in fair agreement with the value of 2.5 found in equilibrium measurements at 25°C.

The amount of data so far available is too small to permit great confidence in the numbers used for the allosteric analysis, but they do show that an internally consistent description is possible.

The velocity constant for dissociation of oxygen from *Riftia* hemoglobin is within the range commonly encountered for many hemoglobins (*15–17*), as is the absolute value of the velocity constant for combination of *Riftia* hemoglobin with oxygen (*15*).

Riftia normally encounters temperature fluctuating from 2° to 23°C at the mouth of the vent. Arp and Childress (*6*) report that the oxygen affinity of *Riftia* hemoglobin changes less than twofold over the range 3° to 14°C. The combination rates change not at all over this range. The relative insensitivity of the oxygen equilibrium to temperature in the face of a drastic increase in the dissociation rate can be compensated for only by a large decrease (50-fold) in the value of L, the conformational equilibrium constant. This effect of temperature on L is in the same direction as has been reported for trout I hemoglobin (*18*) and for menhaden hemoglobin (*19*) and again suggests that *Riftia* hemoglobin is similar to the few others that have been examined in this respect.

JONATHAN B. WITTENBERG*
Department of Physiology, Albert Einstein College of Medicine, Bronx, New York 10461
ROGER J. MORRIS
QUENTIN H. GIBSON
Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853
MEREDITH L. JONES
Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560

References and Notes

1. J. B. Corliss *et al.*, *Science* **203**, 1073 (1979).
2. M. L. Jones, *Science* **213**, 333 (1981).
3. G. H. Rau, *ibid.*, p. 338.
4. H. Felbeck, *ibid.*, p. 336.
5. C. M. Cavanaugh, S. L. Gardiner, M. L. Jones, H. W. Jannasch, J. B. Waterbury, *ibid.*, p. 340.
6. A. J. Arp and J. J. Childress, *ibid.*, p. 342.
7. R. C. Terwilliger, N. B. Terwilliger, E. Schabtach, *Comp. Biochem. Physiol.* **65B**, 531 (1980).
8. J. Breton-Gorius, thesis, Faculty of Sciences, University of Paris (1963); R. E. Weber, in *Physiology of Annelids*, P. J. Mills, Ed. (Academic Press, New York, 1978), p. 369; R. C. Terwilliger, *Am. Zool.* **20**, 53 (1980); R. L. Garlick, *ibid.*, p. 69.
9. Determined as the pyridine hemochromogen taking $\epsilon_{557nm} = 32.0\ mM^{-1}\ cm^{-1}$ (ϵ, extinction).
10. Q. H. Gibson and L. Milnes, *Biochem. J.* **91**, 161 (1964). Hemoglobin (5 μM as heme) in air-equilibrated 0.05M potassium phosphate buffer, pH 7.5, containing 1 mM EDTA, with and without 0.2M NaCl was mixed rapidly with 12 mM sodium dithionite dissolved in anaerobic buffer. First-order plots of the progress of the reaction at 432 nm were linear to about 60 percent completion; in this range the measured dissociation rate should correspond to the R state. With relatively small values of L such as those found in the allosteric analysis, switching from R to T should occur only at low values of saturation.
11. C. A. Sawicki and Q. H. Gibson, *J. Biol. Chem.* **251**, 1533 (1976); *ibid.* **252**, 5783 (1977). The monitoring wavelength was 436 nm, a wavelength near the absorption maximum of deoxyhemoglobin; hemoglobin about 50 μM in 0.05M potassium phosphate buffer, pH 7.5, containing 0.2M NaCl and 1 mM EDTA. Kinetic data were recorded and analyzed with a PDP 8/E computer.
12. N. M. Rumen and J. A. McCray, *Biophys. Soc. Annu. Mtg. Abstr.* **13**, 28a (1973); B. Seamonds, J. A. McCray, L. J. Parkhurst, P. D. Smith, *J. Biol. Chem.* **251**, 2579 (1976).
13. J. Monod, J. Wyman, J. P. Changeux, *J. Mol. Biol.* **12**, 88 (1965).
14. Observations with carbon monoxide as a ligand relate to the R-T change. At 425 nm (isobestic for ligand binding) a small ($\Delta\epsilon_{mM} \simeq 3$) CO independent increase in absorbance with a half-time of about 100 μsec was seen. At high CO (900 μM) the rate of binding at 436 nm was noticeably faster during the first 100 μsec of the reaction (rate of order 10^6 liter mole^{-1} sec^{-1}, half-time 0.7 msec). The effect was much less with lower concentration of CO. If these observations are attributed to a faster rate of CO binding to the R state and to a finite rate of the R to T transition following photolysis, then there must be little interference with oxygen kinetics due to persistence of R state molecules after photolysis.
15. Q. H. Gibson, *Prog. Biophys.* **9**, 1 (1959).
16. ———, *Proc. R. Soc. London Ser. B* **143**, 334 (1955); C. P. Mangum, in *Adaptation to Environment*, R. C. Newell, Ed. (Butterworths, Boston, 1976), p. 191.
17. I. Imamura, A. Riggs, Q. H. Gibson, *J. Biol. Chem.* **247**, 521 (1972); J. B. Wittenberg, C. A. Appleby, B. A. Wittenberg, *ibid.*, p. 527.
18. J. Wyman *et al.*, *J. Mol. Biol.* **109**, 195 (1977).
19. W. A. Saffran and Q. H. Gibson, *J. Biol. Chem.* **253**, 3171 (1978).
20. This report is contribution number 16 of the Galápagos Rift Biology Expedition, supported by the National Science Foundation. This work was supported by NSF grants PCM 80 04472 (to J.B.W.) and BMS 08233 (to Q.H.G); by PHS research grant GM 14276 (to Q.H.G.), and by the Research Fund of the Secretary, Smithsonian Institution (to M.L.J.); J.B.W. is supported by NHLBI Research Career Program Award 1-K6-733. We thank V. L. Goei for assistance and Drs. Arp and Childress for making their data available to us prior to publication.
* Address correspondence to J.B.W.

28 July 1980; revised 6 January 1981

Carcinogenicity in Mice of Mutagenic Compounds from a Tryptophan Pyrolyzate

Abstract. *The compounds 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole, which are potent mutagens in a tryptophan pyrolyzate, are hepatic carcinogens when given orally to mice at concentrations of 200 parts per million in a pellet diet. Female mice showed higher susceptibilities to both compounds than male mice.*

On the basis of the finding that mutagens are formed in the charred parts of broiled meat and fish (*1–4*), series of new heterocyclic amines in pyrolyzate of amino acids, proteins, and proteinaceous foods were found to be highly mutagenic to *Salmonella typhimurium* TA 98 and TA 100 (*5–10*). In fact, some of these compounds have higher specific mutagenic activity than aflatoxin B_1. Among these new heterocyclic amines, which are being subjected to in vitro and in vivo carcinogenicity tests, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]-indole (Trp-P-2) from tryptophan pyrolyzates and 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1) from glutamic acid pyrolyzate have been shown to be carcinogenic in an in vitro transformation system with embryonal Chinese hamster cells (*11–13*). Moreover, Trp-P-1 induced fibrosarcomas locally when injected subcutaneously into hamsters and rats (*14*). We report that Trp-P-1 and Trp-P-2 are carcinogenic to mice and

Table 1. Incidence of hepatic tumors in mice fed on diets with Trp-P-1 or Trp-P-2 (200 ppm) for up to 621 days.

Treatment	Sex	N*	Number of mice with hepatic tumors				
			Hepatocellular tumor		Hemangioma	Total	P‡
			Adenoma	Carcinoma†			
None	M	25	0	0	1	1 (4)§	
	F	24	0	0	0	0	
Trp-P-1	M	24	1	4	0	5 (21)	< .179
	F	26	2	14	0	16 (62)	< .001
Trp-P-2	M	25	1	3	0	4 (16)	< .348
	F	24	0	22 (2)‖	0	22 (92)	< .001

*Number of mice surviving on day 402, when the first hepatic tumor was found. †Mice with both hepatocellular adenoma and hepatocellular carcinoma are included under hepatocellular carcinoma. ‡Statistical significance of the difference in incidence of hepatic tumors between control and Trp-P-1 or Trp-P-2 groups by χ^2 test. §Numbers in parentheses are percentages. ‖Number in parentheses is number of mice with pulmonary metastases of hepatocellular carcinomas.

0036-8075/81/0717-0346$00.50/0

induce hepatocellular carcinomas when given orally in the diet.

Synthetic Trp-P-1 acetate and Trp-P-2 acetate, obtained from Nard Institute, Osaka, Japan, and confirmed to be at least 99.5 percent pure by high-pressure liquid chromatography, mass fragmentometry, and elementary analysis, were given to 7-week-old CDF_1 mice (BALB/cAnN × DBA/2N, Charles River Japan, Atsugi, Kanagawa). Forty mice of each sex were fed concentrations of 200 parts per million (ppm) Trp-P-1 and Trp-P-2 acetate in pellet diets (CE-2 basal diet; CLEA Japan, Tokyo) for 621 days. Forty control mice of both sexes were given the basal diet only. The mice had free access to food and tap water.

The average intakes of food and carcinogens per day per mouse, respectively, were as follows: males on a diet with Trp-P-1, 2.7 g and 0.53 mg; females on a diet with Trp-P-1, 2.2 g and 0.45 mg; males on a diet with Trp-P-2, 2.8 g and 0.56 mg; and females on a diet with Trp-P-2, 2.9 g and 0.57 mg. The average food intake per day per control mouse was 3.3 g for males and 2.9 g for females. Unlike male control mice, males on diets with Trp-P-1 or Trp-P-2 did not gain weight. Female mice on Trp-P-2 diet showed nearly the same increase in body weight as control mice, but females on the Trp-P-1 diet showed only a small increase in body weight. Until about day 480, the survival rates of male mice on the Trp-P-1, Trp-P-2, and basal diets were similar: 48, 48, and 50 percent, respectively. Thereafter male mice fed diets with Trp-P-1 and Trp-P-2 developed liver tumors and showed lower survival rates than controls. Until about day 400, the survival rates of female mice fed on Trp-P-1, Trp-P-2, and basal diets were similar: 65, 63, and 60 percent, respectively; then survival of those on the test diets decreased, because the animals developed liver tumors.

The incidences of tumors in the experimental and control groups are summarized in Tables 1 and 2. The first tumor was found in a male mouse on a Trp-P-2 diet on day 180 and was identified as a thymoma. The first hepatic tumor was found in a female mouse on a Trp-P-2 diet on day 402. From then until the end of the experiment on day 621, hepatic tumors were found in 5 of 24 (21 percent) male mice and 16 of 26 (62 percent) female mice on Trp-P-1 diets and in 4 of 25 (16 percent) male mice and 22 of 24 (92 percent) female mice on Trp-P-2 diets. In control mice, a hepatic tumor was found in only 1 of 25 (4 percent) male mice and none in female mice. Female mice showed higher susceptibilities to Trp-P-1 and Trp-P-2 than male mice. These results are in contrast with those on many other hepatocarcinogens such as dimethylnitrosamine and aflatoxin B_1, which induced more hepatic tumors in male than in female mice (*15–17*). The Trp-P-2 diet showed higher carcinogenicity than the Trp-P-1 diet in female mice.

Table 2. Incidence of nonhepatic tumors in mice fed on diets with Trp-P-1 or Trp-P-2.

Treatment	Sex	Pulmonary tumor		Thymoma	Lymphatic leukemia	Other
		Adenoma	Adenocarcinoma			
None	M	1	3	0	0	0
	F	1	3	0	4	2*
Trp-P-1	M	0	3	0	1	0
	F	0	1	1	0	0
Trp-P-2	M	3	8	1	1	2†
	F	1	3	1	3	5‡

*One pheochromocytoma of the adrenal gland and one mammary adenoma. †One signet-ring cell carcinoma of the small intestine and one histiocytoma of the colon and the mesenteric lymph node. ‡Two leiomyomas of the uterus, one reticulum cell sarcoma of the spleen, one mammary adenocarcinoma, and one histiocytic sarcoma infiltrating into the stomach, pancreas, liver, and intestine.

Macroscopically, multiple yellowish-white, protruding, solid tumors were found in the liver lobes. Histologically, many hepatic tumors were hepatocellular carcinomas. Most had a trabecular structure, but a few were anaplastic. Metastases of hepatocellular carcinomas to the lung were found in two female mice given Trp-P-2. In the control group, a hemangioma was found in one male mouse, but there were no hepatocellular adenomas or carcinomas.

Tumors were also found in the lung, thymus, hematopoietic organs, breast, and a few other organs in mice treated with Trp-P-1 or Trp-P-2 and also in control mice (Table 2). The incidence of pulmonary tumors in male mice fed Trp-P-2 was slightly higher than that in control male mice ($P < .038$ by χ^2 test). No statistically significant difference was found in the incidences of other nonhepatic tumors in the three groups.

The cumulative carcinogen intakes per mouse until the time of the first appearance of liver tumors were as follows: 279 mg of Trp-P-1 for males and 197 mg for females; 304 mg of Trp-P-2 for males and 205 mg for females. Although Trp-P-2 is more mutagenic than aflatoxin B_1, its carcinogenicity is similar to that of 2-acetylaminofluorene.

Both Trp-P-1 and Trp-P-2 are present in broiled sardines cooked in the usual way (*18*), although the sardines also contain other mutagens such as 2-amino-3-methylimidazo[4,5-*f*]quinoline and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (*9, 18*). Thus, we report evidence of the carcinogenicity on oral administration of mutagenic compounds produced by cooking. Many mutagenic compounds have been identified in pyrolysis products of glutamic acid (*6*), phenylalanine (*5*), lysine (*7*), soybean globulin (*8*), and broiled sardine (*9*).

NORIO MATSUKURA
TAKASHI KAWACHI
KAZUHIDE MORINO
HIROKO OHGAKI
TAKASHI SUGIMURA
Biochemistry Division, National Cancer Center Research Institute, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104 Japan
SHOZO TAKAYAMA
Department of Experimental Pathology, Cancer Institute, 1-37-1, Kamiikebukuro, Toshima-ku, Tokyo 170 Japan

References and Notes

1. T. Sugimura *et al.*, in *Origins of Human Cancer*, H. H. Hiatt, J. D. Watson, J. A. Winston, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1977), p. 1561.
2. B. Commoner, A. J. Vithayathil, P. Dolara, S. Nair, P. Madyastha, G. C. Cuca, *Science* **201**, 913 (1978).
3. M. W. Pariza, S. H. Ashoor, F. S. Chu, D. B. Lund, *Cancer Lett.* **7**, 63 (1979).
4. N. E. Spingarn *et al.*, *ibid.* **9**, 177 (1980).
5. T. Sugimura *et al.*, *Proc. Jpn. Acad.* **53** (No. 1), 58 (1977).
6. T. Yamamoto *et al.*, *Proc. Jpn. Acad. Ser. B* **54**, 248 (1978).
7. K. Wakabayashi *et al.*, *ibid.*, p. 569.
8. D. Yoshida, T. Matsumoto, R. Yoshimura, T. Matsuzaki, *Biochem. Biophys. Res. Commun.* **83**, 915 (1978).
9. H. Kasai *et al.*, *Proc. Jpn. Acad. Ser. B* **56**, 278 (1980).
10. T. Sugimura, *Gann Monogr.* **24**, 3 (1979).
11. S. Takayama, Y. Katoh, M. Tanaka, M. Nagao, K. Wakabayashi, T. Sugimura, *Proc. Jpn. Acad. Ser. B* **43**, 126 (1977).
12. S. Takayama, T. Hirakawa, T. Sugimura, *ibid.* **54**, 418 (1978).
13. S. Takayama, T. Hirakawa, M. Tanabe, T. Kawachi, T. Sugimura, *Toxicol. Lett.* **4**, 281 (1979).
14. T. Ishikawa *et al.*, in *Naturally Occurring Carcinogens-Mutagens and Modulators of Carcinogenesis*, E. C. Miller, J. A. Miller, I. Hirono, T. Sugimura, S. Takayama, Eds. (University Park Press, Baltimore, 1979), p. 159.
15. V. S. Turusov and S. Takayama, in *Pathology of Tumors in Laboratory Animals*, V. S. Turusov *et al.*, Eds. (International Agency for Research on Cancer, Lyon, 1979), p. 193.
16. S. D. Vesselinovitch, *Cancer Res.* **29**, 1024 (1969).
17. ——, N. Mihailovich, L. S. Lombard, G. N. Wogan, *Proc. Am. Assoc. Cancer Res.* **13**, 195 (1972).
18. H. Kasai, S. Nishimura, M. Nagao, Y. Takahashi, T. Sugimura, *Cancer Lett.* **7**, 343 (1979).

19 January 1981; revised 30 March 1981