasks. ‡ln (total cell harvest)/ln 2.

in vitro appears to be subject to a proliferative limitation.

Such limitation entails a novel interpretation of late hematopoietic radiation damage. Cellular senescence, being a more recent concept, was not considered when the radiobiological theories were formulated. If valid, the notion of limited division applies to unirradiated cells in such a way that no more than a certain, though perhaps large, amount of terminal offspring is generated by each stem cell. Usual challenges might not burden the system with sufficient stress to make it apparent, whereas radiation would expose this limit as a function of stem cell depletion and thus of radiation dose. Elkind and Whitmore (*9*) showed that an irradiated culture could be restored to its previous growth rate and cellularity by one surviving cell which retained reproductive competence. That irradiated cells can survive with their reproductive competence intact is borne out by observation of large clones in regenerating cultures (*10*) and of endogenous spleen colonies representing up to 20 divisions (*11*). If this is the case, and if there is a limit to the number of divisions, then the remaining proliferative life-span would naturally be restricted to the theoretical maximum of cell output per surviving stem cell.

To test this prediction, we repeated the above experiment using identical techniques and doses. Twenty cultures were irradiated and 1 day later the number of stem cells in the adherent layer was asayed by the spleen colony technique (*7*). The remaining flasks showed the same overall course of recovery as before. Table 1 shows the adherent colony-forming units that survived the irradiation as well as the total number of cells and colony-forming units that were harvested in the supernatant culture media during the first experiment. One could now calculate how many doublings per surviving stem cell were necessary to generate this output. The true cell production was underestimated since not all generated cells were harvested. Also, since the spleen colony assay detects only one out of five colony-forming units (*7*), the surviving stem cells were underestimated. However, actual numbers are less important than the remarkable closeness of values in each of the treatment groups and the controls. This supports the limited division concept and suggests that the postirradiation pattern, as it changes from apparent recovery to failure, may reflect no unique late radiation effect but the inherent limited nature of cellular regeneration in this system. It may be due to chance variation that the most divisions per stem cell were calculated for the cultures which had received the highest doses of radiation. Still, this serves to strengthen the argument that the exhibited late effects of radiation are not attributable to reproductive damage (*12*).

We then conducted a further study using unirradiated cells, thus excluding the possibility of stromal degradation as a contributory factor. We prepared stromal layers [from C3H mouse marrow without hydrocortisone (*13*)] and 5 weeks later, when the cultures showed no trace of hematopoiesis, we overlaid them with limiting dilutions of fresh marrow cells. Some flasks received C57B1

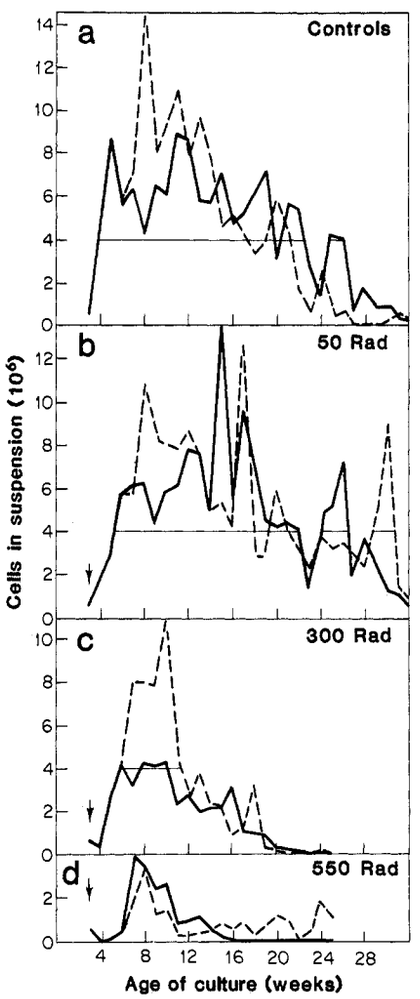

Fig. 1. Cells harvested in supernatant medium of primary, long-term cultures of bone marrow cells. There were four flasks in each group. The solid lines refer to flasks with cells fed once weekly and the broken lines to flasks with cells fed twice weekly by replacement of the supernatant phase with cell-free (one-third conditioned, two-thirds fresh) medium. Only one cell harvest per week is shown. Cultures were terminated at 25 or 33 weeks of age when the adherent stromal layers disintegrated. (a) Untreated cultures; (b to d) cultures from the same batch irradiated at 21 days (arrow) with the doses shown.

(bg/bg^J) marrow which carries the so-called beige mutation, characterized by the presence of easily recognizable, giant lysosomes in all granulocytes (*14*). Other cultures received C57B1(+/+) marrow, or mixtures of both donor types. The presence or absence of hematopoiesis was determined. Any hematopoiesis that could be verified by microscopic (phase contrast) examination, were it one small colony (at least 32 cells) or a fully productive flask with confluent colonies and millions of suspended granulocytes, was judged as positive. The fraction of flasks with positive hematopoiesis corresponded closely to expectations based on assays of colony-forming units in the inoculate, when λ as an estimate for "stem cells per flask" was estimated according to Poisson statistics (*15*). As shown in Table 2, cells in individual flasks remained hematopoietic for some time once they were found to be positive, and the cells in the supernatant media were harvested weekly, counted, and microscopically evaluated for donor type. A single donor type, either beige or C57B1, contributed all of the granulocytes in four hematopoietically active cultures with mixed marrow overlays. The donor type remained the same throughout the active phase which could last for up to 14 weeks. We consider it likely that in each flask a single stem cell accounted for the course of hematopoiesis. As indicated by the λ values, the seeding efficiency in vitro was similar to that of the spleen colony assay in vivo (*7*). That the stromal layers did not contribute to hematopoiesis is implied by the exclusively beige-type granulocytes in cultures which had received only beige marrow. This technique, we believe, might also be applicable to species other than the mouse.

Although one cannot rule out the possibility that irradiation led to dose-dependent degeneration of the culture microenvironment and the eventual failure of granulopoietic activity, such effects are unlikely because clonal granulopoiesis continued for periods ranging from 1 to 14 weeks in a setting quite similar to and indistinguishable microscopically from that in the irradiated primary bone marrow cultures. We attribute the variety in duration and yield to the heterogeneity of the stem cell population as observed previously both in vitro and in vivo (*2*, *16*). A maximum of 21 doublings was documented by clonal hematopoiesis, while 22 doublings were estimated for irradiated mass cultures. One can best explain these results by assuming that murine hematopoietic stem cells undergo limited duplication in vitro wheth-

er they are irradiated or not and whether they are grown in primary mass cultures or in secondary, limiting-dilution overlays. If the same applies to stem cells in vivo, it explains the previous observations of nonsustained recovery in repeatedly irradiated or busulfan-treated animals (*17*), and is in agreement with reports describing regenerative deficits after secondary challenge (*18*). Although the regenerative stem cell reserve may be very large in vivo, if it is limited at all, this needs to be understood in as far as it applies to human beings, and the implications need to be made part of our concepts of cancer treatment with radiation, drugs, and bone marrow transplantation.

URSULA REINCKE
EILEEN C. HANNON
MARGERY ROSENBLATT
SAMUEL HELLMAN
Department of Radiation Therapy, Harvard Medical School, Boston, Massachusetts 02115

References and Notes

1. T. M. Dexter, T. D. Allen, L. G. Lajtha, *J. Cell. Physiol.* **91**, 335 (1977).
2. P. Mauch, J. S. Greenberger, L. Botnick, E. Hannon, S. Hellman, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2927 (1980); U. Reincke, P. Shieh, P. Mauch, S. Hellman, L. B. Chen, *J. Histochem. Cytochem.*, in press.
3. L. Hayflick and P. S. Moorehead, *Exp. Cell Res.* **25**, 585 (1961).
4. L. Hayflick, *ibid.* **37**, 614 (1965).
5. U. Reincke, H. Burlington, E. P. Cronkite, J. Laissue, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 71 (1975); A. Marcieira-Coelho, C. Diatloff, E. Malaise, *Gerontology* **23**, 290 (1977); L. Hayflick, in *Handbook of the Biology of Aging*, C. E. Finch and L. Hayflick, Eds. (Van Nostrand Reinhold, New York, 1977), vol. 1, pp. 159–186; N. Suzuki and H. R. Withers, *Science* **202**, 1214 (1978); E. Bell, L. Marek, S. Sher, C. Merril, D. Levinstone, I. Young, *Int. Rev. Cytol. Suppl.* **10**, 1 (1979); L. E. Botnick, E. C. Hannon, S. Hellman, *Blood Cells* **5**, 195 (1979); A. L. Muggleton-Harris and D. W. DeSimone, *Somat. Cell Genet.* **6**, 689 (1980).
6. J. Ponten, B. Westermark, R. Hugosson, *Exp. Cell Res.* **58**, 393 (1969); Y. LeGuilly, M. Simon, P. Lenoir, M. Bourel, *Gerontology* **19**, 303 (1973); J. G. Rheinwald and H. Green, *Nature (London)* **265**, 421 (1977).
7. L. Siminovitch, E. A. McCulloch, J. E. Till, *J. Cell. Comp. Physiol.* **62**, 327 (1963).
8. U. Reincke, E. C. Hannon, S. Hellman, *J. Cell. Physiol.*, in press.
9. M. M. Elkind and G. F. Whitmore, *The Radiobiology of Cultured Mammalian Cells* (Gordon & Breach, New York, 1967), pp. 334–338.
10. W. K. Sinclair, *Radiat. Res.* **21**, 584 (1964); S. Ban, O. Nikaido, T. Sugahara, *Exp. Gerontol.* **15**, 539 (1980).
11. U. Reincke, E. P. Cronkite, K. Hinkelmann, *Nouv. Rev. Fr. Hematol.* **12**, 255 (1976).
12. V. P. Bond, T. M. Fliedner, T. O. Archambeau, *Mammalian Radiation Lethality* (Academic Press, New York, 1965), pp. 78–81; L. H. Thompson and H. D. Suit, *Int. J. Radiat. Biol.* **15**, 347 (1969).
13. G. I. Cohen, G. P. Canellos, J. S. Greenberger, in *Biology of Bone Marrow Transplantation*, ICN-UCLA Symposium, R. Gale and F. Fox, Eds. (Academic Press, New York, 1980), vol. 17, pp. 491–505.
14. E. D. Murphy, D. E. Harrison, J. B. Roths, *Transplantation* **15**, 526 (1973).
15. A. Boyum and R. Borgstrom, *Scand. J. Haematol.* **7**, 294 (1970).
16. S. Hellman, L. E. Botnick, E. C. Hannon, R. H. Vigneulle, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 490 (1978).
17. E. Lorenz, *J. Chim. Phys.* **48**, 264 (1951); A. Hochmann, Y. Feige, A. Neharin, *Radiol. Clin. Biol.* **36**, 15 (1967); S. J. Baum, M. I. Varnon, D. E. Wyant, *Radiat. Res.* **41**, 492 (1970); A. Morley and J. Blake, *Blood* **44**, 49 (1974).
18. J. B. Storer, *Ann. N.Y. Acad. Sci.* **114**, 126 (1964); J. M. Yuhas, D. Huang, J. B. Storer, *Radiat. Res.* **38**, 501 (1969); J. K. Gong, T. J. MacVittie, J. E. Vertalino, *ibid.* **37**, 467 (1969); A. Morley, K. Trainor, J. Blake, *Blood* **45**, 681 (1975); L. E. Botnick, E. C. Hannon, S. Hellman, *Nature (London)* **262**, 68 (1976); R. Shofield, B. I. Lord, S. Kyffin, C. W. Gilbert, *J. Cell. Physiol.* **103**, 355 (1980); G. E. Huebner, K. H. Wangenheim, L. E. Feinendegen, *Exp. Hematol.* **9**, 111 (1980).
19. Research supported by NIH grants CA 10941 and CA 12662.

28 October 1981; revised 22 December 1981

Interspecies Variations in Mammalian Lens Metabolites as Detected by Phosphorus-31 Nuclear Magnetic Resonance

Abstract. *Multiple interspecies differences were detected between humans and seven other mammals in 15 of the 24 metabolites measured in the intact crystalline lens and lens perchloric acid extracts. Generally, the number of statistically significant metabolite differences among the various species, relative to the human, increase in the following order: cat ~ dog > pig > rat > sheep > rabbit > cow.*

We have reported that there are substantial metabolic differences between the rabbit and human crystalline lens, as measured by phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P NMR) (*1*). The differences appear to represent fundamental divergences in the rate-limiting enzymatic processes that regulate the intermediary metabolism of the lens. Although reports of interspecies differences in tissue metabolite concentrations are not uncommon

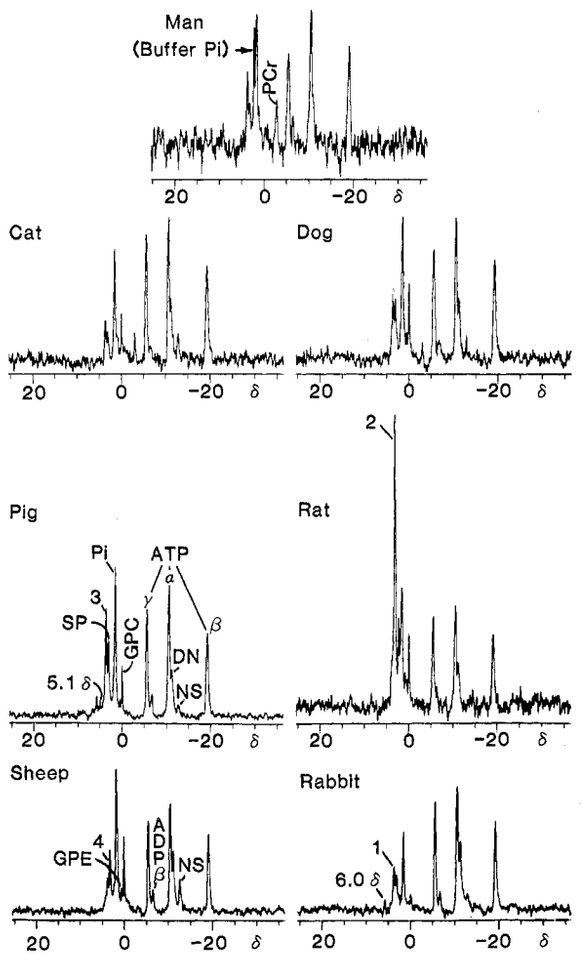

Fig. 1. The ^{31}P NMR spectra of various intact mammalian lenses during incubation in modified Earle's buffer (*p*H 7.4) at 37°C. In accordance with the IUPAC convention, the chemical shift scale is presented as positive downfield. The resonance position of 85 percent orthophosphoric acid corresponds to zero δ. *Buffer Pi* denotes the buffer inorganic orthophosphate resonance; *SP*, sugar phosphate; *GPE*, glycerol 3-phosphorylethanolamine; *GPC*, glycerol 3-phosphorylcholine; *PCr*, phosphocreatine; *ADP* and *ATP*, adenosine di- and triphosphates; *DN*, the dinucleotides, principally nicotinamide adenine dinucleotide; and *NS*, nucleoside diphosphosugars composed of uridine diphosphoglucose, diphosphogalactose, and diphosphomannose. Peak 1 (rabbit) consisted principally of α-glycerol phosphate; peak 2 (rat) represents predominantly phosphorylcholine; peak 3 (pig) denotes an elevated level of ribose-5-phosphate; peak 4 (sheep) reflects elevated inosine 5-monophosphate content. The intact lens ^{31}P spectral profiles were obtained from single lenses in each instance except for the rat; six rat lenses were analyzed simultaneously to provide sufficient tissue mass. The buffer Pi signal, which is discernible in the intact lens spectra for rat and human, is a manifestation of the proportionately low tissue : buffer volume ratio in the detection volume of the spectrometer receiver coil. We find that the enhancement of the buffer Pi signal is an inverse function of tissue volume; this observation explains the presence of this peak in the lens spectra of the rat and human and its apparent absence in the other mammalian lens spectra.

0036-8075/82/0326-1622$01.00/0