P-450 content of the microsomes was observed. In addition, the cytochrome P-450-dependent activities of AHH, ADM and 7-EOC-D were also significantly increased. The pattern of the in vitro inhibition of 7-EOC-D by metyrapone and α-naphthoflavone after irradiation was similar to that found in the controls. Finally, the metabolism of benzo(a)-pyrene in the irradiated mice was clearly enhanced, as shown by the increased amounts of benzo(a)pyrene metabolites found in the liver microsomes. These results indicate that, under the experimental conditions described above, UV-irradiation leads to an induction of the hepatic monooxygenases, particularly AHH, whereas it has no apparent effect on the cutaneous monooxygenases. Pohl and Fouts[3] measured an increased activity of 7-EOC-D in the skin of female hairless mice (strain HRS/J); however, they used short-wave UV-radiation (254 nm) or a sunlamp (280–750 nm), and the 7-EOD-activity was the only parameter they determined.

The interpretation of these findings is difficult. The possibility that the enzyme induction in the liver might be due to a direct effect of the radiation on the hepatocytes seems unlikely, though some photons could have reached the superficial parts of the liver through the extremely thin mouse skin. Alternatively, the UV-radiation may have led to the formation of an inducing substance in the skin or in the cellular elements of the peripheral blood, and the enzyme induction could have been mediated by excited states of oxygen in a sequence of molecular events similar to that suggested by Paine to explain monooxygenase induction in liver cell cultures after exposure to light[12]. The apparent lack of AHH induction in the irradiated skin should be considered with caution, since it may be a temporary and not a representative event. Indeed it is possible that the activity of the cutaneous monoxygenases under UV-irradiation also reveals marked fluctuations, as previously reported with regard to other enzymes[13]. Further studies are now warranted in order to define the behavior of monooxygenase activity in the liver and skin at various intervals under UV-irradiation and also after UV-tumorigenesis has developed.

Table 2. AHH-activity in cpm water-soluble ^{14}C-benzo(a)pyrene/mg protein/min, measured in liver and skin of adult female hairless Ng/-mice by radiometric assay

Organ	Control	Irradiated animals
Liver	504.3	1431.5
Skin	71.5	64.3

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Peroxidase activity and thiocyanate accumulation in salivary glands

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Summary. Salivary glands with high, low, or no peroxidase activity do not differ in [$S^{14}CN^-$] after the i.v. injection of $KS^{14}CN$, nor do the glands differ from blood and muscle in [$S^{14}CN^-$]. The content of SCN^- in a salivary gland does not mirror the gland's participation in the peroxidase-mediated antimicrobial mechanism.

Saliva and tears of some mammals contain the thiocyanate ion SCN^-. It is oxidized by H_2O_2 and lactoperoxidase (LP, E.C.1.11.1.7.) to the antimicrobial hypothiocyanite ion $SCNO^-$ [1,2]. This reaction is supposed to occur in the oral cavity, LP being delivered by the saliva and H_2O_2 by the normal microbial flora[3]. The thiocyanate ion is synthesized by the enzyme thiosulphate cyanide sulphotransferase (E.C.2.8.1.1.). The enzyme is found in many, possibly most, tissues but mainly in the liver of the rabbit[4], the rat[4], and the guinea-pig[5]. There is also a high activity in the kidney[4]. In the guinea-pig the activity of the enzyme in the salivary glands has been reported as $\frac{1}{3}$–$\frac{1}{2}$ of that in the liver and the kidney[5]. After the i.v. injection of thiocyanate the major part is excreted via the kidneys and, as CO_2, via the lungs. A small proportion of injected $^{35}SCN^-$ appears as ^{35}sulphate whereas $^{14}CO_2$ accounts for 30% of $S^{14}CN^-$ [6]. There may also be some contribution of SCN^- from hydrolyzed mustard oil and other thiocyanates of alimentary origin.

The extent of coincidence between peroxidase and SCN^-

Table 1. Lactoperoxidase activity in some excretory glands of 3 rodents, recalculated to give $\Delta A_{470} \times sec^{-1} \times g^{-1}$ wet wt. Four animals per group

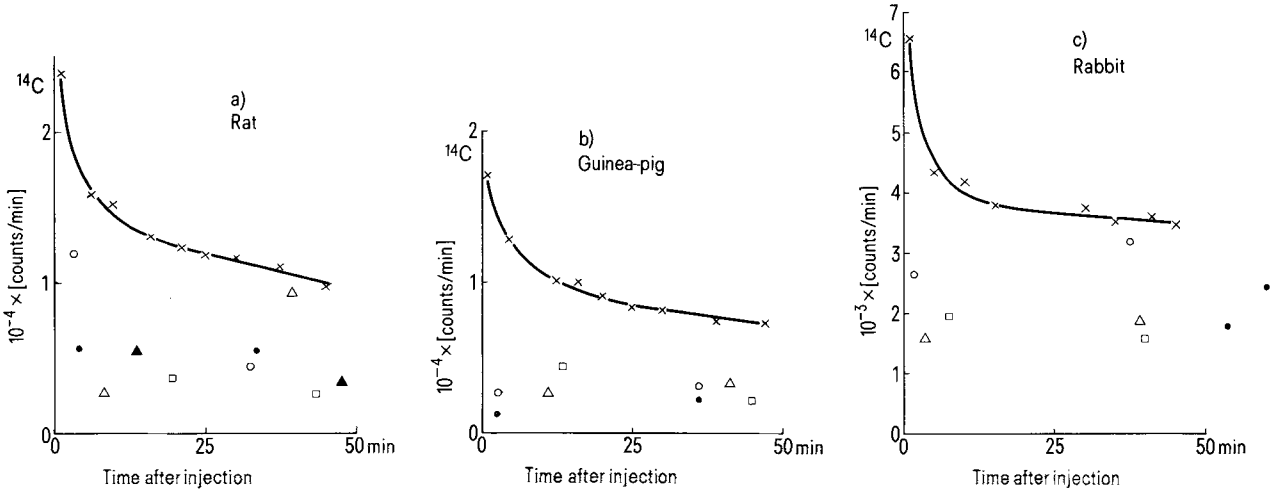
	Submandibular gland	Sublingual gland	Parotid gland	Lacrimatory gland
Rat[a]	0.4±0.2	0.6±0.2	0.5±0.3	15±1
Guinea-pig	12 ±3	13 ±2	4 ±1	
Rabbit	0	0	0	

[a]Two strains.

Table 2. Mean salivary gland, lacrimatory gland, and muscle [$S^{14}CN$]/Serum[$S^{14}CN$] ratios, after i.v. injection of 10 µCi $KS^{14}CN$/rat and guinea-pig, and 20 µCi $KS^{14}CN$/rabbit. A, 0–25 min after and B, 25–50 min after the injection

	Rat[a]		Guinea-pig		Rabbit	
	A	B	A	B	A	B
Submandibular gland	0.58	0.36	0.37	0.40	0.62	0.79
Sublingual gland	0.36	0.37	0.27	0.36	0.49	0.49
Parotide gland	0.26	0.56	0.32	0.27	0.32	0.35
Lacrimatory gland	0.34	0.31				
Muscle tissue	0.23	0.22	0.39	0.38	0.36	0.32

[a]2 strains.



Figures a–c. The contents of $S^{14}CN^-$ in serum (×), the submandibular (○), sublingual (●), parotid (△), and lacrymatory (▲) glands and muscle tissue (□). The left side glands were first removed (t < 25 min) except for the sublingual gland of the rabbit, which required deep dissection. The figures, which show the actual values from a single experiment, are representative for all 4 animals of each species without significant differences.

accumulation in the salivary glands is incompletely explored, and so is the possibility of using the salivary gland [SCN^-] as an indicator of the contribution of a particular gland to the antimicrobial mechanism. There is also some disagreement regarding the occurrence of peroxidase activity in the salivary glands of the rat[7,8].

Materials and methods. Adult guinea-pigs (350–475 g), rats (225–300 g, Sprague Dawley and Wistar) and rabbits (2.3–3.1 kg, New Zealand white) were fed the common diets of the animal house. Fentanyl (0.315 mg/ml) and fluanison (10 mg/ml) in the form of Hypnorm® (Leo, Helsingborg, Sweden) influence the salivation only a little and were used for anesthesia, 1 ml/kg b.wt in a single i.m. injection. $KS^{14}CN$ (Amersham, England) was injected in the jugular vein, 10 μCi/rat or guinea-pig and 20 μCi/rabbit. Blood samples were withdrawn within 1–45 min after the injection from the jugular vein on the opposite side. The parotid, submandibular, and sublingual glands of the animals, and the lacrymatory gland of the rat were removed 1–55 min after the injection. The lacrymatory gland of the rat was positively identified, and assayed for LP, to avoid mistaking this gland for a salivary gland. Biopsies were taken from the pectoral muscle of the rat and the guinea-pig, and from the masseter muscle of the rabbit. Glands and muscle biopsies were carefully freed from contaminating tissues, weighed, and homogenized in an ice-cooled Potter-Elvehjem homogenizer with 9 parts (v/w) of 20 mM sodium phosphate, pH 7.0. 0.1 ml of the homogenate was solubilized with 1 ml of Soluen 350 (Packard Instruments Company Inc., Downers Grove, Illinois 60515) and mixed with 10 ml of Aquasol (New England Nuclear, Boston Mass. 02118) and left overnight before being counted. 0.1 ml of blood serum was treated in the same way. The same homogenates of the salivary glands were also assayed for LP activity, using 20–50 μl of homogenate, 7 mM guaiacol as chromogen[9], 200 μM H_2O_2, and pH 7.0[6]. Four animals of each species were used.

Results and discussion. In bovine sublingual glands LP was connected with fine granules in the cytoplasm of serous cells. The mucous cells were free from LP. Not all immunochemically LP-positive cells were organized as acini, many of them forming caps on the LP-negative acini as 'demilunes'. This observation was not confirmed by direct peroxidase activity staining. In bovine parotid glands LP could only dimly be seen in the apical portions of epithelial cells of the large excretory duct. In bovine lacrymatory glands LP was readily found in the apical portions of acinar cells, weakly seen in apexes of epithelial cells of the small ducts, and more definably detected in the marginal portions of the duct spaces[10].

LP activity and SCN^- were determined in a total of 14 glands. In the 3 main salivary glands of the rabbit no LP activity was found whereas the activity was high in the glands of the guinea-pig. Both rat strains showed a low but definite LP activity in the 3 salivary glands and a high activity in the lacrymatory gland (table 1). No difference was observed between the 2 rat strains. The present results on glandular LP are in accordance with observations on saliva[5].

There was no difference in [SCN^-] between glands with high, low, or no LP activity. The ratios between the serum, gland, and muscle [SCN^-] values were nearly constant in the 3 species (figs. 1a–c, and table 2). The concentrations indicate a uniform distribution between the 3 tissues with no preference for the salivary glands.

The occurrence of LP in the 3 salivary glands of the rat has been unequivocally proven. For the 3 species studied here, the present results clearly show that the concentration of the thiocyanate ion in salivary glands is unrelated to the occurrence of peroxidase activity in the glands. Thus, although limited in extent, this study invalidates the use of the thiocyanate concentration in a gland as an indicator of the gland's contribution to the joint antimicrobial activity.

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