large amounts of histamine from a histidine solution. The samples contained, at the end of incubation, 190 to 210 mg of histamine per 100 g of fresh substance. This spoiled fish meat was fed to dogs, cats, rats, and mice. Guinea pigs do not spontaneously consume fish tissue. These animals received, by stomach tube, 10 ml of a concentrated aqueous extract containing 80 to 100 mg of histamine made from spoiled fish. No symptoms of poisoning could be observed in these experiments, and all the animals survived.

These experiments do not support the contention that histamine present in spoiled fish is the cause of fish poisoning. Instead, they indicate that other toxins produced by certain toxin-forming microorganisms in the course of spoilage may be mainly responsible for toxic symptoms. The possibility that histamine may participate in cases of human fish poisoning cannot be entirely eliminated, because highly seasoned hot dishes prepared from spoiled fish, or simultaneous consumption of alcoholic beverages, may possibly facilitate the intestinal passage of histamine.

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## On the Mechanism of an Anaerobic Exchange Reaction Catalyzed by Succinic Dehydrogenase Preparations


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In the course of studying the mechanism of enzymatic hydrogen transfer reactions, it became necessary to investigate further (*1*) an exchange reaction reported by Weinmann *et al.* in which dideuteriosuccinic acid was converted to normal succinic acid when it was incubated anaerobically with a rabbit kidney succinic dehydrogenase preparation (*2*).

When normal succinic acid was incubated anaerobically in a medium containing $D_2O$ and a Keilin-Hartree heart-particle preparation made as is described by Slater (*3*), the exchange reaction was extremely slow compared with the possible rate of aerobic succinic acid oxidation (Table 1, experiment 1). However, it can be seen that initially added fumaric acid greatly stimulated the incorporation of deuterium into succinic (Table 1, experiment 2).

Table 1. Influence of fumarate on deuterium incorporation into succinate. The reaction mixture contained 2.44 mM of potassium phosphate buffer, *p*H 7.29; 11.0 mg of cytochrome *c*; 1.0 ml of an enzyme capable of oxidizing, under similar conditions, 418 μM of succine acid per hour with succinate as the sole substrate or 319 μM of succinic acid per hour when succinate plus fumarate were present; the fumarase equilibrium was established about the fourth hour. The total volume was 16.5 ml, having $D_2O$ concentration by volume of 88.2 and 86.3 percent for experiments 1 and 2, respectively. Succinate was tipped into the main body of the Thunberg tube after the reaction mixture had been flushed with purified nitrogen gas and the tubes had been evacuated with an oil pump. Incubation time, 8 hr; temperature, 37°C.

| Expt. | Acids added initially | Acids isolated at end of reaction | Deuterium (atoms/molecule) |
|---|---|---|---|
| 1 | 1 mM succinic | Succinic | 0.0310 |
| 2 | 1 mM succinic | Succinic | 0.460 |
| | + | Fumaric | 0.0188 |
| | 1 mM fumaric | Malic | 0.779 |

These results are most readily explained by the following equations.

$$^{-}OOC—CH_2—CH_2—COO^{-} + 2X \rightarrow {}^{-}OOC—CH{=}CH—COO^{-} + 2X^{-} + 2H^{+} \quad (1)$$

$$^{-}OOC—CH{=}CH—COO^{-} + 2X^{-} + 2D^{+} \rightarrow {}^{-}OOC—CHD—CHD—COO^{-} + 2X \quad (2)$$

Equation 1 represents a reduction of a natural carrier $X$ by succinate, and Eq. 2 represents the reversal of this reaction in heavy water. Alternatively, the reduction of $X$ would yield $XH_2$, the hydrogens of which would be rapidly exchangeable with the medium $D_2O$. The resulting $XD_2$ would subsequently be reoxidized by fumarate, yielding $X$ and deuteriosuccinate. Spectrophotometric evidence for the reversal of reaction 1 with a partially purified enzyme preparation has recently been presented by Green *et al.* (*4*). According to this scheme, the addition of fumarate would promote the incorporation of deuterium into succinate by establishing a new equilibrium position in which the rates of both reactions 1 and 2 would be increased. The deuterium appearing in the succinate should, therefore, arise directly from the medium and not from a reduction of labeled fumarate.

The enzyme preparation used in these experiments contained sufficient fumarase to establish the fumarate-malate equilibrium. Hence, the finding of deuterium in succinate and the stimulation of this deuterium incorporation by fumarate may have reflected an initial incorporation of deuterium into fumarate via the fumarase equilibrium, provided that this enzyme behaved in a nonstereospecific manner with respect to the hydrogen atoms on the methylene carbon of malate. To test this possibility, all three acids from the reaction mixture, in which both succinate and fumarate were incubated anaerobically

with the succinic dehydrogenase preparation in $D_2O$, were isolated, and their respective deuterium content was determined. The results are outlined in Table 1, experiment 2. Thus, while the malate exhibited a high deuterium content, as was anticipated, the fumarate had an exceedingly low deuterium content. Since the reaction proceeded long after the fumarase equilibrium had been reached, ample opportunity had been allowed for the fumarate to become more highly labeled if the fumarase had behaved nonstereospecifically.

Similar results have been obtained by Fisher (*5*) using crystalline fumarase. These experiments thus establish the strict stereospecific behavior of fumarase and add strength to our proposed mechanism of the anaerobic exchange reaction as catalyzed by succinic dehydrogenase.

The afore-described results with fumarase, as well as the recent studies on alcohol dehydrogenase (*6, 7*), have shown that these enzymes are endowed with the property of distinguishing between two hydrogen atoms on a methylene carbon. In order to test the possibility of a similar behavior by succinic dehydrogenase, deuteriosuccinate, obtained from an anaerobic incubation of succinate and fumarate in $D_2O$ with a succinic dehydrogenase preparation under conditions similar to those reported in Table 1, experiment 2, was oxidized aerobically with the same enzyme, and the deuterium content of the resulting fumarate was measured. The results are given in Table 2.

The results clearly indicate that the fumarate obtained from the enzymatic oxidation of the enzymatically exchanged deuteriosuccinate retains about one-half of the deuterium, leading to the conclusion that succinic dehydrogenase is incapable of distinguishing between the hydrogens on either methylene carbon. This conclusion is based on the assumption that a single enzyme is responsible for the exchange reaction represented by Eqs. 1 and 2, and that this enzyme is also the one concerned in the aerobic oxidation of succinate. The seeming discrepancy between the deuterium content of the succinate before and after 50 percent of it had been oxidized is probably the result of an isotope effect that causes preferential oxidation of the unlabeled succinate molecules (*8*) and leads to an apparently higher deuterium content of the residual succinate and a somewhat lower than theoretical content of the resulting fumarate.

Table 2. Enzymatic oxidation of enzymatically labeled succinate.

| Acids | Deuterium (atoms/molecule) |
|---|---|
| Deuteriosuccinate obtained from an anaerobic enzymatic exchange reaction | 0.100 |
| Succinate after 50 percent of above deuteriosuccinate was enzymatically oxidized | 0.135 |
| Fumarate resulting from the deuteriosuccinate oxidation | 0.044 |

The small amount of deuterium found in the fumarate in experiment 2 of Table 1 probably arose from the initially formed deuteriosuccinate by a nonstereospecific reversal of reaction 2, and its low value probably reflects the dilution of the isotope by the initially added normal fumarate. However, in other experiments not reported here, a greater incorporation of label into fumarate was observed, the reasons for which are not clear at present. It was found, however, that this increased labeling depended on the addition of succinate, as would be expected from the scheme proposed here.


**References and Notes**

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## Fertilization and Normal Development of Follicular Oocytes in the Rabbit


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The growth, maturation, and activation of rabbit follicular oocytes *in vivo* and *in vitro* (*1*) and the nuclear maturation of rabbit oocytes in culture and their fertilization in Fallopian tubes (*2*) have been studied. In the rat, the fertilization and development of follicular oocytes when transferred into the ovarian bursa of mated rats have been reported (*3*). This paper is concerned with the fertilization and normal development of follicular oocytes of rabbits recovered at various times after gonadotropic hormone injection (*4*).

The donor rabbits were intravenously injected with sheep pituitary extracts to induce ovulation, which occurs, as in the case of mating, about 10 hr later. At various intervals after injection, the ovaries were removed and suspended in serum-saline medium (equal volumes of rabbit serum and 0.9 percent NaCl) in a watch glass. Under a dissecting microscope, the follicles were broken with a needle, and the oocytes surrounded by mucous follicular cells were picked up with a pipette. In order to facilitate the manipulation of oocytes, they were treated with 0.05 percent hyaluronidase in serum-saline medium for 5 to 10 min and then were resuspended in serum-saline medium. In addition, the oocytes recovered from uninjected ani-