slower since only 4.3% of P_2 had accumulated by the end of the 3-h test. The insignificant amount of O detected in this test indicates that hydrolysis of P to O is negligible under these conditions. This may explain the infrequent detection of O in the environmental studies of P. On the other hand, the detection of O_1 indicates partial hydrolysis of P_1 to O_1. Since the quantities of both P_1 and O_1 do not increase with time, further oxidation of P_1 to P_2 and O_1 to O_2 may be proceeding subsequently. Of all the degradation products identified in Table I, only P_2 shows a slow, but consistent increase in quantity with time. This suggests that P_2 is the major, stable product derived from P under the conditions studied.

Degradation of P at pH 10 and 4 °C. The mechanism of the degradation of P at pH 10 was further elaborated by a 5-day time course study as shown in Table I. The hourly results showed the approximate difference in rates of the oxidation of P to P_1 and P_1 to P_2. The daily results show that the two-step oxidation of P to P_2 is complete after the first 24 h, even at 4 °C. This rapid oxidation was confirmed by testing [^{14}C]P_1 under the same conditions. The daily results demonstrated that after 24 h, both P and P_1 had disappeared and that P_2 was the major product. The secondary residue, O_2, may be derived in two ways. It may come from the direct hydrolysis of P_2 or by hydrolysis of P_1 to O_1 and subsequent oxidation of O_1 to O_2. The hydrolysis of P_2 as the principal mechanism for producing O_2 was proved by testing [^{14}C]P_2 under the same conditions. On the other hand, the O_1 present in the hourly study was absent in the daily study, indicating rapid oxidation of O_1 to O_2 at least within 24 h, under the conditions studied. The infrequent detection of O_1 in environmental studies involving thiofanox may then be explained by the observation that O_1 was produced in limited quantities by partial hydrolysis of P_1, followed by the rapid oxidation to O_2 as shown here. In addition, a small quantity of K_2 was detected during the fifth day of the daily test. This indicated subsequent deoximation of O_2 under the conditions studied.

Kinetic Hydrolysis of P_2 at pH 10. Based on the information obtained in the above experiments, it is seen that the oxidation of P to P_2 and the partial hydrolysis of P_1 to O_1 and subsequent oxidation of O_1 to O_2 are all complete in 24 h at pH 10 and 4 to 25 °C. The remaining reaction rate of interest that can be determined from these data is the hydrolysis of P_2 to O_2. The pseudo-first-order rate constants for hydrolysis of P_2 to O_2 at pH 10 and 4, 25, and 45 °C were determined to be 0.0036, 0.0653, and 0.2427 per day with half-lives of 192.5, 10.6, and 2.9 days, respectively. An overall summary of the degradation of P at pH 10 is given in Figure 1. Because P_2 is the most significant degradation product of P and has the highest toxicity of the three carbamates, the hydrolysis of P_2 to O_2, which toxicity is 386 times less than that of P_2 (Chin et al., 1975), may be regarded as a detoxifying process.

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Selenium Content of Bangladeshi Rice by Chemical and Biological Assay

Rice samples from three districts of Bangladesh were analyzed for selenium by bioassay (prevention of liver necrosis in rats) and by chemical analysis. The latter gave a range of 0.06–0.17 ppm of selenium, whereas a U.S. sample had 0.46 ppm. Bioassay of samples from both countries indicated that not all the selenium was biologically active.

Selenium is now recognized as an essential trace element for many species, including man, and its biochemical role as a cofactor for the enzyme glutathione peroxidase has been established (Rotruck et al., 1973). The selenium content of foodstuffs has thus taken on new significance, and is particularly important in populations in which usual diets rely heavily on a few commodities. Such a situation exists in Bangladesh, where rice provides 85% of the calories and 68% of the protein in the rural population (Nutrition Survey of East Pakistan, 1966).

METHODS

Rice samples were purchased in the market in three different districts of Bangladesh: Barisal, Mymensingh, and Comilla. In Barisal, samples included white rice, brown rice, and bran; the samples from the other districts were white or brown rice only. For comparison, a sample of U.S. Louisiana white rice was included.

The bioassay for selenium was based on the prevention of liver necrosis in rats by dietary selenium. Female Fisher strain rats with litters 1 week old were kept in plastic cages with wood chips. Over a 3–4 day period, a selenium and vitamin E deficient torula yeast diet (Schwarz, 1951; Bieri and Poukka Evarts, 1974) was mixed in increasing proportions into the ground stock ration until the mothers were consuming only the deficient diet. The young rats were weaned when 21 days old and were caged individually in suspended cages and fed the experimental diets ad libitum. Addition of finely ground rice was made at the expense of the sucrose component. To estimate the selenium content of rice samples, the incidence of death due to liver necrosis or hemorrhage and the average survival

Table I. Prevention of Dietary Liver Necrosis by U.S. Rice or Inorganic Selenium

Group	Dietary supplement	Incidence[a]	Av days survival[b]
1	None	6/6	15.2 ± 1.0
2	5% U.S. white rice	5/6	23.0 ± 4.8
3	10% U.S. white rice	4/6	30.5 ± 4.0
4	15% U.S. white rice	2/6	39.2 ± 3.7
5	0.01 ppm of Se[c]	6/6	19.2 ± 0.8
6	0.02 ppm of Se	5/6	26.2 ± 4.0
7	0.04 ppm of Se	1/6	42.2 ± 2.8
8	0.1 ppm of Se	0/6	45.0

[a] Number of deaths over number of rats. [b] Mean ± standard error. Experiment was terminated at 45 days. [c] Provided as sodium selenite.

Table II. Prevention of Dietary Liver Necrosis by Rice from Bangladesh or the U.S.

Group	Supplement	Incidence[a]	Av days survival[a]
1	None	5/5	18.6 ± 1.2
2	10% U.S. white rice	2/6	32.0 ± 1.8
3	15% U.S. white rice	3/6	31.5 ± 1.3
4	20% U.S. white rice	0/6	35.0
5	15% Bang. white rice[b]	6/6	21.8 ± 1.5
6	30% Bang. white rice[b]	6/6	20.2 ± 0.5
7	15% Bang. brown rice[b]	6/6	22.0 ± 1.2
8	30% Bang. brown rice[b]	5/5	21.0 ± 0.8
9	0.02 ppm of Se	5/6	30.0 ± 1.5
10	0.04 ppm of Se	4/6	30.2 ± 1.5
11	0.06 ppm of Se	0/6	35.0

[a] See footnotes to Table I. Experiment terminated at 35 days. [b] From Barisal district.

time of the experimental groups were compared with the performance of groups receiving known amounts of selenium as sodium selenite. For chemical assay, the rice samples were burned by the oxygen-flask technique, and the selenium determined fluorimetrically after reacting with diaminonaphthalene (Allaway and Cary, 1964). The analyses were kindly performed by Dr. W. H. Allaway, U.S. Department of Agriculture Laboratory, Ithaca, N.Y.

RESULTS AND DISCUSSION

In the first experiment with U.S. rice (Table I), each addition of rice decreased the incidence of liver necrosis and increased the average survival time. Ten percent rice was slightly more effective than 0.02 ppm of selenium, and 15% was slightly less active than 0.04 ppm of selenium. These results indicate that the biologically active selenium content of the rice was greater than 0.20 ppm and less than 0.27 ppm.

In the second experiment, both U.S. and Bangladeshi rices were used (Table II). Ten and fifteen percent U.S. rice gave similar responses, slightly better than 0.04 ppm of selenium. Twenty percent U.S. rice gave complete protection, as did 0.06 ppm of selenium. These results indicate that this rice had at least 0.3 and perhaps >0.4 ppm of biologically active selenium.

With the Bangladeshi rices, neither the Barisal white nor the brown at 15 or 30% prevented liver necrosis in any of the rats, and also had little effect on survival time (20–22 days vs. 18.6 days for unsupplemented rats). Thus, it can be estimated that 30% of either white or brown rice provided less than 0.02 ppm of selenium to the diet, and the biologically active selenium of these rices must be less than 0.07 ppm. Chemical analyses (Table III) revealed that U.S. white rice contained 0.46 ppm of selenium; thus, the bioassay value of from 0.3 to 0.4 ppm indicates that all of the selenium was not biologically active. The Barisal white and brown rice contained 0.06 and 0.07 ppm of selenium, respectively, which agree with the bioassay value of less than 0.07 ppm. The bran from the Barisal rice had about twice the content as the endosperm. Brown and white rice from two other districts contained 0.16 and 0.17 ppm of selenium, about twice the content of the Barisal rice but only about one-third that of the U.S. rice.

Table III. Selenium Content of Rice from Bangladesh and the U.S. Determined by Chemical Analysis

	ppm of Se
Bangladesh	
Barisal white	0.06
Barisal brown	0.07
Barisal bran	0.12
Mymensingh brown	0.17
Comilla white	0.16
U.S.	
Louisiana white	0.46

The selenium content of plants is related to the type and amount of selenium in the soil in which they are grown. Morris and Levander (1970) reported selenium values for U.S. rice of 0.31 and 0.38 ppm for white and brown rice, respectively. In comparison with these values, the rice from Bangladesh is very low in selenium. Based on an average consumption by rural adults of 500 g of rice daily, the selenium intake from this source can be estimated to be in the range of 30–85 μg. Since animal protein, the richest source of selenium in human diets, is consumed in very small quantities (ca. 40 g wet weight/day) by these rural populations, it appears that in many areas the total diet may provide less than 0.1 ppm of selenium, the value considered to be a satisfactory human intake (National Academy of Sciences, 1971). Calculation of the total dietary selenium, using published values for other components of the diet (Morris and Levander, 1970; National Academy of Sciences, 1971), gives a range of 63–122 μg of selenium daily. In view of the reported low vitamin E status in a significant fraction of the Bangladeshi population (Rahman et al., 1964), this area could provide valuable information on the human requirement for selenium, at present not established.

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