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.

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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

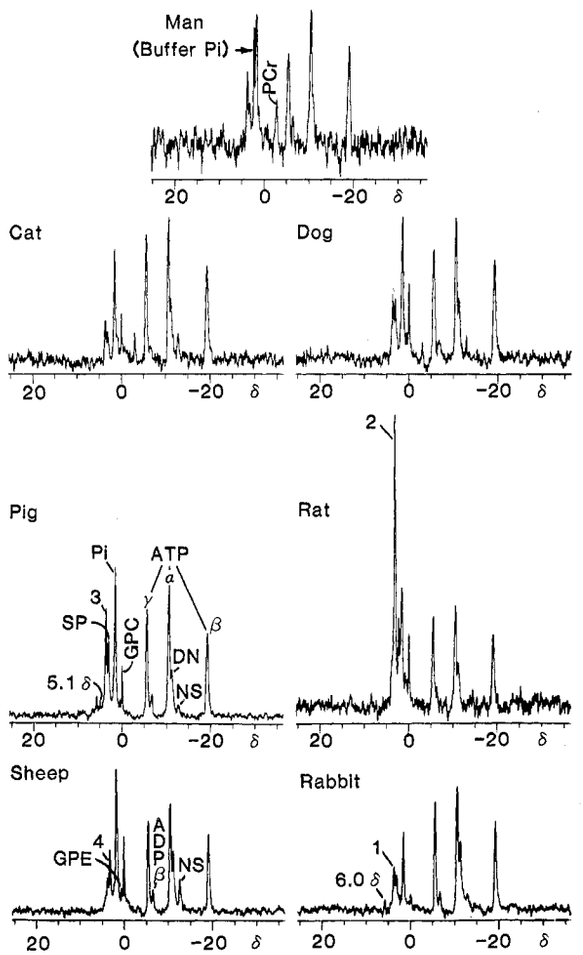

Fig. 1. The ^{31}P NMR spectra of various intact mammalian lenses during incubation in modified Earle's buffer (*p*H 7.4) at 37°C. In accordance with the IUPAC convention, the chemical shift scale is presented as positive downfield. The resonance position of 85 percent orthophosphoric acid corresponds 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.

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(*2*), the magnitude and extent of the differences were not anticipated from experiments with rabbits, which are widely used to investigate lens metabolism related to cataractogenic mechanisms in the human. Therefore, we analyzed the concentrations of phosphorus-containing metabolites from the lenses of a variety of mammals for two reasons: (i) if there are significant metabolic differences between human and other mammalian lenses, the animal should be identified that most closely approximates the human, and (ii) interspecies metabolic differences may provide evidence of phylogenetic adaptations that have evolutionary significance.

Within 2 hours after death, intact human lenses (N = 9) from donors 24 to 30 years of age were excised from eyes from which the corneas had been removed and preserved for transplant (*1*). Intact lenses from young adult New Zealand White rabbits (*Oryctalagus cuniculus*, 5 to 7 months, N = 20), cats (*Felis catus*, 1 to 2.5 years, N = 16), dogs (*Canis familiaris*, 2 to 4 years, N = 16), and Sprague-Dawley rats (*Rattus norvegicus*, 60 to 80 days, N = 30), of both sexes were excised from eyes within 1 hour after the administration of a lethal sodium pentobarbital dose. Eyes from cows (*Bos*, 2 to 2.5 years, N = 20), pigs (*Sus*, 1.3 to 1.6 years, N = 20), and sheep (*Ovis*, 6 to 9 months, N = 20) were obtained from an abattoir, and the lenses were excised within 2 hours after death. The lenses were then analyzed in one of two ways: (i) excised lenses were frozen immediately in liquid nitrogen and lens perchloric acid (PCA) extracts were prepared and analyzed by ^{31}P NMR or (ii) intact lenses were placed in modified Earle's buffer and analyzed by ^{31}P NMR (*1*, *3*).

The relative contents of phosphorus-containing metabolites were determined from the ^{31}P NMR spectra for both intact lens and PCA extracts by computerized peak-area analysis. (Intact, incubated cow lenses were not analyzed.) Intralenticular pH values were calculated from the shift in the internal inorganic orthophosphate resonance; the glycerol 3-phosphorylcholine resonance at −0.13 δ was used as the internal reference (*4*). Twenty-four different resonance signals detected in lens PCA extracts, which correspond to separate phosphorus-containing metabolites, were evaluated. Separate ^{31}P NMR analyses were performed on five different groups of lenses from each species. Statistical inferences are based on one-way analysis of variance and the Scheffé comparison procedure (*5*). The chemical assignments of the various resonance signals were based on multiple criteria, including comparison of the resonance signals with those obtained when known phosphatic metabolites are added to the extracts under varied conditions of pH and ionic strength, and other chemical properties and theoretical considerations (*6*).

Data from intact lenses and PCA extracts were comparable within each species analyzed; however, significant interspecies variations were apparent (Figs. 1 and 2). Metabolites for which significant interspecies differences were observed are summarized in Table 1. Certain variants were peculiar to specific mammals. These differences included (i) the high phosphorylcholine and low adenosine diphosphate and inorganic orthophosphate content of the rat lens; (ii) the elevated levels of the 5.98 δ unassigned resonance, α-glycerol phosphate, and nicotinamide adenine dinucleotide, and the low inorganic orthophosphate and phosphodiester (glycerol 3-phosphorylcholine and glycerol 3-phosphorylethanolamine) content of the rabbit lens; (iii) the relatively high phosphocreatine and low phosphodiester content of the human lens; (iv) the apparently low adenosine triphosphate content and high level of the 5.98 δ unassigned resonance detected in the cow lens; and (v) the elevated ribose-5-phosphate levels in the pig and cow lenses.

Although the lens metabolite levels show pronounced interspecies fluctuations, there are some overall species similarities. Metabolites for which interspecies variations were not detected include uridine diphosphoglucose, diphosphogalactose, and diphosphomannose; nicotinamide adenine dinucleotide phosphate; glucose-6-phosphate and galactose 6-phosphate; glucose-1-phosphate; fructose-1, 6-diphosphate; and the unassigned resonances at 10.69, 9.97, 5.14, and 0.92 δ. Slight species variations were evident in the calculated intralenticular pH values (human, 6.9; cat, 6.8; dog, 6.8; pig, 6.8; rat, 6.8; sheep, 6.9; and

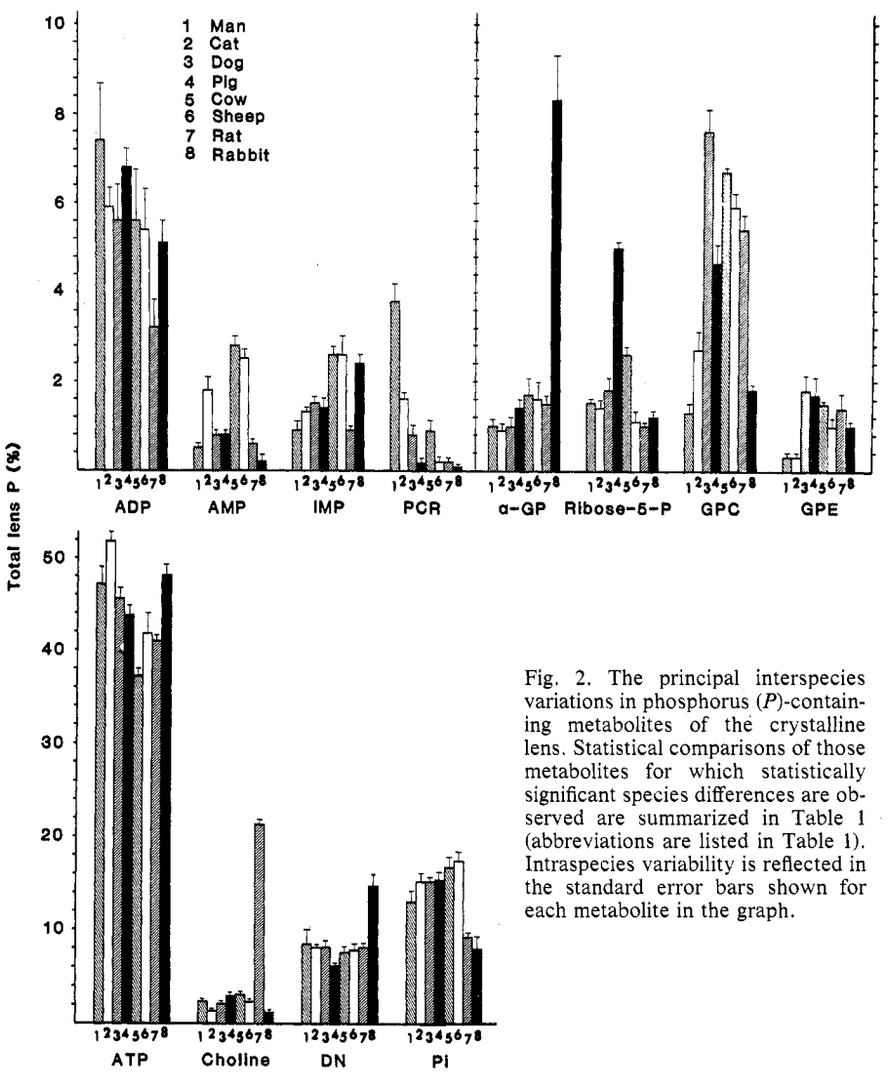

Fig. 2. The principal interspecies variations in phosphorus (*P*)-containing metabolites of the crystalline lens. Statistical comparisons of those metabolites for which statistically significant species differences are observed are summarized in Table 1 (abbreviations are listed in Table 1). Intraspecies variability is reflected in the standard error bars shown for each metabolite in the graph.

Table 1. Multiple comparisons of species differences in crystalline lens metabolites. (Redundant combinations are excluded.) Only those mammalian species are listed in each metabolite category for which statistically significant interspecies differences exist ($P < 0.01$, unless otherwise indicated). Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; DN, nicotinamide adenine dinucleotide; PCR, phosphocreatine; Pi, inorganic orthophosphate; GPC, glycerol 3-phosphorylcholine; GPE, glycerol 3-phosphorylethanolamine; α-GP, α-glycerol phosphate; ribose-5-P, ribose-5-phosphate; IMP, inosine 5-monophosphate; AMP, adenosine 5-monophosphate; and Pcholine, phosphorylcholine.

	ATP	ADP	DN	PCR	Pi	GPC	GPE	α-GP	Ribose-5-P	IMP	AMP	Pcholine	Unassigned resonances (δ)		
													10.69	9.97	5.98
Human	Cow Rat*	Rat Rabbit*	Rabbit	Cat Dog Pig Cow Sheep Rat Rabbit	Cow* Sheep Rat* Rabbit	Dog Pig Cow Sheep Rat Rabbit*	Dog Pig Cow Sheep* Rat	Rabbit*	Pig Cow	Cow Sheep Rabbit*	Cat Cow Sheep Rabbit*	Cow* Rat Rabbit	Cat Cow Sheep	Cat Cow* Sheep	Pig* Cow Rabbit
Cat	Pig Cow Sheep Rat		Pig Rabbit	Sheep Rat Rabbit	Rat Rabbit	Dog Cow Sheep* Rat	Dog Pig Cow Sheep* Rat Rabbit*	Rabbit	Pig Cow	Cow Sheep	Dog* Pig Cow Sheep* Rat	Dog Pig Cow Sheep Rat	Dog Pig Rat Rabbit	Dog Pig Rat Rabbit	Pig Cow Rabbit
Dog	Cow		Rabbit		Rat Rabbit	Pig Rat* Rabbit	Sheep Rabbit	Rabbit	Pig Cow* Rat*	Sheep	Cow Sheep	Pig Cow Rat Rabbit	Cow Sheep	Cow Sheep	Cow Rabbit
Pig			Rabbit		Rat Rabbit	Cow* Rabbit	Sheep* Rabbit*	Rabbit	Cow Sheep Rat Rabbit	Cow* Sheep	Cow Sheep	Sheep* Rat Rabbit	Cow Sheep	Sheep	Cow Sheep Rat Rabbit
Cow	Rabbit		Rabbit		Rat Rabbit	Rabbit		Rabbit	Sheep Rat Rabbit	Rat	Rat Rabbit	Sheep Rat Rabbit	Rat Rabbit	Rat* Rabbit*	Sheep Rat
Sheep	Rabbit*		Rabbit		Rat Rabbit	Rabbit		Rabbit		Rat	Rat Rabbit	Rat Rabbit	Rat Rabbit	Rat Rabbit	Rabbit
Rat	Rabbit		Rabbit			Rabbit		Rabbit		Rabbit*		Rabbit			Rabbit

*$P < .05$.

rabbit, 6.8); however, these differences are not statistically significant. Overall results indicate that the cat and dog lenses most closely resemble the human lens in that they have the fewest number of significant metabolite differences, four and three, respectively, relative to the human. The rabbit and cow lenses are the least similar to the human with 10 and 12 significant metabolite differences, respectively. Generally, the rank order of lens metabolism from most to least similar to human is as follows: cat ~ dog > pig > rat > sheep > rabbit > cow.

The detected interspecies differences in lens metabolism represent divergent phylogenetic modifications in specific rate-limiting enzymatic reactions that regulate lens intermediary metabolism. The perplexing differences between uncommonly related metabolites—specifically, α-glycerol phosphate, phosphocreatine, ribose-5-phosphate, phosphorylcholine, glycerol 3-phosphorylcholine, and glycerol 3-phosphorylethanolamine—cannot be rationalized in terms of a single metabolic pathway. However, consideration of known metabolic pathways operant in the lens (*2, 7, 8*) indicates that these metabolites are branch points connecting carbohydrate, amino acid (particularly glycine and serine), and phospholipid metabolic pathways. The differences in activity of these pathways in the various mammalian lenses may represent genetic differences that could be associated with specialized evolutionary adaptations. The modified activity of the various metabolic pathways among these species may contribute to the reported interspecies differences in the primary structure of lens macromolecules such as proteins and phospholipids (*2, 8, 9*).

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3. A Nicolet NT-200 NMR system equipped with

deuterium stabilization, variable temperature, and Fourier-transform capabilities operating at 80.987663 MHz for ^{31}P interfaced to a wide bore (89 mm) Oxford superconducting magnet was used. Intact, incubated lenses placed in 12-mm NMR tubes were analyzed at 37°C under the following spectrometer conditions: pulse sequence, 1 pulse; pulse width, 8 μsec (45° flip angle); acquisition delay, 200 μsec; cycling delay, 250 μsec; number of scans, 4400; number of data points per free induction decay, 8192; acquisition time, 819.4 msec; sweep width, ± 2500 Hz. In addition, a computer-generated filter time constant introducing 10-Hz line broadening was applied. The chemical shifts are reported in field-independent units denoted δ (hertz per megahertz). Lens PCA extracts were analyzed as described in (*1*).

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9. The results reported represent metabolite levels present in biochemically and physiologically mature lenses excised from young adult mammals. The contribution of age as a factor in the interspecies differences cannot be completely discounted; however, the biologic ages of the animals studied are relatively close, and the markedly divergent lens metabolic differences encountered among the various species of animals are not consistent with reported differences attributable to age. As a consequence, the results reported appear predominantly to represent interspecies differences in lens metabolism, rather than age-dependent changes.

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Lower Devonian Gametophytes: Relation to the Phylogeny of Land Plants

Abstract. *Three gametophytic plants now known from the Lower Devonian of Scotland and Germany show common features in their fertile parts. The morphological and anatomical structures indicate affinities to bryophytic gametophytes, although there is no evidence for a parasitic sporogonium-like sporophyte as in the Bryophyta. Several of the vascular plant sporophytes from the Rhynie Chert also have a few characteristics reminiscent of bryophytes. But these ancient gametophytes, if related to the sporophytes, indicate a closer relation of Bryophyta to Tracheophyta than would the study of sporophytes alone.*

In 1980 we described (*1*) a gametophyte with anatomically preserved tissues, gametangia, and antherozoids in different stages of development from the Lower Devonian Rhynie Chert in Scotland. We described it in detail (*2*) under the name *Lyonophyton rhyniensis* (Fig. 1). We now report a new gametophyte (Fig. 2), with anatomically preserved tissues, also from the Rhynie Chert (*3*).

Eight specimens of this new gametophyte have similar specific and generic characteristics, which are different from *Lyonophyton*. We found five gametangiophores bearing antheridia only and three bearing organs only, which we interpret as archegonia. The new gametophytes seem to form separate antheridiophores and archegoniophores. The antheridiophores exhibit the following features. (i) A round axis widens terminally into a disklike antheridiophore head. (ii) The axis and the underside of the gametangiophore head are covered with scalelike enations. (iii) The margin of the antheridiophore lacks antheridia, is thin, and seems to consist of a few upturned lobes (possibly involucral bracts). (iv) Closely spaced antheridia are borne on the convex surface of the antheridiophore. (v) The antheridia are stalked, globular, or club-shaped and mostly contain sperm (antherozoids) (Fig. 2, B and C) in different stages of development, demonstrating the gametophytic nature of the plant. (vi) The antheridia are slightly sunken or intermingled with multicellular, branched, paraphysis-like sterile tissue. (vii) A central strand of hydroids passes through the stalk of the antheridiophore and diverges into a funnel-like structure in the basal part of the antheridiophore head.

The probable archegoniophores are built similarly; the archegoniophore head may be lobed in axis-like structures, and the hydrom splits up into terete, separate strands. There are some indications that male and female gametangiophores are seated on common axes or a protocorm, as in *Sciadophyton*.

The morphological and anatomical characteristics of *Lyonophyton* enabled us (*4*) to show that another Lower Devonian plant, *Sciadophyton*, previously believed to be a sporophyte, is probably a gametophyte (Fig. 3). *Sciadophyton* has so far only been found as impressions or compressions with conducting strands partly mineralized, but in its morphological features we see a nearly complete gametophyte. From a flat central initial structure (protocorm) axes (up to 10 cm long) radiate. It may be that all the upright subaerial axes terminate in gametangiophores which are bowl- to funnel-

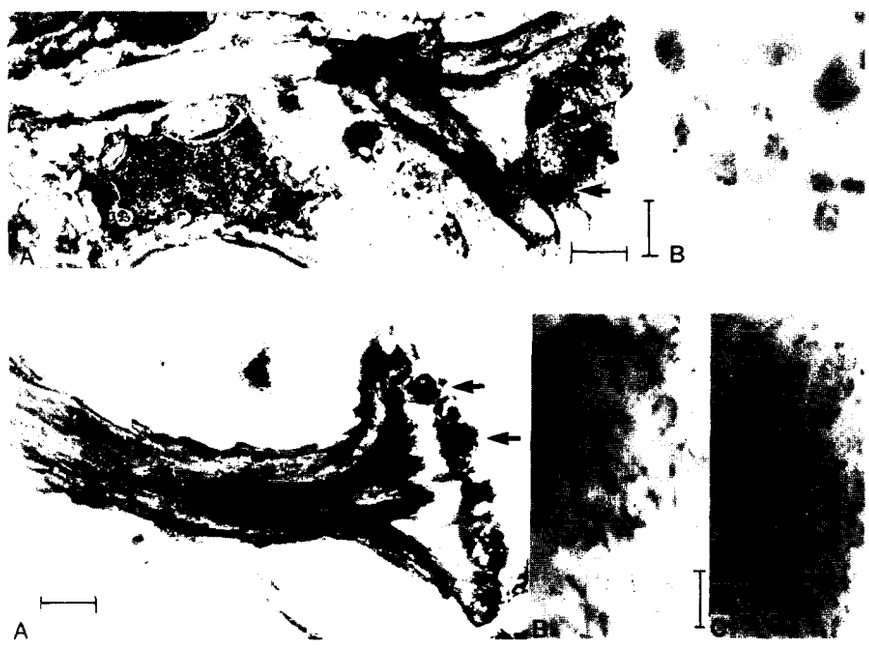

Fig. 1 (top). *Lyonophyton rhyniensis*. (A) Longitudinal section through the stalked gametangiophore showing two antheridia on the upper (inner) surface (arrows) (scale bar, 1 mm); (B) section through an antheridium with mature sperm (scale bar, 10 μm). Fig. 2 (bottom). Sections through the new gametophyte. (A) Longitudinal section through the stalked antheridiophore showing antheridia on the upper surface (arrows) (scale bar, 1 mm); (B and C) sections through two antheridia with mature sperm (scale bar, 10 μm).

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