raphy. No significant difference was observed between 2-naphthylamine and 1-naphthylamine. However, when the dose was reduced to 5 mg/kg, a dose at which 2-naphthylamine is carcinogenic and 1-naphthylamine is not, negligible quantities of *N*-oxidation products were found in dogs given 1-naphthylamine and considerable quantities of *N*-oxidation products (0.2 percent of the administered dose) were found in dogs given 2-naphthylamine (Fig. 2). The same dogs given 5 mg of 4-aminobiphenyl per kilogram of body weight produced yet higher amounts of *N*-oxidation products averaging about 1.5 percent of the administered dose.

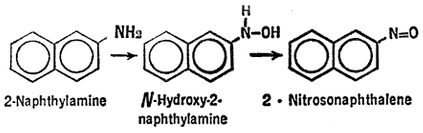

Fig. 1. Oxidation of 2-naphthylamine.

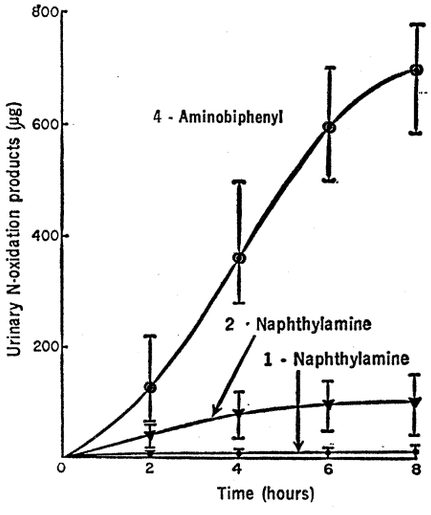

Fig. 2. Mean cumulative excretion of total *N*-oxidation products of four beagle dogs given single doses (5 mg/kg) of 1-naphthylamine, 2-naphthylamine, or 4-aminobiphenyl. Ranges indicated.

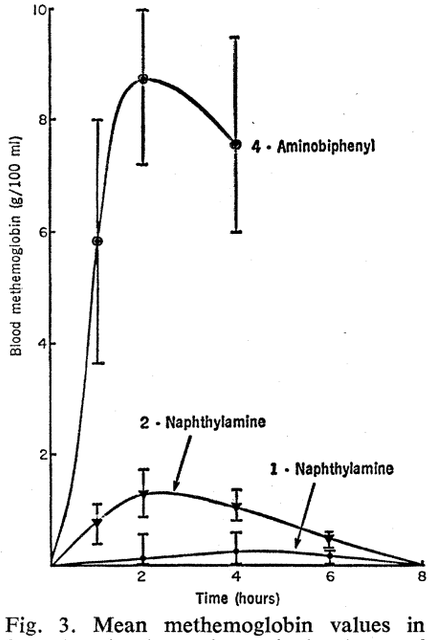

Fig. 3. Mean methemoglobin values in four beagle dogs given single doses of 1-naphthylamine, 2-naphthylamine (70 mg/kg), or 4-aminobiphenyl (25 mg/kg). Ranges indicated.

We believe that the *N*-hydroxy derivatives of aromatic amines are responsible for the methemoglobinemia commonly observed in man and experimental animals exposed to these substances (*6*). In working with large doses of 1-naphthylamine, 2-naphthylamine, and 4-aminobiphenyl in dogs, it became apparent that the most potent carcinogen, 4-aminobiphenyl, produces severe methemoglobinemia more readily than the other two substances. When the production of methemoglobinemia by 1- and 2-naphthylamine was compared quantitatively at a dose of 70 mg/kg, methemoglobin concentrations in the dogs given 2-naphthylamine reached a mean of 1.3 g/100 ml and concentrations in the dogs given 1-naphthylamine were consistently lower (Fig. 3). A dose of 70 mg of 4-aminobiphenyl per kilogram of body weight was rapidly fatal to dogs; and even when the dose was reduced to 25 mg/kg, three of the four dogs died. The maximum methemoglobin levels observed at this dose (Fig. 3) ranged between 7.2 and 10.3 g/100 ml.

When interest first began to develop in the role of *N*-hydroxylation in bladder cancer, we initiated an experiment to test the carcinogenicity of *N*-hydroxy-2-naphthylamine by direct repeated application to the dog bladder mucosa. A dimethylsulfoxide solution of *N*-hydroxy-2-naphthylamine (5 mg in 5 ml) was instilled by catheter into two male and two female beagles twice a month for 30 months. When these animals were killed 45 months after the first instillation, three had bladder tumors. Similar groups of four dogs each treated with 2-naphthylamine in dimethylsulfoxide or dimethylsulfoxide alone developed no tumors.

Thus the case for the role of *N*-hydroxylation in the production of bladder cancer by aromatic amines is complete. A correlation has been established between the carcinogenicity of these three aromatic amines, their methemoglobin-producing capabilities (a measure of *N*-hydroxylation in the blood), and the amount of *N*-hydroxy metabolites in the urine. Although the noncarcinogen 1-naphthylamine is *N*-hydroxylated, and the *N*-hydroxy metabolite is carcinogenic, negligible amounts occur in the urine at the doses usually given to dogs, or to which humans would be exposed. It, therefore, seems clear that *N*-hydroxy-2-naphthylamine or perhaps the closely related nitroso derivative is the active urinary carcinogens.

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References and Notes

1. J. W. Cramer, J. A. Miller, E. C. Miller, *J. Biol. Chem.* **235**, 885 (1960); J. A. Miller, J. W. Cramer, E. C. Miller, *Cancer Res.* **20**, 950 (1960).
2. W. Troll and N. Nelson, *Fed. Proc.* **20**, 41 (1961); E. Boyland, D. Manson, R. Nery, *J. Chem. Soc.* **114**, 606 (1962).
3. E. Brill and J. L. Radomski, *Life Sci.* **6**, 2293 (1967).
4. S. Belman, W. Troll, G. Teebor, F. Mukai, *Cancer Res.* **28**, 535 (1968).
5. W. B. Deichmann, J. L. Radomski, W. A. D. Anderson, E. Glass, M. Coplan, F. Woods, *Ind. Med. Surg.* **34**, 640 (1965).
6. M. Kiese, *Pharmacol. Rev.* **18**, 1091 (1966).

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Beta Glucuronidase Activity in Skin Components of Children with Cystic Fibrosis

Abstract. *As compared to that in normal children a decreased activity of beta glucuronidase, a lysosomal enzyme associated with mucopolysaccharide metabolism and with salt transport, has been detected in the epidermis and sweat gland tissues of children with cystic fibrosis.*

We have found a decreased activity of beta glucuronidase in certain skin components in children with cystic fibrosis. Cystic fibrosis is an inherited disease characterized by excretion of an abnormally viscous mucus from various exocrine glands and by excretion of abnormally high concentrations of salt in the sweat (*1*). The disease usually produces pulmonary infection and exocrine pancreatic deficiency and is usually fatal.

Beta glucuronidase is a lysosomal enzyme widely distributed in animal tissues. Linker *et al.* (*2*) observed that this enzyme along with other hydrolytic enzymes, degrades acid mucopolysaccharide fragments derived from hyal-

Table 1. Activity of beta glucuronidase in skin components from 15 patients with cystic fibrosis (mean age 8.5 years) and 15 controls (mean age 11 years). Units are expressed as micrograms of phenolphthalein released per gram of dry tissue per hour of incubation; t is the difference between the means divided by the standard error of the difference. The t values (P associated with this t value): sweat gland, $t = 2.9$ ($P = .01$); epidermis, $t = 2.6$ ($P = .02$); dermis, $t = 0.77$.

Patients with cystic fibrosis			Controls		
Sweat gland (units $\times 10^3$)	Epidermis (units $\times 10^3$)	Dermis (units $\times 10^2$)	Sweat gland (units $\times 10^3$)	Epidermis (units $\times 10^3$)	Dermis (units $\times 10^2$)
1.2	1.8	1.8*	2.7*	2.0	0*
	2.7	5.9*	3.1*	3.2	0*
2.9	2.9	5.0	2.2*	2.2	0*
1.9	2.0	1.4	1.3*	3.3	0*
1.5*	2.0	2.5	4.2	2.6	2.7
	1.5	3.7*	2.2	2.6	5.0
0.5*	1.2	2.8	2.3	2.0	4.8
1.5	1.6	4.1	2.9	2.5	3.1
1.4*	1.7	3.2	2.2*	1.9	1.5
2.1	2.4	1.0*	2.2	3.3	3.2
3.3	1.9	4.9	2.7	2.6	5.7
1.4	2.2	3.3	2.8	3.4	5.3
2.2	1.9	4.3	1.7	3.2	2.7
1.6	1.8	2.2	4.1	1.5	5.2
1.8	2.5	4.0	1.9	1.7	2.8
		Means ± standard deviations			
1.8 ± 0.7	2.0 ± 0.46	3.3 ± 1.4	2.6 ± 0.8	2.5 ± 0.6	2.8 ± 2.1

* This figure is not the average of three separate determinations.

uronic acid and chondroitin sulfate. Others have implicated the enzyme both in the synthesis and degradation of acid mucopolysaccharides. Montagna (*3*) found high activities of beta glucuronidase in human sweat gland, and Ballantyne and Wood (*4*) observed increased activity of the enzyme in the actively secreting salt glands of ducks that had been given a load-dose of salt. Thus, beta glucuronidase may be a necessary component of the salt-secretion mechanism, and may have some role in mucopolysaccharide metabolism.

We have determined the activities of several enzymes, including acid and alkaline phosphatase, ouabain-sensitive adenosine triphosphatase activated by Na^+ and K^+, phosphatidic acid phosphatase, and lactate, malate, glucose-6-phosphate, isocitrate, and succinic dehydrogenases (*5*) in components of freeze-dried skin isolated by microdissection from children with cystic fibrosis. We found no differences in enzyme activities between normal children and those with cystic fibrosis, with respect to enzyme activities until we assayed beta glucuronidase activity.

Skin biopsies were taken with a high speed drill and imbedded in tragacanth gel. The samples were frozen, cut into thin sections on a microtome, and dried in a vacuum. Fragments of epidermis, sweat gland, and dermis were isolated by microdissection and weighed on a quartz-fiber balance (*6*). Average weights for dermis, epidermis, and sweat gland were 9, 6, and 3 μg, respectively. Beta glucuronidase activity of the fragments was determined with phenolphthalein glucuronide as the substrate (*7*). The liberated phenolphthalein was measured on a spectrophotometer after the mixture was incubated for 24 hours. Kinetic studies indicated that the rate of release of product during the entire incubation period was linear. There is a statistically significant ($P = .05$, Student's t-test) decrease in beta glucuronidase activity in the epidermis and sweat gland of patients with cystic fibrosis (Table 1).

We do not know whether this decrease in enzyme activity represents decreased synthesis, abnormal enzyme structure, or enzymatic inhibition by some other substance. The decreased activity of beta glucuronidase would seem to indicate some defect more fundamental than general debilitation, inasmuch as a number of other enzymes assayed had normal activities (*5*). The finding (*8*) of metachromatic material (presumably mucopolysaccharide) accumulating in cultured cystic fibrosis fibroblasts is interesting in view of the connection between beta glucuronidase and mucopolysaccharide metabolism.

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References and Notes

1. P. A. di Sant' Agnese and R. C. Talamo, *N. Eng. J. Med.* **277**, 1287 (1967).
2. A. Linker, K. Meyer, B. Weissmann, *J. Biol. Chem.* **213**, 237 (1955).
3. W. Montagna, *J. Biophys. Biochem. Cytol.* **3**, 343 (1957).
4. B. Ballantyne and W. G. Wood, *J. Physiol. (London)* **191**, 89P (1967).
5. G. E. Gibbs, *Bibliogr. Paediat.* **10**, 95 (1967).
6. O. H. Lowry, *J. Biol. Chem.* **140**, 183 (1941).
7. G. E. Gibbs and G. D. Griffin, *J. Invest. Dermatol.* **51**, 200 (1968).
8. B. S. Danes and A. G. Bearn, *J. Exp. Med.* **129**, 775 (1969).
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Horseshoe Crab Lactate Dehydrogenase: Tissue Distribution and Molecular Weight

Abstract. *Lactate dehydrogenase from* Xiphosura (Limulus) polyphemus *is* D(−)*-lactate specific. It does not use* L(+)*-lactate, α-hydroxybutyrate, α-hydroxyvalerate, or α-hydroxyisocaproate as substrate. In most tissues lactate dehydrogenase is composed of five isozymes with a molecular weight of 140,000 for each, as judged by gel-filtration chromatography. This suggests that the isozymes are tetramers comprised of varying amounts of two physicochemically distinct subunits.*

Long and Kaplan observed that horseshoe crab, *Xiphosura* (*Limulus*) *polyphemus,* lactate dehydrogenase (LDH) is D(−)-lactate specific (*1*). Also, they reported that the molecular weight of this enzyme, as judged by gel filtration and ultracentrifugation, was approximately 70,000 or one-half that of vertebrate lactate dehydrogenase [E.C. 1.1.1.27 : L-lactate : nicotinamide-adenine dinucleotide (NAD) oxidoreductase] (*2*). This report confirms that *Limulus* LDH is D(−)-lactate specific. However, its molecular weight, as judged by gel filtration, is essentially identical to that of vertebrate LDH (approximately 140,000).

Because of the discrepancy between the molecular weights obtained for *Limulus* LDH, I compared it with a D(−)-lactate specific LDH from another invertebrate. It has been reported (*1*) that a D(−)-lactate specific LDH is present in the moon snail (*Gastro-*