malabsorption of nutrients could produce floral changes or inhibit certain bacterial enzymes. In this regard, 7 α-dehydroxylase, the bacterial enzyme responsible for the anaerobic and irreversible dehydroxylation, which affects the bulk of intestinal bile acids, is inhibited by dehydroxylated fatty acids[17]. However, results in the 4 CF children without pancreatic insufficiency (CF-1) do not favour that hypothesis. Although the present studies were carried out while patients were off antibiotics, it is possible that the intestinal flora could be chronically altered because of intermittent but prolonged periods of antibiotic therapy. This would best account for the observation that the percent lithocholic acid generated by CF-1 stools incubated with chenodeoxycholic was lower than the percent obtained in controls.

Bile acids, especially in their unconjugated form, are inhibitory to anaerobic intestinal microorganisms[18]. Because of the large fecal losses of bile acids in CF with pancreatic insufficiency[14], it is reasonable to suppose that the anaerobic flora responsible for the 7 α-dehydroxylation of cholic and chenodeoxycholic acid[18] is reduced. In view of the reported inhibition of 7 α-dehydroxylase by an excess of substrate[19], the possibility that the decreased dehydroxylation could be due to large concentrations of free primary bile acids in CF stool homogenates cannot be dismissed. However, the concentrations necessary to achieve substrate inhibition[15] are far in excess of concentrations we have found in CF.

The pathogenesis of bile acid malabsorption in CF remains obscure. Although free bile acids are absorbed faster than conjugated ones[20], the present findings do not suggest that the hydroxylase bacterial enzymes could be a limiting factor. The impaired capacity of CF stool homogenates to dehydroxylate bile acids is not likely to be responsible for the large fecal sequestration of bile acids in CF, since free and dehydroxylated bile acids are probably equally well absorbed by the ileum[20]. In fact, impaired dehydroxylation would favour bile acid absorption in the colon since deoxycholic acid, readily bound to dietary residues and bacteria, and lithocholic, precipitated out of solution, are largely unavailable for absorption[21].

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Olfactory discrimination between glycine and deuterated glycine by fish

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Summary. Electrophysiological and behavioural experiments showed that whitefish (Coregonus clupeaformis) were able to discriminate between glycine and fully deuterated glycine by olfaction, while both chemicals stimulated the olfactory receptors to induce bulbar responses of similar magnitude.

Fish may detect various chemical stimuli and respond by changing their behavioural patterns. These may be characterized as either avoidance or preference depending upon the stimuli perceived. These chemical stimuli are detected through at least 2 different sensory channels, olfaction and taste, as in terrestrial vertebrates. In fish, both olfaction and taste take place entirely in the aquatic environment. The carrier of stimulant molecules is water, therefore chemicals that are detected olfactorily need not be volatile. Thus the spectra of chemicals detected by fish could be entirely different from those detected by terrestrial animals. One of such chemical classes is the amino acid. Electrophysiological studies indicate that certain amino acids, which are normally non-odourous to humans, are extremely effective olfactory stimuli and may play an important role, such as acting as chemical signals, in olfactory communication in fishes[2–5]. Recent investigation of the specificity of olfactory stimulation by amino acids and analogues has led to the establishment of definite structure-activity relationships, and further suggested the nature of a possible receptor membrane binding site which involves 2 charged subsites, one cationic, one anionic, capable of interacting with ionized α-amino and α-carboxyl groups of amino acid molecules[6,7]. The substitution of an isotopic atom such as deuterium for protium in a molecule containing hydrogen should leave essentially unchanged those molecular properties that are associated with electronic structure and force fields. In contrast, molecular properties dependent upon mass are changed[8]. If the olfactory receptor for amino acid detection involves an electric field that fits the receptor site mentioned, the replacement of hydrogen with deuterium should not change the stimulatory effectiveness of a molecule. If it involves molecular motions or dipole moments[9,10], the isotopic effects should cause

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changes. Here I present evidence that the amino acid glycine (Gly) and fully deuterated glycine (Gly-d_5) (both obtained from Aldrich Chemical Co., 98% purity) have similar stimulatory effectiveness indistinguishable electrophysiologically, but that fish are able to discriminate both compounds olfactorily when tested behaviourally. The electrical responses were measured in the olfactory bulb of whitefish (Coregonus clupeaformis) when the paired nares were stimulated with solutions of Gly and Gly-d_5 according to the method described previously[11,12]. Gly is the smallest of amino acid molecules that fulfill most of the requirements for olfactory stimulation in fish[6]. The integrated bulbar responses to Gly and Gly-d_5 increased exponentially with a logarithmic increase in stimulus intensity until the response reached a maximum. No significant difference in the stimulatory effectiveness was observed between 2 compounds over the concentration range tested (figure 1). The cross-adaptation experiments performed indicate that both Gly and Gly-d_5 may stimulate the same recptor site.

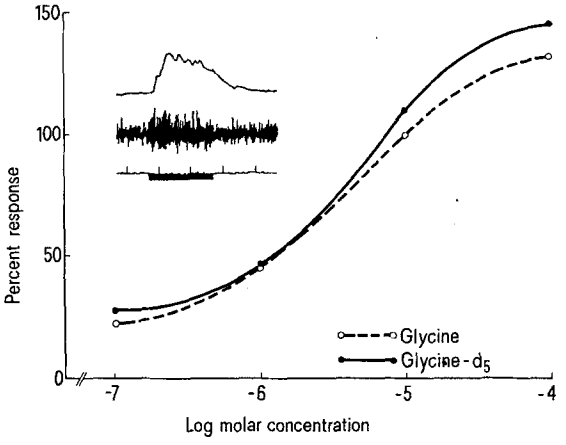

Fig. 1. Relations between the olfactory bulbar responses and the concentrations of glycine and glycine-d_5. The inserted record shows a typical response induced by 10^{-6} M glycine, where the top record indicates an integration of the below. The period of stimulation and time marker indicating 1-sec-intervals is shown at bottom.

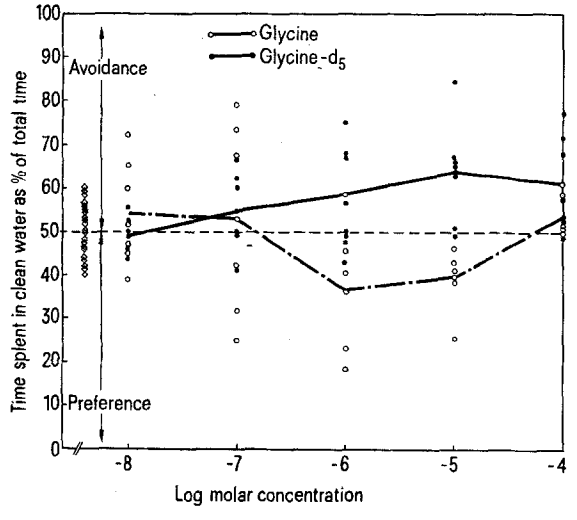

Fig. 2. Avoidance and preference reactions of whitefish to glycine and glycine-d_5, with performance of each fish treated as a graded response. The lines represent mean reactions at each concentration. Diamonds at far left represent the control response of fish with clean water in both halves of the trough.

The behavioural reactions of whitefish to Gly and Gly-d_5 were tested in an avoidance/preference trough (plexiglass, 17 × 17 × 120 cm). Details of the setup and experimental procedures are described elsewhere[13,14]. Briefly, water flows into each end and out the centre of the trough, with test solution on one or other side. With appropriate flow velocity (0.6 cm/sec in the present experiment), a distinct separation between the 2 bodies of water at the centre was obtained. Following the initial accustomization for about 5 min, whitefish generally swam back and forth across the centre line, with only occasional stops. In each test the following procedure was adopted: a) with clean water in both halves of the trough, the time which a fish spent in one side was calculated as a percentage of the total test time of the 5 min (control recording), b) upon introduction of a test chemical in either side, the time spent by the fish in the clean side was calculated as in a. The mean value of time-responses during control recording for all tests was close to 50% (neutral reaction; diamonds in figure 2). Time-responses over 50% were considered to be avoidance, and time-responses less than 50% were considered to be preference. Test solutions were introduced into either side at random. Each fish was tested only once to one concentration of chemical at a time.

Whitefish avoided Gly-d_5 at all concentrations tested, except 10^{-8} M at which no appreciable reaction was observed (figure 2). On the contrary, they showed a diverse reaction pattern to Gly over the concentration range tested. At 10^{-8} M, some fish definitely avoided Gly, but others did not avoid it. 3 fish avoided Gly at 10^{-7} M, but 3 preferred it, resulting in a neutral reaction. Clear preference was observed at 10^{-6} and 10^{-5} M. All fish showed slight avoidance to Gly at the highest concentration, 10^{-4} M. Such biphasic reactions of fish to chemicals are not unusual[14]. Since the preference reactions to Gly were eliminated after the nares of fish were cauterized, it is clear that the behaviour reactions observed are mediated primarily through olfaction.

The results clearly indicate that whitefish were able to discriminate between Gly and Gly-d_5 by olfaction, in spite of the fact that both chemicals stimulated the olfactory receptors to induce bulbar responses of similar magnitude. Previous studies have suggested that stimulatory effectiveness depends upon interaction of an amino acid molecule with a receptor membrane structure of definite shape, size and charge distribution[5,6]. Furthermore, a receptor site can be considered that involves 2 charged subsites capable of interacting with ionized amino and carboxyl groups of amino acid molecules. Since deuteration should leave essentially unchanged molecular shape and those molecular properties associated with electronic structure and force fields, but alter molecular motions, the present results suggest that olfactory stimulation by amino acids in fish involves an electric field that fits a receptor site mentioned, and further that the discriminatory process may be associated with other molecular properties such as molecular motions.

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