The results suggest that this gecko contains in its retina a photosensitive pigment comparable to that found in other geckos (*1*). This unusual group of visual pigments is characterized by absorption spectra located, not in the general region of 500 mμ, the typical position for the visual pigments of terrestrial animals, but in the general region of 520 mμ. The pigment from *Oedura* is very probably a visual pigment. This is indicated by its photosensitivity, by the presence of retinene, and by the fact that the difference spectrum agrees well with the construction based on Dartnall's nomogram (*2*) for the visual pigments. The possible biological significance of this group of visual pigments has been discussed in a previous report (*1*).

The spectrum obtained (Fig. 1, top, curve 2) after the initial bleaching with light at 606 mμ is very informative because it possesses a distinct upward inflection in the region of 460 mμ. This is an important point because it suggests the presence, in the solution from which the 518_1 pigment had been removed, of a blue-absorbing component. The next two exposures show that this inflection was abolished by light of wavelength shorter than 606 mμ. The first of these exposures was to light at 560 mμ. This caused a very small change, but the important information conveyed by this bleaching was that the 518_1 pigment had, in fact, all been removed by the previous long exposure to light at 606 mμ. The final exposure was to white light. This led to a disappearance of the inflection (Fig. 1, top, curve 4). The difference spectrum which resulted from these two final bleachings (Fig. 1, bottom, curve 3) revealed the occurrence of a small but definite density loss, maximal at about 457 mμ, and a density gain at about 360 mμ.

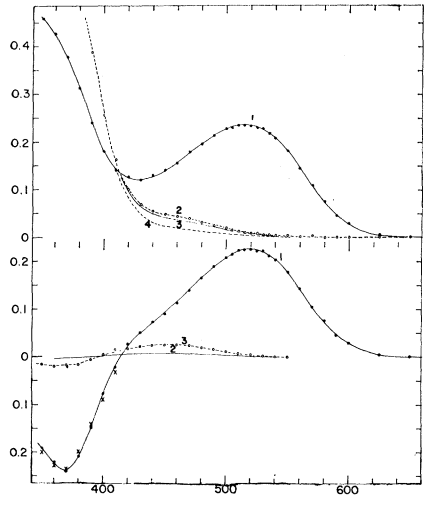

Fig. 1. (Top) Curve 1, absorption curve of unbleached extract; curve 2, result of exposure to light at 606 mμ; curve 3, result of exposure for 125 minutes to light at 560 mμ; curve 4, result of exposure to tungsten light (40 watts) for 10 minutes. (Bottom) Corresponding difference spectra. Curve 1 is the 1–2 difference spectrum. The points indicated as *X* show the data obtained with a retinene$_1$ oxime in 2-percent digitonin. Curve 2 is the 2–3 difference spectrum; curve 3, the 2–4 difference spectrum.

The evidence supports the idea that a blue-sensitive pigment was in fact a component of the original retinal extract of this gecko. The results of both experiments point to the fact that, following removal of the 518_1 pigment, there occurred in response to appropriate illumination a selective change as described. The important feature of this finding is that it was obtainable in an extract containing NH_2OH, a substance which is known to be useful in preventing isomerizing or other side reactions of the products of bleaching. Moreover, the presence of a clear inflection in the spectrum following the removal of the 518_1 pigment is evidence which cannot be easily ignored. The only point in question in this case is whether the blue-absorbing pigment was a component of the original unbleached extract or was a product formed as a result of bleaching the 518_1 pigment. It could, for example, have been a regenerated pigment. Hydroxylamine, by combining with retinene, effectively prevents regeneration in extracts of the retina. In any case no evidence was obtained of regeneration during the 45-minute period which was required to determine the absorption curve. The argument that a secondarily formed photosensitive pigment was present is not easily disposed of; to refute it would require experiments in which the main pigment is left untouched. Whether such experiments can ever be satisfactorily carried out is questionable; lack of animals has thus far prevented attempts in this direction.

A comparison of the difference spectra of this presumed blue-sensitive pigment (a pigment with peak absorption at 457 mμ was assumed) with the curve constructed from Dartnall's nomogram (*2*) shows that the data fit well, considering the small magnitude of the difference spectra. This agreement accords with the idea that the *Oedura* retina contains a visual pigment with absorption in the blue region of the spectrum. The photolabile substance riboflavin has been detected in the vertebrate retina (*3*). It is clear, however, that the blue-sensitive pigment of *Oedura* is not riboflavin. A solution of riboflavin in 2-percent digitonin yielded, after illumination, a difference spectrum with two peaks, one at 373 mμ and the second at about 453 mμ. The 453 mμ was significantly narrower than the 457 mμ spectrum of *Oedura*.

The literature on visual pigments includes a number of references (*4*) which suggest the occurrence of blue-sensitive pigments in the retinae of various vertebrates. Some of these claims are based on inadequate or even questionable experimental procedures, so it is not surprising that the claims have provoked criticism. This report, which is the first account of a blue-sensitive component in the retinae of lizards, is unique for two reasons: (i) Isomerizing actions, which could confuse the interpretation, were reduced to a minimum, and (ii) the pigment in question was demonstrated to be present in the extract before the bleaching employed to remove it.

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5. This study was aided by a grant from the Division of Research Grants and Fellowships, National Institutes of Health, U.S. Public Health Service, and by a grant from the University of California Board of Research.

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A Mechanism for Pyridine-Nucleotide-Dependent Dehydrogenases

Most dehydrogenases, which utilize DPN (*1, 2*) or TPN as hydrogen acceptors, can be classified as either alcohol or aldehyde dehydrogenases. A large body of evidence, accumulated in recent years, indicate that fundamental differences exist between these two classes of enzymes. The enzymes which can be classified as "alcohol" dehydrogenases oxidize primary alcohols to aldehydes, secondary alcohols to ketones, primary amines to ketones and ammonia, and hemiacetals to lactones (*3*). Examples of each subgroup are alcohol dehydrogenase, lactic dehydrogenase, glutamic dehydrogenase, and glucose-6-phosphate dehydrogenase, respectively. The mechanism proposed in this paper is meant to be applicable only to this group of enzymes and is not to be applied to aldehyde dehydrogenases.

In recent years great effort has been expended in elucidating detailed properties of these dehydrogenases. Among the many significant findings, some require special enumeration. First, the enzymes act by direct hydrogen transfer in a stereospecific fashion both with respect to the substrate and with respect to the pyridine ring (*4*). The reversible oxidation-reduction site in the coenzyme molecule is the *para* position of the pyridine ring (*5*). Second, it has been shown that

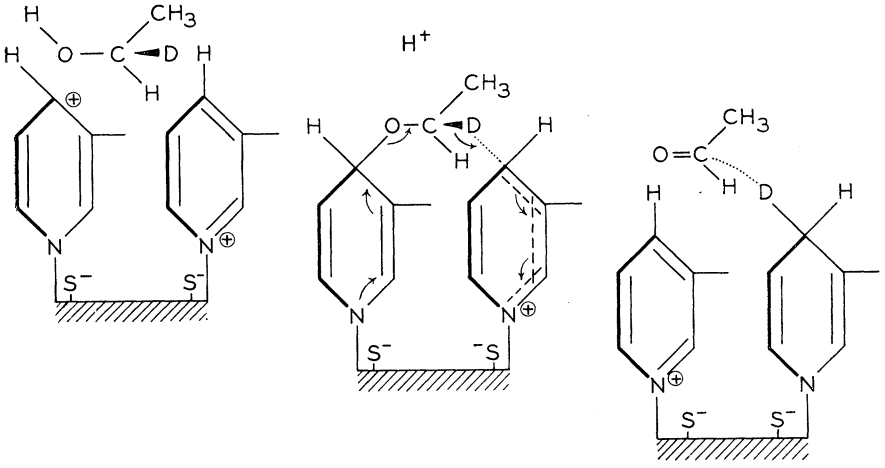

Fig. 1. Proposed mechanism of hydrogen transfer from substrate to coenzyme. The substrate is depicted, for clarity, as deutero-ethanol.

in many instances the presence of a dehydrogenase will promote a much more favorable addition reaction between nucleophilic substances and the pyridine coenzymes, as compared with the extent of addition in the absence of enzyme (*6, 7*). The nucleophilic substances which show this favored addition fall into two classes (*7*): (i) ions, such as sulfide, bisulfite, and cyanide, are bound with a stoichiometry of 1 mole per mole of DPN bound to the enzyme, and (ii) substances which resemble the substrate in structure are bound with a stoichiometry of 1 mole per 2 moles of DPN bound maximally. All substances which show this favored addition on a dehydrogenase are competitive inhibitors of the corresponding substrates. Finally, a number of these dehydrogenases are known to be zinc proteins (*8*), though some do not appear to contain this metal, notably rat liver lactic dehydrogenase (9).

To explain the above observations, the following unified theory for the mechanism of action of the "alcohol" group of dehydrogenases is offered for consideration. This theory is schematically summarized in Fig. 1. The enzyme binds 2 moles of DPN or a multiple thereof. The substrate, depicted here as monodeuteroethonal, binds through the hydroxyl group (or amino group) to the *para* position of one of the DPN molecules, with the release of a hydrogen ion. The second molecule of DPN will then accept a hydride ion (in this case a deuteride ion) from the substrate, thereby being converted to the reduced form. This transfer of a hydride ion can be realized by a simple electron shift from the nitrogen of one DPN molecule to the nitrogen of the second molecule. When the product is released, the first DPN molecule is regenerated. Both DPN molecules are equally effective in accepting substrate or hydrogen. It must be emphasized that no bond between substrate and protein is required.

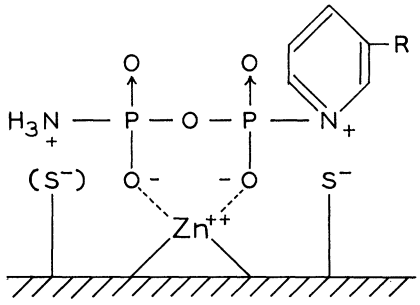

Fig. 2. Suggested generalized mode of binding of DPN to the dehydrogenases. The adenine amino group may or may not be bound to a sulfhydryl grouping. The zinc binds the pyrophosphate, and the pyridinium nitrogen is linked to a sulfhydryl group.

This mechanism is consistent with the experimental observation of direct hydrogen transfer between substrate and coenzyme. Since addition complexes between nucleophilic agents and DPN form at the same position of the DPN molecule as does the substrate complex, the ternary complexes between enzyme, coenzyme, and substrate analog are understandable. This would also explain the competitive inhibition observed between the substrate and the complexing agents. The mechanism is in accord with the discrepancy observed in stoichiometry between ions such as cyanide and bisulfite and agents which resemble the substrate in structure. Lastly, the theory takes into account the known site of reduction of DPN and also the position of the pyridine ring where formation of the complex occurs.

Kinetically, this picture results in a complex series of equilibria, which can only be approximately treated by simplified forms of rate equations. The abnormal kinetic behavior of the dehydrogenases, especially their substrate inhibition, would be a logical consequence of this mechanistic picture.

As indicated above, this mechanism would imply the following sequence of reactions:

$$E + \text{DPN} \rightarrow E\text{-DPN} \quad (1)$$

$$E\text{-DPN} + \text{DPN} \rightarrow E\text{-DPN}_2 \quad (2)$$

$$E\text{-DPN}_2 + S \rightarrow E\text{-DPN-DPN}.S + \text{H}^+ \quad (3)$$

$$E\text{-DPN-DPN}.S \rightarrow E\text{-DPN-DPNH} + P \quad (4)$$

$$E\text{-DPN-DPNH} + \text{DPN} \rightarrow E\text{-DPN}_2 + \text{DPNH} \quad (5)$$

$$E\text{-DPN-DPNH} + S \rightarrow E\text{-DPN}.S\text{-DPNH} + \text{H}^+ \quad (6)$$

$$E\text{-DPN-DPN}.S + S \rightarrow E\text{-DPN}.S_2 + \text{H}^+ \quad (7)$$

where E stands for enzyme, S for substrate, and P for product. There will be a competition between reactions (*5*) and (*6*) and reactions (*4*) and (*7*). The product from reactions (*6*) and (*7*) will result in an inactive species of enzyme; thus, the results of reactions (*6*) and (*7*) appear as substrate inhibition. Reaction (*3*) depends largely on the affinity of the coenzyme for the substrate. For this reason the affinity will differ with different coenzymes. From reactions (*2*) and (*3*) it is clear that the affinity for the substrate depends on the DPN concentration.

Zinc is not an integral part in the actual catalysis, but its presence can be easily explained on a functional basis by assigning to the radical the role of binding site of the pyrophosphate moiety of the coenzyme (Fig. 2).

In this mechanism the role of the protein in the over-all reaction is to impart specificity to the dehydrogenase action by determining the relative distance and spatial arrangement of the DPN molecules. A specific geometrical configuration of the DPN pair will also result in

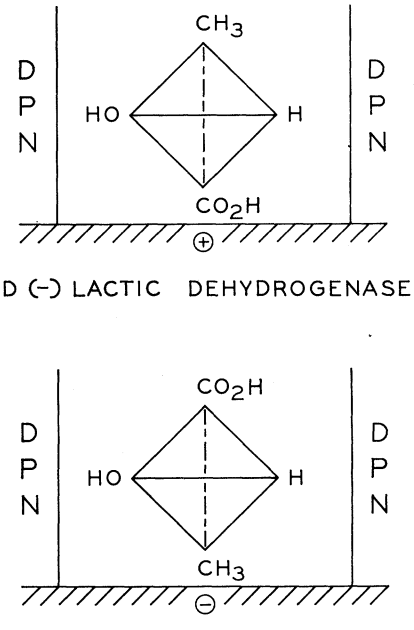

Fig. 3. Possible role of the protein in determining enzyme specificity. The relative arrangement of the DPN molecules determines substrate specificity. The protein itself partially determines stereospecificity.

stereospecificity, both with regard to substrate and with regard to coenzyme. A further role of the protein can be envisioned and is illustrated in Fig. 3. Given identical spatial arrangement of the DPN molecules, the presence of a negatively charged grouping on the enzyme would convert a D-lactate specific enzyme into an L-specific enzyme.

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References and Notes

1. Abbreviations: DPN and DPNH, oxidized and reduced diphosphopyridine dinucleotide, respectively; TPN, triphosphopyridine dinucleotide.
2. Contribution No. 226 of the McCollum-Pratt Institute. This work was supported in part by grants from the American Cancer Society, the American Heart Association, and the National Cancer Institute, National Institutes of Health (grant No. 2374C).
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Production of Sterility in Mice by Deuterium Oxide

The present availability of D_2O at a reasonable price has stimulated an increased investigation of its physiological effects. The inhibition of ascites tumor growth and algal reproduction has recently been reported by this laboratory (*1, 2*) and others (*3*).

We have demonstrated the production of sterility in mice by the substitution of D_2O for a part of the drinking water (*4*). In the first experiment, six female and six male C_{57} mice that had been maintained on 30 percent (*5*) D_2O in the drinking water for 4 weeks were mated (*6*). The animals were housed, three females and three males to a cage. Administration of D_2O was continued for 10 weeks. Since there were no pregnancies at the end of this time, the D_2O was discontinued. At the end of another 8 weeks, there still being no pregnancies, three of the treated females were mated with three normal males, and three of the treated males were mated with three normal females. Although the mating of D_2O-treated females with normal males resulted in litters at the end of 3 weeks, all offspring died within 24 hours, and two of the mothers died. The mating of D_2O-treated males with normal females did not produce offspring until the end of 13 weeks, when one female littered. From the three D_2O-treated males and three D_2O-treated females remaining together, one female littered in 4 weeks, one in 10 weeks. This experiment is graphically represented in Fig. 1.

In the second experiment, both C_{57} and Swiss mice were used. A minimum of ten mice of each sex of each strain were maintained on 5, 20, or 30 percent D_2O in the drinking water for 8 weeks, as is indicated in Table 1. At the end of this treatment period, administration of D_2O was discontinued and each mouse was individually mated with a normal mouse of the same strain. At the same time, normal mice of each strain were individually mated as controls. The animals were not handled or disturbed during the first week after parturition, since handling of the young can lead to cannibalism by the parents. Offspring were counted and sexed 2 weeks after birth.

Those pairs which did not produce a litter during a 28-day period after the beginning of mating are considered to be a sterile pair (Table 1). This period allowed 1 week more than the average 21-day gestation period.

Our data, summarized in Table 1, indicate that 30 percent D_2O causes 100 percent sterility (*7*) in both C_{57} and Swiss males. Some of the C_{57} females were also sterile. These data and the unpublished results of another series suggest that 20 percent D_2O in the drinking water for C_{57} males produces almost complete sterility and that 5 percent D_2O in the drinking water of C_{57} mice appears to produce a degree of sterility comparable to that which 20 percent D_2O achieves in Swiss mice.

Those pairs in which the males had received 20 or 30 percent D_2O and which had no litters during the first 28 days began having litters after 45 days of mating, indicating that the sterility produced by D_2O is slowly reversible (*8*).

We found no significant difference in the litter size or the sex ratio from that of the controls. This is in contrast to the effect of radiation which shows, in addition to the sterility effects (*9*), reduction in the litter size and a change in sex ratio (*10*). The failure of Hansen and Wülfert (*11*) to observe sterility in mice as a result of administration of D_2O is probably due to the low concentration employed by them.

Table 1. Effect of D_2O on the fertility of C_{57} and Swiss mice. D_2O was added to the drinking water.

Sex	D_2O concn. (%)	Pairs (No.)	Pairs Sterile (%)	Offspring Av. No.*	Offspring Av. No. per mating
		C_{57} mice			
Controls	0	24	17	6.4	4.4
Male	5	10	30	5.4	3.8
Female	5	10	30	7.6	3.8
Male	30	19	100	0	0
Female	30	10	40	5.2	2.6
		Swiss Mice			
Controls	0	19	5	9.0	8.5
Male	20	10	40	9.3	5.6
Female	20	11	0	9.1	9.1
Male	30	10	100	0	0
Female	30	10	0	9.1	7.3

* Calculated as the total number of offspring surviving 2 weeks divided by the number of litters containing live offspring at 2 weeks.

Several physiological mechanisms by which D_2O produces sterility in mice can be suggested. D_2O could interfere with maturation of the ova or sperm, or possibly reduce sperm motility. There is also an indication from our results that D_2O may interfere with the proper development of the fertilized ovum. It has been shown that D_2O inhibits the cell division of algae (*2*). This observation suggests that the most likely points of susceptibility would be the development of the sperm and the division of the fertilized ovum. That the former may be the more probable is supported by the greater susceptibility of the males to D_2O and, theoretically, by the known difference between males and females in the generation of germ cells.

On the biochemical level, hydrogen bonding is important for the maintenance of the secondary and tertiary structure of many biologically important macromolecules. Even a small difference in the properties of a proton bond and a deuteron bond might be expected to induce

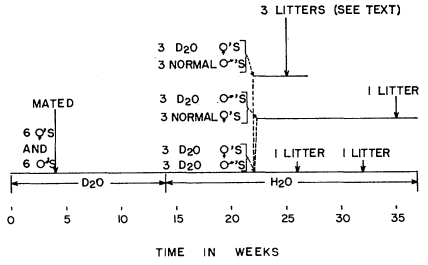

Fig. 1. Production of sterility in C_{57} mice by administration of 30 percent D_2O in the drinking water. Horizontal arrows indicate the length of time animals were given D_2O or H_2O. Vertical arrows indicate the time of mating or littering. Dotted lines indicate the time of cross-mating of treated and normal animals, as described in the text.