stained in the lysozyme PAP preparations. Omission of the primary antisera, substitution of rabbit antisera to unrelated human antigens (α-lactalbumin, casein), or of inappropriate PAP reagents abolished the immunostaining.

Absorption of the antisera to human lysozyme with neat and 1:10 dilutions of human tears, saliva and 1 mg human lysozyme in 1 ml saline completely abolished immunostaining (fig. b). Hen's egg lysozyme and α-elastin peptides were without effect.

Trypsin digestion was a necessary preliminary step; no lysozyme immunostaining of elastic fibers in the paraffin sections was found with either antiserum in its absence. Only relatively weak elastic-fiber immunoreactivity was found with the indirect fluorescence and PAP methods on normal vessels in snap-frozen sections.

Discussion. Use of the techniques described here have confirmed the immunolocalization of lysozyme in the other tissue components described previously[1,2]. It seems likely that the unmasking of tissue antigens by preliminary trypsinization[5], and the sensitivity of the PAP method[3] are responsible for the demonstration of lysozyme in vascular elastic fibers of formalin-fixed, paraffin-embedded tissue.

Lysozyme is a cationic protein. Charge attraction appears to be responsible for its interaction with mucin[10] and cartilage proteoglycan[11]. Only limited anionic groups are found in elastin[12]. They may contribute to the affinity of lysozyme for elastic fibers, but the presence of adjacent glycoprotein microfibrils with a greater proportion of diacidic aminoacids[13] and anionic glycosides seem more likely to account for the selective localization of lysozyme in elastic fibers.

The biological role of lysozyme in elastic fibers is currently completely unknown. In the absence of an appropriate glycosidic substrate it is tempting to speculate the lysozyme may assume a nonenzymatic function[14]. In high concentration lysozyme inhibits the activity of collagenase[15], and has been shown to inhibit the proteolysis of proteoglycans by elastase[16]. Possibly, lysozyme may thus protect elastic-fiber components from in-vivo proteolysis.

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On the distribution of plasma L-asparaginase[1,2]

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Summary. The guinea-pig, *Cavia porcellus,* is unusual in possessing plasma L-asparaginase, an enzyme with anti-tumor activity. 21 additional species have been examined as to the presence of this enzyme: the results confirm and extend its remarkably limited species distribution.

Recently we drew attention to the fact that there are several significant biochemical differences between the guinea-pig and a number of other mammals[4]. Two of the most striking of these are the presence of plasma L-asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) in the guinea-pig, and an insulin amino acid sequence that is strikingly different from that of porcine and bovine insulin. These biochemical peculiarities are shared by the guinea-pig (and a few other members of the same superfamily, i.e., Cavoidea) and New World monkeys, species which otherwise have little in common except that their evolutionary ancestors arrived in South America during the late Eocene or early Oligocene[5-10].

The appearance of similar traits in unrelated species sharing the same biospace might logically be considered as convergent evolution. One can speculate, for example, that New World parasites might have existed which lacked the capacity to synthesize asparagine, and that guinea-pigs and New World monkeys independently developed resistance to these parasites by evolving a serum asparaginase, although there is no evidence to support this suggestion.

No obvious biochemical purpose is met by plasma L-asparaginase but the enzyme is of interest, aside from questions about its limited distribution, because of its anti-tumor activity (see Wriston, Jr et al.[11] for review). In fact, it was the anti-tumor activity of guinea-pig serum against certain transplanted mouse tumors that led to the discovery and isolation of L-asparaginase from guinea-pig serum[12-15] and indirectly to the discovery of an *E. coli* asparaginase with anti-tumor activity[16].

22 species were examined for plasma asparaginase in connection with the early work on the guinea-pig serum enzyme. We recently became interested in screening additional species because of the possibility that the results might shed light on the still unsettled question as to whether the ancestors of present day South American rodents and primates came from North America or Africa. These results, together with those of the earlier workers, are summarized in the table.

Methods. Asparaginase activity was determined by direct nesslerization as previously described[17]. Samples were obtained from the frozen sera collections of the National Zoological Park/Smithsonian Institution, Washington, D.C., and the Philadelphia Zoological Garden, Philadelphia, PA.

Results. Because most of the recent assays were done on a single serum sample which had often been stored frozen for

Distribution of plasma L-asparaginase

Order	Family	Species	Plasma L-asparaginase	References
Marsupialia	Didelphidae	*Didelphis marsupialis virginianus* (opossum)	-	this work
Marsupialia	Phascolomidae	*Phascolomis ursinus* (wombat)	-	this work
Marsupialia	Macropodidae	*Dendrolagus* (tree kangaroo)	-	this work
Insectivora	Macroscelididae	*Macroscelides proboscideus* (elephant shrew)	-	this work
Primate	Lorisidae	*Perodicticus potto* (potto)	-	this work
Primate	Cebidae	*Alouatta caraya* (black howler)	+	this work
Primate	Cebidae	*Saimiri sciureus* (squirrel)	+	18
Primate	Cebidae	*Lagothrix Lagotricha* (woolly)	+	this work
Primate	Cercopithecidae	*Macaca mulatta* (rhesus)	-	18
Primate	Cercopithecidae	*Macaca speciosa* (stump-tailed macaque)	-	18
Primate	Cercopithecidae	*Cercopithecus talapoin*	-	18
Primate	Cercopithecidae	*Nasalis larvatus*	-	this work
Primate	Cercopithecidae	*Colobus polykanos*	-	this work
Primate	Pongidae	*Pan troglodytes* (chimpanzee)	-	18
Primate	Hominidae	Man	-	18-20
Edentata	Bradypodidae	*Choloepus didactylus* (2-toed sloth)	-	this work
Lagomorpha	Leporidae	Rabbit, not specified	-	12, 20
Rodentia	Castoridae	*Castor canadensis* (beaver)	-	this work
Rodentia	Sciuridae	*Marmota monax* (woodchuck)	-	this work
Rodentia	Cricetidae	Hamster, not specified	-	21
Rodentia	Muridae	*Rattus norvegicus*	-	22, 23
Rodentia	Muridae	*Mus musculus* (mouse, ICR strain)	-	this work
Rodentia	Erethizonthidae	*Coendou,* 18 species, not specified*	-	21
Rodentia	Caviidae	*Cavia porcellus* (guinea-pig)	+	13, 15, 18, 19, 21
Rodentia	Caviidae	*Dolichotis patagonum* (mara, or Patagonian hare)	+	21; and this work
Rodentia	Caviidae	*Dolichotis salinicola* (lesser Mara)	+	this work
Rodentia	Hydrochoeridae	*Hydrochoerus hydrochaeris* (capybara)	+	21
Rodentia	Dasyproctidae	*Cuniculus paca* (paca)	+	19, 21
Rodentia	Dasyproctidae	*Dasyprocta,* 13 species, not specified (agouti)	+	19, 21
Rodentia	Chinchillidae	*Chinchilla*	-	21
Rodentia	Capromyidae	*Capromys pilorides* (Cuban hutia)	-	this work
Rodentia	Capromyidae	*Myocastor coypus* (coypu or nutria)	-	16, 18
Carnivora	Canidae	*Canis familiaris* (dog)	-	17, 19
Carnivora	Procyonidae	*Procyon lotor* (raccoon)	-	this work
Carnivora	Mustelidae	*Lutra canadensis* (otter)	-	this work
Carnivora	Viverridae	*Arctictis binturong* (binturong)	-	this work
Carnivora	Viverridae	*Atilax paludinosus*	-	this work
Carnivora	Felidae	*Felis domesticus* (cat)	-	19
Tubulidentata	Orycteropodidae	*Orycteropus afer* (aardvark)	-	this work
Perissodactyla	Equidae	Horse, not specified	-	9, 17
Perissodactyla	Tapiridae	*Tapirus terrestris* (Brazilian tapir)	-	this work
Artiodactyla	Suidae	Pig, not specified	-	17
Artiodactyla	Camelidae	*Lama guanacoe* (guanaco)	-	this work
Artiodactyla	Bovidae	Cow, not specified	-	17
Artiodactyla	Bovidae	Sheep, not specified	-	17

*Described in Old et al.[21] as a prehensile-tailed porcupine, and member of the Hystricoidea superfamily; but these are Old World porcupines, and the prehensile-tailed porcupines are New World, thus listed here as a member of the family Erethizontidae.

some time, no attempt was made to determine accurately the number of units of asparaginase activity/ml; the results are reported simply as + or −, as shown in the table. It may be seen that we have added 3 more orders of placental mammals, and Marsupials, to the list of orders examined; and have added additional species to the list of Carnivores, Primates, Rodents, Artiodactyla and Perissodactyla. The results confirm and extend the previous findings: a) 2 more New World monkeys contained the enzyme in their sera, 2 more Old World monkeys and a member of the Lorisidae family did not; b) 2 species in a Rodent suborder not previously examined (i.e., beaver and woodchuck, both Sciuromorphs) did not contain plasma asparaginase; c) of the other rodents examined in this study, the 2 that were positive (lesser Mara and Patagonian hare) belong to the superfamily Cavoidea, as do all the other rodents so far found to contain this enzyme; the other rodent examined here, found not to contain the enzyme (Cuban hutia), is a member of the superfamily Octodontoidea.

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Influence of chronic UV-light exposure on hepatic and cutaneous monooxygenases

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Summary. Hairless female Ng/-mice were irradiated by UV-light for 16 h daily over a period of 24 weeks. Monooxygenase activities were measured in liver and skin, and an induction of the aryl-hydrocarbon hydroxylase was detected in liver by both fluorometric and radiochemical methods, whereas no induction of this enzyme could be demonstrated in the skin.

Monooxygenases are membrane-bound enzymes which play an important role in the oxidative metabolism of numerous chemical carcinogens, drugs and lipophilic endogenous substrates. They have been found in the microsomal fractions of different tissues in various mammalian species. Apart from genetic factors[1], environmental agents may also be capable of influencing the levels of monooxygenases and their inducibility.

To our knowledge, the effects of radiation on the activity of different monoxygenases, especially aryl-hydrocarbon hydroxylase (AHH; EC 1.14.14.2), have not yet been published. Tredger and Chhabra[2] postulated that the light cycle may be related to the circadian variations in monooxygenase activities found in the liver, lungs and other extrahepatic tissues of experimental animals. An increased 7-ethoxycoumarin-deethylase(7-EOC-D)-activity was found in the skin of female hairless mice after exposure to short-wave UV-radiation (254 nm) and to a sun-lamp (280–750 nm)[3].

In the present study, we report the effects of chronic UV-light exposure on the activity of cutaneous and hepatic monooxygenases in hairless mice.

Materials and methods. Adult female hairless Ng/-mice (mean weight 33 g) were kept at a constant room temperature of 21 °C and a relative humidity of 55% and were allowed free access to food and water. 10 mice were irradiated for 16 h daily, using assemblies of TL 40/W 09 (Phillips) fluorescence tubes (mean daily doses: UVA = 106 J/cm^2; UVB = 0.62 J/cm^2). A detailed description of the experimental procedure has been published elsewhere[4]. 24 weeks after the start of the experiment, irradiated and nonirradiated control animals (10 mice) were sacrificed by decapitation, and liver and skin microsomes were prepared as previously described[5]. In the microsomal fractions, the following parameters were determined: protein content[6], cytochrome P-450 content[7], 7-EOC-D[8], AHH[9] and aminopyrine-N-demethylase(ADM)[10]. The benzo(a)pyrene metabolism and AHH-activity were studied by the radiometric assay described by Van Cantfort et al.[11].

Results and discussion. The results of our investigation are shown in tables 1 and 2. The values of all measured parameters in the skin microsomes of the irradiated animals revealed no significant differences as compared to controls. On the contrary, in the liver of the irradiated animals, a significant rise in the protein and cytochrome

Table 1. Protein, cytochrome P-450 content and cytochrome P-450-dependent enzyme activities in skin microsomes (values represent a pool of 10 animals) and in liver microsomes (values represent means and SD ($\bar{x} \pm$ SD)) of 8 animals

	Protein[a]	Cytochrome P-450[b] content	7-EOC-D[c]	AHH[d]	ADM[e]
Skin					
Irradiated	2.7	n.d.	0.02	0.2	n.a.
Controls	2.9	n.d.	0.02	0.2	n.a.
Liver					
Irradiated	22.1 ± 0.4	0.78 ± 0.01	2.70 ± 0.66	5.7 ± 0.2	210.0 ± 10.0
Controls	13.1 ± 0.4	0.37 ± 0.02	1.00 ± 0.21	1.6 ± 0.1	66.9 ± 11.2
p-Values	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

n.d., not detectable; n.a., not analyzed. [a]mg × g tissue^{-1}; [b]nM cytochrome P-450 × mg protein^{-1}; [c]nM umbelliferone × min^{-1} × mg protein^{-1}; [d]nM 3-OH-benzo(a)pyrene × 20 min^{-1} × mg protein^{-1}; [e]nM formaldehyde × min^{-1} × g tissue^{-1}.