a single pigment, rhodopsin, initiates both excitation and light adaptation at moderate light intensities.

JUDITH STRONG*
JOHN LISMAN

Department of Biology,
Brandeis University,
Waltham, Massachusetts 02154

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9. In order to have adapting lights that were too dim to significantly alter the pigment concentration, but which were still bright enough to have a desensitizing effect, it was necessary to use sensitive cells. None of the cells used showed any signs of facilitation. Some cells never became sensitive enough to be used; one of these did show an increase in sensitivity when exposed to a dim background light. M. Hanani and P. Hillman [*J. Gen. Physiol.* **67**, 235 (1976)] report that such facilitation is seen more often in less sensitive cells, while light adaptation as we have described it is seen more often in sensitive cells. The background lights desensitized the cell by about a 0.6 log unit but did not significantly alter the pigment photoequilibrium established at the beginning of the experiment. We verified this point at the end of the experiment by demonstrating that exposing the cell to ten times as much monochromatic light as had been used during the experiment did not, at any wavelength, measurably alter the pigment concentration as assayed by the early receptor potential. It was necessary to maintain an essentially constant pigment concentration; only under this condition does an action spectrum approximate the relative absorbance spectrum of the pigment initiating the process in question. The monochromatic background lights used to produce a criterion desensitization evoked responses with a plateau phase of approximately 10 mV amplitude. This potential was independent of wavelength within $\pm$0.5 mV.
10. The action spectrum of excitation was determined by finding, at each wavelength, the intensity of a brief monochromatic flash that would elicit a criterion response in a dark-adapted cell. The relative attenuation of the different monochromatic flashes and background lights used was readily determined since calibrated neutral-density filters were used to attenuate the light beam.
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* Present address: Department of Physiology, Yale University, New Haven, Connecticut 06520.

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Nitrite and Nitrate Are Formed by Endogenous Synthesis in the Human Intestine

Abstract. *Studies of nitrate balance in humans and analyses of fecal and ileostomy samples indicate that nitrite and nitrate are formed de novo in the intestine, possibly by heterotrophic nitrification. These findings significantly alter our previous conceptions of human exposure to nitrite and suggest an even wider role for nitrite in the etiology of human cancer.*

Nitrite is a precursor of the putatively carcinogenic *N*-nitroso compounds that may be formed in situ in humans (*1*). These compounds are thought to form in the stomach (*2*), the bladder (*3*), and, as recent evidence suggests, in the lower intestinal tract (*4*). The origin of this nitrite is significant, since it might be related to the etiology of several types of cancer in humans (*5*).

An earlier investigation of nitrate metabolism (*6*) indicated that bacterial reduction of nitrate in saliva was the major mode of nitrite formation in humans (*7*). Further studies with vegetables and vegetable juices rich in nitrate indicated that levels of salivary nitrite could reach hundreds of milligrams per liter in a process of recirculation of unmetabolized nitrate through the blood via the salivary glands (*8, 9*). Thus, to assess the extent of nitrate intake in a population through measurements of nitrate in urine and saliva (*10*), nitrate balance studies were undertaken in humans. The results of these studies, and of subsequent studies of effluent samples from the intestinal tract, led to the discovery that nitrite and nitrate are formed by endogenous synthesis in humans.

Six Caucasian males, 68 to 72 years old, weighing 57 to 105 kg, participated in the nitrate balance study. All were judged to be healthy on the basis of a medical history, physical examination, and clinical and laboratory tests. Procedures for the selection of subjects and their initial characteristics have been described (*11*).

The subjects were studied as inpatients at the Clinical Research Center (CRC), Massachusetts Institute of Technology (MIT). After having a diet of their own choosing for at least 1 week, the subjects were given a protein-free diet (6 mg of nitrogen per kilogram of body weight per day) for 10 days and then a diet containing 0.8 g of egg protein per kilogram of body weight per day (based on a liquid formula) for 10 days. The compositions of these diets have been described (*11–13*). The diets were consumed under the supervision of the CRC research dietician to ensure adherence to the experimental protocol. Only minor fluctuations in body weight occurred during the diet periods. Urine and dietary components were analyzed for nitrate and nitrite by a modified Griess microprocedure (*9*). Complete daily urine collections were made throughout the diet periods and the samples were preserved with HCl (*14*).

Since nitrate is known to be absorbed from the proximal small intestine and cleared from plasma by the kidney (*15*), we expected the subjects to reach equilibrium rapidly and to excrete in urine a steady-state concentration of nitrate that would represent some fraction of that consumed. Instead, we found wild fluctuations in urinary nitrate on a day-to-day basis on both the 0.8-g and protein-

Table 1. Urinary nitrate in subjects consuming a protein-free diet.

Subject	Total daily nitrate in urine (μmole $NaNO_3$)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
1	985	462	978	1610	1350	2270	279	325	289	275
2	4050	1410	1330	791	2400	1720	1000	1390	815	
3	1140	5150	1260	1140	3040	2210	1220	918	840	1070
4			1550	2640	1960	355	1140	981		883
5	406	378	219	1550	833	233	861	579	530	971
6	1130	1720	635	1550	1430	1320	1380	448	2210	462

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Table 2. Total daily dietary nitrate intake compared with average daily urinary nitrate excretion.

Subject	Diet	Intake* (μmole $NaNO_3$)	Excretion (μmole)
1	Protein-free	72	884
	0.8 g/kg	140	551
2	Protein-free	54	1660
	0.8 g/kg	86	1300
3	Protein-free	72	1800
	0.8 g/kg	155	1830
4	Protein-free	61	1360
	0.8 g/kg	117	1350
5	Protein-free	85	655
	0.8 g/kg	140	1230
6	Protein-free	60	1230
	0.8 g/kg	117	814

*Daily intake was constant over the experimental period.

free diets. The complete pattern for each individual during the protein-free diet period is shown in Table 1; there is clearly no tendency to equilibrium.

Table 2 shows a comparison of total nitrate intake and average output for both dietary regimens. The differences are so large for each individual on both diets that there must have been some significant source of nitrate other than the diet. Since urinary nitrate is apparently unrelated to the level of protein in the diet, it is unlikely that muscle catabolism is involved. That leaves the intestine as the most likely site of endogenous nitrate or nitrite synthesis.

In a second study, eight healthy Caucasian males, 25 to 40 years old, submitted single fecal samples for analysis of nitrate and nitrite. All were eating free-choice, Western-style diets containing about 2200 calories per day, approximately 43 percent as fat, 18 percent as protein, and 39 percent as carbohydrate. Samples were collected in washed plastic containers and frozen at − 20°C within 1 hour after collection. Frozen samples were then freeze-dried and submitted for analysis. In addition, six Caucasian males, 25 to 30 years old, submitted samples of ileostomy effluent. Each had undergone a total colectomy for ulcerative colitis approximately 2 years earlier, and each was in good general health. The samples were collected at midmorning, approximately 3 hours after breakfast, and were prepared by the same method as the feces.

Table 3 shows the nitrate and nitrite contents of the eight fecal and six ileostomy samples. Great variations were found among individuals, but all samples contained nitrite. Five of the eight fecal samples also contained nitrate. The absence of nitrate in the ileostomy samples in the presence of such high nitrite concentrations is surprising and suggests that nitrite is synthesized from more reduced nitrogen compounds.

Fecal samples from two of these individuals were analyzed on several separate occasions and with varying dietary regimens (Table 4). The pattern was similar to that shown in Table 3: nitrite was always present, whereas nitrate was sometimes present and sometimes absent. Substances that react with nitrite, such as ascorbic acid (*16*), do not appear to have a significant effect on nitrite concentration at $pH > 5.5$, although they may influence the mutagenicity of fecal extracts (*4*). This phenomenon may be explained by the fact that it is N_2O_3, not nitrite ion, that reacts with ascorbate. While ascorbate is present, it will scavenge N_2O_3 and effectively prevent amine nitrosation by virtue of its greater reactivity with N_2O_3 (*17*).

A logical explanation for these results is that heterotrophic nitrification of ammonia or organic nitrogen compounds takes place in the upper, aerobic portion of the intestine. In effect, the small intestine behaves like other ecosystems—such as sewage, river and lake water, and soils (*18*)—in which fungi and heterotrophic bacteria participate in the nitrification process. Ample evidence of this type of microbial ecology has been found in studies of ileostomy effluents, which contained many aerobic organisms (*19*) and the nutrients required for heterotrophic nitrification (*20*).

As material passes through the intestine, the environment gradually becomes more anaerobic (*20*); this leads to the accumulation of nitrate as well as nitrite. A large portion of these compounds may be utilized by other fecal microorganisms. Some may be absorbed through the intestinal wall, and some of the nitrite may react to form *N*-nitroso compounds in the more acidic environment of the cecum and colon.

Table 3. Nitrate and nitrite levels in human feces and intestinal contents.

Subject	Sample	NO_3 (μmole/kg)	NO_2 (μmole/kg)
A	Fecal	174	77
B	Fecal	124	112
C	Fecal	N.D.*	407
D	Fecal	71	106
E	Fecal	N.D.	186
F	Fecal	34	48
G	Fecal	487	339
H	Fecal	N.D.	425
I	Ileostomy	N.D.	1510
J	Ileostomy	N.D.	1540
K	Ileostomy	N.D.	350
L	Ileostomy	N.D.	880
M	Ileostomy	N.D.	530
N	Ileostomy	N.D.	760

*N.D., not detectable (< 2 μmole/kg).

Table 4. Variation in nitrate and nitrite content of human feces with time and diet.

Subject	Day*	NO_3 (μmole/kg)	NO_2 (μmole/kg)
A	18	260	183
	20	174	77
	C3	N.D.†	242
	C13	N.D.	296
	C15	N.D.	425
B	4	N.D.	213
	8	N.D.	330
	15	N.D.	552
	C3	14	307

*Subjects followed a normal diet for 20 days, then consumed a diet supplemented with ascorbic acid (C) (4 g/day) for 20 days. †N.D., not detectable (< 2 μmole/kg).

Thus, human exposure to nitrite is much greater than previously recognized. Absorbed nitrite is rapidly destroyed in the blood, but the nitrate is recycled into saliva via the salivary glands, the stomach via the parietal glands, and the bladder via the urine. In this way, intestinal nitrification may contribute to the etiology of cancer in humans.

S. R. TANNENBAUM
D. FETT
V. R. YOUNG
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139
P. D. LAND
W. R. BRUCE
Ontario Cancer Institute, Toronto, Ontario M4X 1K9, Canada

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Mercury Concentrations in Pacific Hake, *Merluccius productus* (Ayres), as a Function of Length and Latitude

Abstract. *Mercury concentrations in Pacific hake increase with fish size and with the latitude of collection. While the mercury-size trend is consistent with data for other species, the latitudinal trend is opposite to that reported for other fishes over the same geographical area. Consequently, latitudinal trends of mercury concentrations in fishes do not necessarily indicate trends of mercury concentrations in water. Food habits and metabolism may cause the observed variations.*

The discovery that mercury concentrations in some fishes exceeded levels allowed by regulatory agencies, and that mercury-polluted fishes caused many deaths in Japan, focused great concern on the use and control of mercury. We report a study of mercury in Pacific hake collected off the western U.S. coastline in 1969 to 1970 (*1*).

Mercury occurs in the environment both as a result of natural processes and as a pollutant from human activities. Jenne (*2*) concluded that high concentrations in western U.S. waters were more closely associated with the occurrence of mercury in rocks than with industrial discharges. However, even though northern California and Oregon have been the principal mining regions for mercury in the United States (*3*), Jenne also observed that the mercury in western waters was generally lower than in U.S. waters as a whole. On a global scale, Weiss *et al.* (*4*) estimated that natural degassing of the earth mobilizes more mercury than mining, smelting, or fossil fuel burning. They also suggested that man has increased the mercury content of the surface ocean by increasing the exposure of earth crust to the atmosphere. Nonetheless, it appears that increased mercury concentrations in marine fish by pollution has been proved only for relatively restricted bodies of water such as Minimata Bay (*5*) or local coastal areas (*6*).

Conversely, Young (*7*) reported that flatfish trawled from the vicinity of submarine outfalls in southern California showed no unusual accumulation of mercury even though the mercury content of discharge is many times higher than seawater, and sediments near the outfalls contain much higher mercury concentrations than similar sediments nearby. Barber *et al.* (*8*) concluded that the concentration of mercury in benthic fish depended more on species-specific metabolic factors and individual size than on the water content of mercury. A positive correlation of mercury concentration with size (age) has been reported for many fishes (*9–11*). This has generally been explained as an inability of fish to eliminate mercury at the rate at which it is assimilated.

Both the ecological availability of mercury to fish and the kinetics of turnover depend on chemical form. Methylmercury(II) (CH_3Hg^+) appears to be much more mobile in aquatic systems (*12*) and more readily assimilated by fish (*13*) than inorganic forms. Biological retention times are longer for methylmercury than inorganic mercury (*13, 14*) and a large proportion of the mercury found in fish is methylated (*15*).

Pacific hake, *Merluccius productus* (Ayres), spawn in winter along the coast of southern California (*16*). The young feed primarily on crustaceans, although a wide variety of prey have been identified in stomach contents (*17*). Adults migrate northward along the coastline during late spring and summer, feeding largely upon euphausiids supplemented by fish and pandalid shrimp (*18*). Many individuals migrate as far northward as the coast of Washington. During fall, hake appear to disperse offshore and to migrate southward. Adults are estimated to attain an age of 10 to 12 years and are presumed to migrate annually. A distinct nonmigratory population of the same species resides in Puget Sound.

Pacific hake were collected during their summer migration (*19*). Whole fish were taken directly from the trawl nets, placed in plastic bags, and frozen immediately after capture. They were stored on board ship at about −10°C and in the laboratory at about −25°C. Care was taken to avoid thawing the samples or rupturing the plastic bags during storage and transport.

For analysis the individual fish were selected at random, their lengths were

Table 1. Mercury concentrations in Pacific hake and results of linear least squares regressions on length for different latitudes. Standard errors are shown for means and for regression slopes.

North latitude	Number of fish analyzed	Mean mercury concentration [μg (g wet)$^{-1}$]	Regression on length	
			Slope (μg g^{-1} m^{-1})	r^2
32°52′	6	0.085 ± .007	0.42 ± .03	.98
37°03′	13	0.179 ± .008	0.81 ± .07	.94
38°41′	17	0.168 ± .007	0.37 ± .04	.86
39°16′	37	0.130 ± .007	0.58 ± .02	.94
40°08′	29	0.180 ± .008	0.77 ± .05	.91
41°32′	20	0.197 ± .010	0.80 ± .11	.74
42°23′	22	0.239 ± .005	0.59 ± .04	.93
43°23′	17	0.242 ± .007	0.67 ± .05	.93
45°49′ to 45°59′	30	0.286 ± .006	0.99 ± .07	.87
46°14′ to 46°55′	57	0.371 ± .005	0.83 ± .04	.88
47°10′	8	0.371 ± .007	0.45 ± .07	.88
48°00′ to 48°28′	15	0.383 ± .006	1.09 ± .03	.56
Puget Sound*	10	0.323 ± .011	0.70 ± .03	.98

*Not included in pooled data.

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