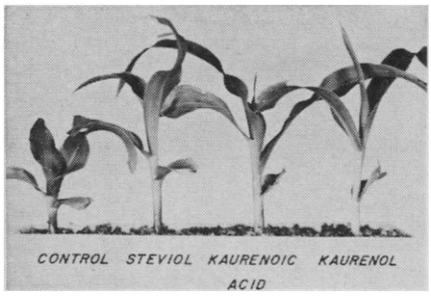

Fig. 2. Growth response of *d-5* mutants of maize to 100 μg of kaurenol, kaurenoic acid, and steviol. Seedlings were treated at the time of emergence of the first leaf from the coleoptile.

for biological activity and compared with gibberellic acid (GA-3) (*3*) and steviol. Two of these eight compounds, a kaurenoic acid (*4*) and a kaurenol, have been found to stimulate leaf sheath elongation for the *d-5* and *an-1* dwarf mutants of *Zea mays L.* Steviol, reported to be biologically active for the *d-5* mutant (*5*), was also active for the *an-1* mutant. Kaurene has been reported to be inactive as a gibberellin-like substance (*6*), although it is incorporated into GA-3 by the fungus *Fusarium moniliforme* Sheldon (see *7*).

All of the kaurene derivatives tested (Fig. 1, compounds 2–10) were found to be inactive in other gibberellin-elongation assays—tests on *d-1* and *d-2* mutants of maize (*2*), the rice seedling (*8*), the cucumber hypocotyl (*9*), the dwarf pea (*10*), and the morning glory seedling (*11*). Each compound was routinely tested in doses of 50 μg or 100 μg. All compounds were applied in aqueous solution or in 95 percent ethanol.

At a dosage of 100 μg, the amount of seedling elongation for the *d-5* mutant was approximately the same for the kaurenoic acid, the kaurenol, and steviol (Fig 2). The lengths of leaf sheaths of treated mutants were on the average 3.5 cm greater than those of non-treated dwarf controls. In this test, a response 1.2 cm over the mean shown by leaves of dwarf controls was significant at the 5-percent level. When the responses to 50-μg doses were compared to a standard curve of response to GA-3, the kaurenoic acid and the kaurenol were approximately 1 percent as active as GA-3 for the *d-5* mutant. Steviol has also been reported to be appreciably less active than GA-3 for the *d-5* mutant (*4, 5*). The type of growth response associated with the kaurenoic acid and the kaurenol was visually indistinguishable from a gibberellin response; 10-μg dosages applied at daily intervals over a period of 10 days resulted in *d-5* seedlings which appeared very similar to nontreated normals.

Comparison of the three biologically active kaurene derivatives with kaurene suggests that neither oxygenation of the C-19 position to the acid, nor hydroxylation at the C-13 position (as in steviol) is necessary for biological activity in this system (*d-5* and *an-1* mutants). It would also appear that the exocyclic ethylenic bond of the kaurene ring system is necessary for biological activity in the *d-5* and *an-1* assays, since the norketo derivatives of the kaurenoic acid and the kaurenol (I-4 and I-5) were inactive, as were the kauranoic acid (II-6) and the C-17-oxygenated derivatives of the kauranoic acid (II-7, II-8, and II-9).

The biological activities of the kaurene derivatives and the gibberellins may reflect similarities in active sites. However, it is also possible that these compounds are metabolically related, such that the kaurenoic acid, the kaurenol, and steviol are precursors that can be converted to gibberellins by the *d-5* and *an-1* mutants of maize.

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Intestinal Disaccharidases: Absence in Two Species of Sea Lions

Abstract. *Pups of both the California and Stellar sea lions have no intestinal enzymes for hydrolysis of sucrose, lactose, cellobiose, or trehalose. If these animals were given lactose or sucrose, they developed severe fermentative diarrhea and weight loss similar to the clinical syndrome encountered in infants with either hereditary or acquired intolerance to disaccharides.*

In 1962 Pilson and Kelley (*1*) reported that lactose was absent from the breast milk of the California sea lion (*Zalophus californianus*). This milk contained no detectable carbohydrate (*2*). Because of the possible role for substrate as an inducer of enzymes during development, it was of some interest to ascertain whether lactase or any other disaccharidase was present in the intestine of these animals and whether the sea lion could digest various disaccharides.

Sea lion pups (6 to 8 weeks old) were captured on the Coronado Islands, Mexico, and were housed at the hospital of the San Diego Zoo in a cage containing a pool of fresh water (*3*). Although feeding with nipple and bottle has been successful for pups of the Stellar sea lion (*Eumetopias jubata*) (*4*) and of the walrus (*Odobenus divergens*) (*5*), our pups of the California sea lion were force fed through a gastric tube which was passed through a hole in a rubber mouth gag, a method similar to that employed in feeding the sand seal (*Phoca vitulina*) (*6*). Initially, a mixture containing 10 percent casein and 32 to 35 percent cod liver oil was used for the diet. However, since casein contains approximately 0.75 percent lactose, boiled eggs were substituted to supply the needed protein; vitamins B and C were added daily. In more recent studies a diet containing 10 percent meat-base protein (*7*) and 35 percent corn oil has been used.

The animals were tested for their capacity to digest and absorb carbohydrates. Either glucose, lactose, or sucrose (1.75 to 2.0 g per kilogram of body weight) was administered by gastric tube as a 15 percent solution. Initially, blood was obtained from the brachial vein just lateral to the axilla, but in later studies samples were taken by cardiac puncture. Glucose was measured by the glucose oxidase method on a Somogyi filtrate of blood (*8*).

In order to assay for intestinal disaccharidases, some animals were killed and the intestine was removed quickly and washed with isotonic potassium chloride. The mucosa was scraped free and homogenized in 0.65*M* mannitol. The disaccharidases were assayed by the method of Doell and Kretchmer (*9*). Protein was determined by the method of Lowry (*10*). Alkaline phosphatase was assayed by the method of Moog (*11*). Carbamyl phosphate synthetase and ornithine transcarbamylase were measured by the method of Brown and Cohen (*12*). The animals were fed three to four times daily with a total of 1.5 to 2.0 liters of formula per 24 hours. They were also weighed daily. Regardless of diet, the animals showed only slight increases in weight.

Figure 1 illustrates the clinical course of one of the animals. After lactose and sucrose were given the animals developed lassitude, anorexia, vesicles on their flippers and skull, severe fermentative diarrhea, and weight loss. The sea lion pups had clinical signs similar to those described for infants with either hereditary (*13*) or acquired (*14*) intolerance to disaccharides. Some, but not all, of the animals developed a very mild and transient diarrhea of three to four loose stools after the ingestion of glucose.

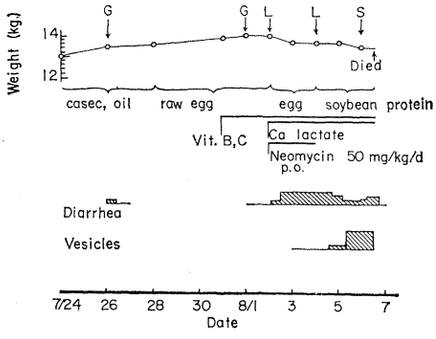

Fig. 1. Clinical course: *Zalophus californianus*. *G*, *L*, and *S* refer, respectively, to administered glucose, lactose, and sucrose.

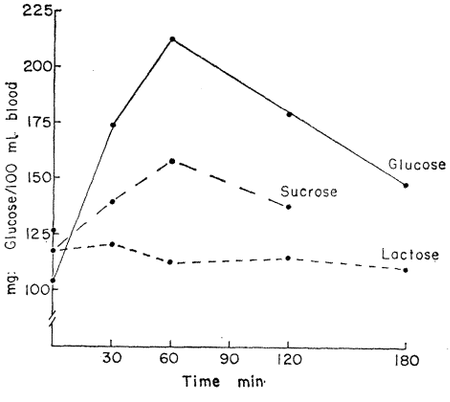

Fig. 2. Oral carbohydrate tolerance tests in *Zalophus californianus*; 1.75 to 2.0 g/kg of each carbohydrate was administered orally.

Table 1. Activity of intestinal enzymes in human, rat, and sea lion.

Enzyme	Human	Rat	*Zalophus californianus*
Lactase *	10.8	10.2	0
Invertase *	7.2	3.0	0
β-galactosidase *	1.3	3.5	0
Trehalase *		2.8	0
Cellobiase *		1.7	0
Alkaline phosphatase †		12.4	3.4
Carbamyl phosphate synthetase ‡		14	7.0
Ornithine transcarbamylase ‡		1372	314

* Units expressed as micromoles of glucose per 60 minutes per milligram of protein. † Units expressed as micromoles of phosphate per 10 minutes per milligram of protein. ‡ Units expressed as micromoles of critrulline per 60 minutes per gram of wet tissue.

Figure 2 illustrates this same animal's response to oral administration of carbohydrates. After the adminstration of glucose there was a rapid elevation of the glucose concentration in the blood. No elevation of glucose occurred after the administration of lactose.

The animal was ill when the sucrose tolerance was determined and the test showed only a minimal elevation (32 mg/100 ml) in the concentration of glucose in the blood.

We have attached no particular significance to this minimal elevation and have attributed it to a nonspecific stress reaction in a sick animal. It may have also been due to the fact that these animals have a unique capacity to alter their peripheral blood flow, and we were, on occasion, measuring the concentration of glucose in a pooled area of blood.

In subsequent studies with animals who were not ill and where blood was obtained by a direct cardiac puncture, there was no elevation of glucose after administration of lactose or sucrose.

Activities of various intestinal enzymes of sea lion pups, baby rats, and infant humans are compared in Table 1. Even though up to 50 times the amount of tissue ordinarily required for similar assay of enzyme in rat or human intestine was used, no lactase, sucrase, trehalase, cellobiase, or β-galactosidase (with orthonitrophenol galactoside as the substrate) was detected in the intestine of the sea lion. Alkaline phosphatase, carbamyl phosphate synthetase, and ornithine transcarbamylase were present and active. Also, no activity of lactase or sucrase could be demonstrated in the intestine from an adult *Zalophus californianus*.

Initial studies performed on the intestinal mucosa of the Stellar sea lion pup (*Eumetopias jubata*) (*15*) also indicated the absence of lactase, sucrase, trehalase, and cellobiase. However, the mucosa contained a very low activity of maltase, about 5 to 10 percent of that found in the baby rat. When lactase was fed to these animals there was no elevation of glucose in the blood but the animals developed severe diarrhea and excreted large amounts of lactose in stools and urine.

These animals (*16*) can be used for the investigation of the aberrant physiology and biochemistry encountered in infants with a hereditary or acquired intolerance to disaccharides.

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