SPECIAL ARTICLES

A DEFECT IN THE METABOLISM OF AROMATIC AMINO ACIDS IN PREMATURE INFANTS: THE ROLE OF VITAMIN C[1]

IN the course of studies of the nitrogen metabolism of premature infants, a number of the infants were found to excrete substances in their urine which gave the Million reaction.[2] Further studies demonstrated the absence of appreciable amounts of tyrosine, dihydroxyphenylalanine, homogentisic acid and melanin, thus exempting them as the hydroxyphenyl compounds responsible for the reaction. Dr. Hans Clarke, professor of biochemistry at Columbia University, extracted from these urines crystals of l-p-hydroxyphenyllactic acid, identification being made by elementary analysis, melting point and optical rotation, the urinary substance conforming in all respects to the pure compound. Qualitative tests (a positive but transitory response to dilute ferric chloride, a precipitate with 2, 4-dinitrophenylhydrazine) also established the presence of a keto-acid, probably p-hydroxyphenylpyruvic acid in these urines, but it has not yet been isolated in a pure state. Reduction of phosphomolybdic acid by Medes' technique[3] has been employed for quantitative assay.

Since these substances were absent from the urine of premature infants receiving human milk and since their excretion was enhanced by the feeding of phenylalanine and tyrosine, it seemed reasonable to relate the defect to the higher content of these amino acids in cow's milk. The possibility that this defect in oxidation and decarboxylation of aromatic amino acids above a threshold level of intake might be related to an enzymatic deficiency remediable by administration of vitamins was suggested by the observation of Closs and Fölling[4] that the urinary excretion of phenylpyruvic acid following injection of phenylalanine was increased in rats by deficient intake of vitamin B_1. Such therapy with thiamin chloride in liberal dosage (20 mg daily) had no effect on the spontaneous excretion of hydroxyphenyl compounds in several of the infants. The effect of vitamin C therapy, however, was striking.

Shortly after birth five male premature infants were placed on diets of powdered cow's milk of adequate caloric, fluid and protein (5 gm per kg) content supplemented with vitamin A and D concentrates (20 drops of percomorph oil daily) but without added vitamins B and C. Complete 24-hour urines were quantitatively assayed for hydroxyphenyl derivatives.[5] The infants received from 50 to 200 mg of ascorbic acid parenterally at the ages indicated in Table 1, and in four of the five there resulted a prompt decline with virtual disappearance in excretion of hydroxyphenyl compounds within 48 to 72 hours. In the fifth infant (G. A.) 50 mg was ineffective, but the administration of 100 mg several days later was accompanied by a prompt response. In another infant (G. B.) the cycle of appearance of urinary hydroxyphenyl derivatives with omission of dietary vitamin C and disappearance with its resumption was demonstrated on two occasions. These results gain added interest in view of a recent report relating the production of experimental alcaptonuria to vitamin C deficiency[6] and the demonstration *in vitro* of the relation of dehydroascorbic acid to the breakdown of amino acids, including phenylalanine, to aldehydes.[7]

TABLE 1

THE EFFECT OF VITAMIN C ON THE EXCRETION OF HYDROXYPHENYL COMPOUNDS

Age days	Vitamin C supplement mg	Tyrosine* in urine mg	Age days	Vitamin C supplement mg	Tyrosine* in urine mg
	A. B.			G. A.	
38		953	20		448
39	200	940	21		477
40	200	67	22	50	524
41	100	33	23		401
42	25	37	24		436
			25	100	176
	A. C.		26		36
45		1,079			
46		1,067		G. B.	
47	200	324	18		606
48	200	38	19		654
49	100	32	20		534
			21	100	322
	J. P.		22		48
11		525	23		39
12		627	28		281
13	75	619	29		469
14	75	251	30		619
15		30	31	200	494
16		24	32		340
			33	200	170
			34		40

* Million reaction.[5] Figures represent hydroxyphenyl compounds expressed as tyrosine.

Analysis of the blood in three of the infants in the control periods showed no ascorbic acid.[8] After administration of vitamin C in amounts sufficient to suppress the excretion of hydroxyphenyl compounds, the blood ascorbic acid was still low, varying from 0.13 to 0.32 mg per ml, indicating that although a change in cellular oxidations had taken place, the blood had not reached normal levels. Roentgenograms of the long bones revealed no signs of manifest scurvy. The evidence suggests that the metabolic aberration in premature infants is an interrelated function of the level of intake of the aromatic amino acids, phenylalanine

[1] Assistance in this work was given by the Children's Bureau, U. S. Department of Labor.

[2] S. Z. Levine, *Am. Jour. Dis. Child.*, 58: 674, 1939.

[3] G. Medes, *Biochem. Jour.*, 26: 917, 1932.

[4] K. Closs and A. Fölling, *Ztschr. f. physiol. Chem.*, 254: 258, 1938.

[5] O. Folin and V. Ciocalteu, *Jour. Biol. Chem.*, 73: 627, 1927.

[6] R. R. Sealock and H. E. Silberstein, SCIENCE, 90: 517, 1939.

[7] E. Alderhalden, *Fermentforschung*, 15: 522, 1938.

[8] R. L. Mindlin and A. M. Butler, *Jour. Biol. Chem.*, 122: 673, 1938.

and tyrosine, and the degree of saturation of the tissues with vitamin C. Whether the same metabolic error is present in full-term infants under similar dietary conditions or in older infants with manifest scurvy is at present being investigated. Studies are also under way to determine the specificity of vitamin C in remedying the defect. The spontaneous occurrence of hydroxyphenyl compounds in the urine of premature infants fed cow's milk deficient in vitamin C affords the opportunity of studying the intermediary metabolism of aromatic amino acids in the growing human organism.

S. Z. LEVINE
ELEANOR MARPLES
H. H. GORDON

NEW YORK HOSPITAL AND THE
DEPARTMENT OF PEDIATRICS,
CORNELL UNIVERSITY MEDICAL COLLEGE

REACTION OF VARIOLA VACCINE VIRUS TO ROENTGEN RAYS[1]

ROENTGEN ray irradiation of variola vaccine virus particles causes a reduction in number of active particles. The rate of inactivation proceeds exponentially.

Vaccinia virus[2] for irradiation was extracted from infected rabbit testis preserved in glycerine, by grinding the testis in sand and diluting it 1:10 with Locke's solution. After centrifuging, 0.75 cc of the supernatant solution which carried the virus was exposed to x-rays from a copper target tube.[3] The mean effective wave-length of this radiation was found to be 1.5 Å. The reduction in the velocity of virus inactivation was determined by titration experiments in which 0.1 cc of the irradiated and the control virus suspensions were inoculated intradermally into normal rabbits. Estimates of the infectious particles, remaining after irradiation, were made from those tests in the dilution series which gave both positive and negative takes.[4]

The inactivation curves of three experiments, in which vaccinia virus was irradiated with increasing dosages of x-rays, are plotted in Fig. 1. The logarithms of the numbers of infectious particles are the ordinates of the graph. The abscissae represent the Roentgen units of irradiation incident to the surface of the virus solution. Each curve is evidently linear

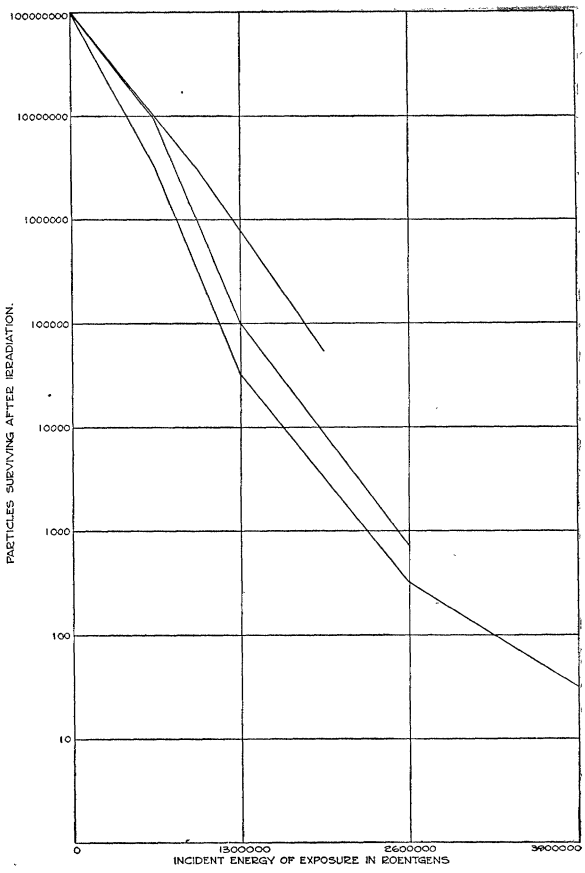

FIG. 1. Survival ratios of vaccinia virus treated with Roentgen rays of 1.5 Å.

in its trend; the inactivation of virus particles begins when irradiation commences. The small quantitative differences in the slope constants which do occur are partly accounted for by small variations in the Roentgen units in each experiment.

The linear nature of the raw data when plotted on the semi-logarithmic coordinates, and the absence of any initial lag during the inactivation of the virus is expected if we assume that one unit of radiant energy is sufficient to inactivate an infectious unit of the virus. The curve form would further postulate that the ultimate virus particles are held separate and distinct from each other in the water suspension when irradiation takes place—not in any conglomerate of particles—for if they were conglomerate the entities would require more than one unit of x-ray energy for inactivation and consequently lead to raw data having an initial lag period and a convex curvature instead of the straight lines observed.

The experimental observations are due to the inability of certain virus particles to reproduce after they have absorbed a unit of irradiation. The portion of the virus particle with which we are dealing is therefore only that which has to do with reproduction.

[1] Journal paper No. J-676 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 573. We are indebted to the Fuller Fund and the International Cancer Research Foundation for assistance in these researches.

[2] We are pleased to acknowledge our indebtedness to Dr. A. B. Sabin for the dried virus from which these experiments were started.

[3] L. E. Pinney, *Iowa State College Jour. Sci.*, 13: 269–273, 1939. We are indebted to Mr. Pinney for much other assistance.

[4] K. Iwaszkiewicz and J. Neyman, *Acta Biol. Exp.*, 6: 101–142, 1931.