Table 1. Hemoglobin components in members of Thai family "K."

Subject	Sex	Age (yr)	Hemoglobin types*			Percentage of hemoglobin F†	Remarks
			E	A	F		
Mrs. K.	F		−	+	−	Normal	Mediterranean trait
Mr. K.	M		+	+	−	Normal	Hemoglobin-E trait
D. K.	F	2½	+	+	+	13.6	Mediterranean-hemoglobin E disease (transfused)
C. K.	M	1	+	−	+	33.0	Mediterranean-hemoglobin E disease
P. K.	F	7	+	+	−	Normal	Hemoglobin-E trait

* By paper electrophoresis method.
† By alkali denaturation technique.

osmotic fragility, and only a few target cells. Electrophoretic analysis of his hemoglobin revealed hemoglobin E in association with hemoglobin A; he was regarded, therefore, as having the hemoglobin-E trait. Two children (D. K. and C. K.) were found to have Mediterranean-hemoglobin E disease, a severe hemolytic anemia, indistinguishable from Mediterranean anemia but with both the E and F types of hemoglobin present. The small amount of hemoglobin A found in D. K. almost certainly resulted from a recent transfusion. Some of the characteristics of the disease are evident in the following data on C. K. This boy has hepatosplenomegaly, and on the day of examination he had 2.24 million erythrocytes per mm^3, 5 g of hemoglobin per 100 ml, 19 percent packed red-cell volume, 8.2 percent reticulocytes, 17,450 leucocytes per mm^3 with a shift to younger forms of granulocytes, and 40 nucleated erythrocytes per 100 white-blood cells. The third child, P. K., is an example of the hemoglobin-E trait.

Of the remaining four subjects who were found to have hemoglobin E, three were unrelated, while the fourth subject was the maternal half-sister of the children listed in Table 1; she presumably inherited the hemoglobin-E gene from her father who was not available for study. One of the four subjects had the hemoglobin-E trait, while the other three, including the maternal half-sister, had Mediterranean-hemoglobin E disease.

Among the cases so far encountered, it is interesting to note that, if the intensity of the hemoglobin spots on the paper is taken as a rough guide to the amount present, hemoglobin E forms the major component in Mediterranean-hemoglobin E disease and the minor component in the hemoglobin-E trait.

Two additional families in which Mediterranean anemia has occurred have been examined; one of these was included in the report by Minnich *et al.* (*2*) in their study of Mediterranean anemia in Thailand. Only hemoglobins A and F were found, the expected pattern in Cooley's anemia. Further studies of the occurrence and significance of hemoglobin E are currently in progress in Thailand.

References and Notes

1. This work was supported by Grant No. H-22 C(7) from the National Heart Council of the U.S. Public Health Service and by the Mark A. Edison Memorial Fund. The authors wish to acknowledge their appreciation for help given by Donald Ruchnagel and Su-ed Kochaseni.
2. V. Minnich *et al.*, *Blood* **9**, 1 (1954).
3. J. V. Neel and W. N. Valentine, *Genetics* **32**, 38 (1947).
4. H. A. Itano, *Science* **117**, 89 (1953).
5. E. W. Smith and C. L. Conley, *Bull. Johns Hopkins Hosp.* **93**, 94 (1953).
6. T. H. Spaet, *J. Lab. Clin. Med.* **41**, 6 (1953).
7. Hemoglobin A refers to the normal adult type of hemoglobin; hemoglobin F, to the normal fetal variety; hemoglobin S, to sickle cell hemoglobin; and hemoglobins C and D, to more recently discovered varieties. *Blood* **8**, 386 (1953).
8. H. A. Itano, W. R. Bergren, and P. Sturgeon, *J. Am. Chem. Soc.* **76**, 2278 (1954).
9. W. R. Bergren, personal communication.
10. K. Singer, A. I. Chernoff, and L. Singer, *Blood* **6**, 413 (1951).
11. A. I. Chernoff, *ibid.* **8**, 413 (1953).

20 April 1954.

Experimental Production of Renal Glycosuria, Phosphaturia, and Aminoaciduria by Injection of Maleic Acid*

Harold E. Harrison and Helen C. Harrison

Department of Pediatrics, Johns Hopkins University School of Medicine and *Pediatric Division, Baltimore City Hospitals*

Berliner, Kennedy, and Hilton (*1*) reported that the intravenous injection of maleate into acidotic dogs interfered with the renal tubular mechanisms necessary for excretion of acid urine. Impairment of other renal tubular functions also resulted, and the urinary excretion of phosphate was increased. In a footnote the authors commented on a possible reduction of phosphate Tm. We have suggested that one of the effects of vitamin D is to increase renal tubular reabsorption of phosphate (*2*) and became interested in maleic acid as a possible inhibitor of a renal tubule system that is influenced by vitamin D.

When maleic acid, neutralized to *p*H 7.0 with NaOH, was injected intraperitoneally as a 0.1*M* solution into rats fed a low phosphate diet, an increased phosphate loss in the urine occurred. In the following experiments, 6-wk-old rats that had been maintained on a high-calcium, low-phosphorus, rachitogenic ration for 3 wk were placed in metabolism cages permitting quantitative collection of urine uncontaminated with feces or diet. The following substances were determined in the urine by the methods listed: phosphorus, Fiske and Subbarow (*3*); calcium, Kramer and Tisdall (*4*); citrate, Natelson *et al.* (*5*); and

Table 1. Effect of intraperitoneal injection of maleate upon excretion of calcium, citrate, phosphorus, and amino acids by rachitic rat; No. 85A, wt. 112 g.

Day	Maleate (ml of 0.1*M* soln.)	Urine excretion Ca	Citrate (mg/24 hr)	P	Amino acids (μM/24 hr)
1	0	18.2	18.7	0.05	62
2	0	16.6	18.2	.04	65
3	1.0	6.5	11.8	1.53	481
4	1.0	6.9	8.8	0.63	382
5	2.0	5.3	8.5	.45	147

amino acids by an adaptation to urine of the ninhydrin spectrophotometric technique of Troll and Cannan (*6*).

Table 1 shows the results of a typical experiment in which the given dose of maleate was injected intraperitoneally once daily starting on the third day. In the control period only traces of phosphorus were excreted in the urine, which is to be expected of rats fed this low phosphate diet. Following maleate there was a marked increase in urine phosphorus and concomitantly there was a manyfold increase in urinary amino-acid excretion. The urinary excretion of calcium and citrate, on the other hand, were both diminished. Control rats injected with succinate or malate, in equimolar amounts, showed no increase in urinary phosphate or amino-acid excretion and no decrease in urine calcium or citrate excretion.

Resistance to the maleate effect develops rapidly. In the experiment described, the phosphaturia and aminoaciduria had decreased by the third day of maleate treatment, even though the dose was increased. In several of the rats treatment was discontinued at this point and then resumed after a 5- to 7-day interval. The maleate effect could be elicited again with redevelopment of resistance after several days of the second course of treatment. No evidence of permanent renal injury was found in rats receiving 1.0 ml of 0.1*M* maleate per 100 gm body weight daily for periods of 2 to 3 wk.

Table 2. Glycosuria, aminoaciduria, and phosphaturia following intraperitoneal injection of maleate; rat No. 81A, wt. 70 g.

Day	Maleate (ml of 0.1*M* soln.)	Urine excretion Ca	Citrate (mg/24 hr)	P	Glucose	Amino acids (μM/24 hr)
1	0	23.0	25.4	0.02		80
2	0	19.1	20.0	.03		63
3	0	18.5	20.4	.05	< 6	69
4	1.0	9.4	16.5	1.54	168	566
5	2.0	10.0	16.5	0.93	116*	344
6	2.0	12.2	12.1	.13	38	177

* Blood sugar on this day, 80 mg/100 ml.

Tests of the urine with Benedict's reagent revealed the presence of a reducing substance in the urine of rats injected with maleate, and this was identified as glucose. The concentration of glucose in urine and blood was determined quantitatively by the Somogyi-Nelson method (*7*). Table 2 gives the results of one such study showing the glycosuria appearing simultaneously with the increased urinary excretion of phosphate and amino acids.

It was thought that the increased urinary losses of glucose, phosphate, and amino acids were most likely caused by impairment of renal tubular reabsorption of these solutes and it could readily be shown that the plasma levels of glucose or phosphate were not elevated by maleate injection. In two rats urine was collected for 6 hr following a single injection of maleate, and blood samples were obtained at the end of this period. The blood-glucose levels were 102 and 104 mg/100 ml, and the urinary excretions of glucose were 34 and 3.7 mg, respectively, over the 6-hr period. The serum-phosphorus concentrations were 1.9 and 1.5 mg/100 ml, and the urine contained 0.19 and 0.08 mg of phosphorus. Blood-glucose levels were also determined in four rats on the second day of maleate treatment. The animals were not fasted. The blood-sugar concentrations ranged from 77 to 90 mg/100 ml. In three of the maleate-treated animals the serum-phosphorus levels were found to be 2.0, 2.1, and 2.4 mg/100 ml in comparison with the average value of 2.9 mg/100 ml for seven control rats on the same diet.

Plasma amino acids were not determined. Identification of the urine amino acids by paper chromatography revealed that the excretion of leucine, iso-leucine, valine, methionine, histidine, alanine, and glutamic acid was increased following maleate. Since amino acids of the leucine, iso-leucine, valine group have been shown to be reabsorbed almost completely by the renal tubules in the normal dog even at elevated plasma levels (*8*), their excretion in increased amounts also suggests interference with renal tubule mechanisms.

In a total of 18 rats thus far studied, the maleate effect described here has been consistently elicited. In three rats 20,000 units of vitamin D were given 3 days prior to maleate injection. The increased loss of phosphate, glucose, and amino acids in the urine was not prevented by this large does of vitamin D.

The triad of renal glycosuria, phosphaturia, and aminoaciduria produced by maleate is characteristic of a congenital metabolic defect in man known as the Fanconi syndrome (*9, 10*). In this inborn error of metabolism, rickets or osteomalacia resistant to ordinary treatment with vitamin D is also found. If the locus of maleate effect on renal tubular functions can be determined, it may be possible to characterize the defective enzymatic mechanisms in the Fanconi syndrome and determine whether a single defect or multiple defects are present. Investigation of the basis for resistance to vitamin D in subjects with impairment

of renal tubular function will help in understanding the physiology of vitamin D. The experiments reported suggest an interrelationship of the metabolism of the polycarboxylic acids and specific renal tubular functions.

References and Notes

* Aided by a grant from the Playtex Park Research Institute.

1. R. W. Berliner, T. J. Kennedy, and J. G. Hilton, *Proc. Soc. Exptl. Biol. Med.* **75**, 791 (1950).
2. H. E. Harrison and H. C. Harrison, *J. Clin. Invest.* **20**, 47 (1941).
3. C. H. Fiske and Y. Subbarow, *J. Biol. Chem.* **66**, 375 (1925).
4. B. Kramer and F. F. Tisdall, *ibid.* **47**, 475 (1921).
5. S. Natelson, J. B. Pincus, and J. K. Lugovoy, *ibid.* **175**, 745 (1948).
6. W. Troll and R. K. Cannan, *ibid.* **200**, 803 (1953).
7. N. A. Nelson, *ibid.* **153**, 375 (1944).
8. K. H. Beyer *et al., Am. J. Physiol.* **151**, 202 (1947).
9. G. Fanconi, *Jahrb. Kinderheilk.* **147**, 299 (1936).
10. D. J. McCune, H. H. Mason, and H. T. Clarke, *Am. J. Diseases Children* **65**, 81 (1943).

1 June 1954.

Influence of Hydrogen Ion Concentration on Radiation Effects

Tikvah Alper and M. Ebert

Experimental Radiopathology Research Unit, Medical Research Council, Hammersmith Hospital, London

When ionizing radiation acts on chemical or biological systems by "indirect effect," that is, through the medium of free radicals, the changes are very often oxidations or reductions. It is commonly supposed that the majority of radiobiological changes are oxidative, because the presence of oxygen during irradiation enhances the effects (*1, 2*). Evidence was presented earlier by one of us (*3–5*) that the inactivation of phages S13 and T3 by indirect action of radiation is due to reduction, so that oxygen in the suspension protects the phage, whereas removing oxygen and bubbling hydrogen during irradiation enhances "immediate" radiation effects (*6*). More recently, Bachofer and Pottinger (*7*) found a protective effect of oxygen on T1, and it may be that all phages are sensitive, in the free state, to inactivation by reducing agents.

We have shown (*6*) that, under electron or x-irradiation, the mechanisms of hydrogen peroxide formation in water and immediate phage inactivation in aqueous suspensions can to some extent be regarded as complementary. Under a wide variety of gas treatments, the inactivation of phage proceeded fastest when the hydrogen peroxide formed was least, and vice versa. We showed that the likely reactions leading to formation of hydrogen peroxide and phage inactivation could be fitted into a simple theory of radical formation and reaction. This theory would lead to a dependence on hydrogen ion concentration of the yield for both oxidative and reductive changes, and we are now able to show that in this respect, too, phage inactivation and hydrogen peroxide formation proceed in complementary fashion.

The *p*H dependence of these reactions arises from the step

$$HO_2 \rightleftarrows H^+ + O_2^-,$$

reductive changes being due to the radical ion O_2^-, so that oxidation yields are increased, and reduction yields decreased, in more acid solutions. However, the extent of reaction with O_2^- will depend on *p*H only in the presence of oxygen, since the formation of the HO_2 radical is a preliminary step. As is shown by Fig. 1a, phage is protected against inactivation in acid

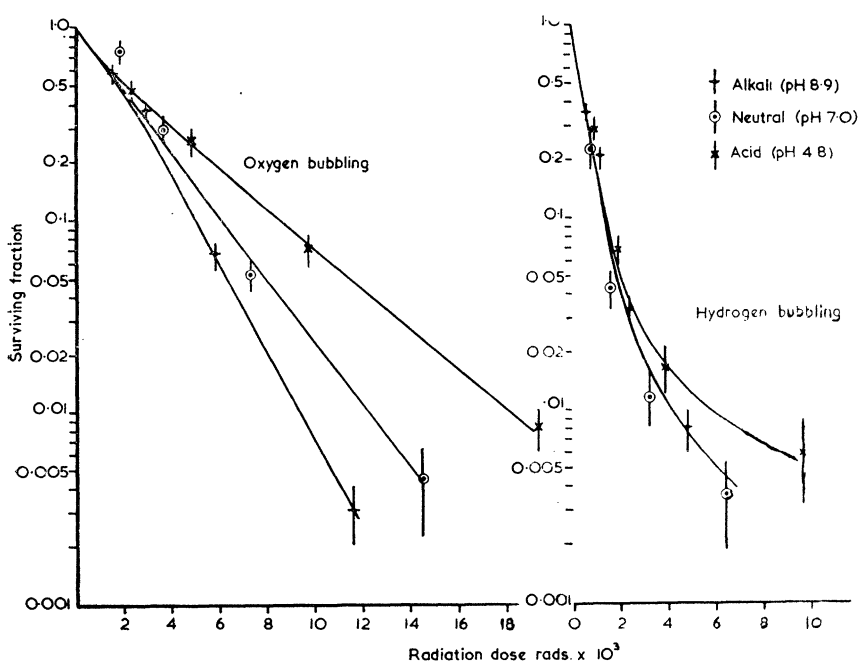

Fig. 1. Survival curves of bacteriophage S13, x-irradiated under (*a*, left) oxygen, (*b*, right) hydrogen bubbling, at three hydrogen ion concentrations.