Oxygenation and Ion Transport in Red Cells

Abstract. *Ion transport in red blood cells may depend on the binding of 2,3-diphosphoglycerate (DPG) to deoxyhemoglobin.*

McManus (*1*) discusses the "surprising" finding that deoxygenation of red cells leads to an increased rate of ion transport. This observation, originally made by Tosteson and Robertson (*2*), was interpreted in terms of changes in cell permeability.

We suggest an alternative explanation based on our finding that 2,3-diphosphoglycerate (DPG) and inositol hexaphosphate (IHP), the major organic phosphate esters of mammalian and avian erythrocytes, respectively, are bound preferentially to the deoxygenated form of hemoglobin (*3*). In the case of DPG, binding at *p*H 7.3 in 0.1*M* NaCl occurs only on deoxyhemoglobin in a ratio of 1 mole of DPG to 1 mole of hemoglobin, while no interaction with oxyhemoglobin takes place at all (*3*).

As a result, DPG and, in the case of avian red blood cells, IHP would be continually removed by hemoglobin under anaerobic conditions, thus stimulating glucose uptake by the red cells to replenish the stores of free organic phosphate (*4*). Asakura *et al.* (*5*) have actually shown that, while the rate of glycolysis and the rate of formation of 2,3-DPG is the same in human red cell suspensions in oxygen or in carbon monoxide, it is considerably greater in suspensions kept under nitrogen where the hemoglobin is in the deoxy form.

The hypothesis that altered ion transport under anaerobic conditions is due to removal of DPG by deoxyhemoglobin and consequent stimulation of glycolysis is in agreement with two further observations:

1) The aerobic incubation [by Tosteson (*1*, p. 1822)] of cells in the presence of respiratory inhibitors *does not* cause an increase in influx of potassium. Under these conditions, hemoglobin would be still in the oxygenated state and would therefore not interfere with the levels of free organic phosphate.

2) It is equally significant that there is also no increase in sodium transport under completely anaerobic conditions, if they are created by incubation in 100 percent CO (*1*). This again bears out the above interpretation, since carbon monoxy hemoglobin, like oxyhemoglobin, will not bind polyphosphates under physiological conditions.

Schacter (*6*) suggested to us that the red cells of ruminants with their negligible content of DPG (*7*) should provide an ideal control for testing our proposal. Thus, in the case of sheep erythrocytes, for example, ion transport should not be affected by deoxygenation.

The question of the mechanism whereby the level of free organic phosphate esters such as DPG might control normal ion transport, remains open, but it suggests a fruitful approach to this important problem.

The dramatic effect of these compounds in lowering the oxygen affinity of hemoglobin to bring it into the normal physiological range is probably but one example of the powerful regulatory role of these substances within the red cell.

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Milk and Lactose Intolerance in Healthy Orientals

Abstract. *Nineteen of 20 healthy Oriental adults living in the United States developed abdominal cramps and diarrhea after ingesting an amount of lactose equivalent to that in one quart of milk; 14 reported similar symptoms after one or two glasses of milk; all had consumed milk as infants without having such symptoms. Two of 20 Caucasians tested were intolerant to milk and lactose. Many Orientals therefore may have a genetically determined lactase deficiency that may lead to intolerance to milk. Since lactase deficiency is also common among Negroes, the bulk of the world's adult population is probably intolerant to milk.*

Milk-induced abdominal cramps or diarrhea (probably caused by low levels of intestinal lactase, resultant maldigestion of the milk-sugar lactose, and a subsequent osmotic and fermentative diarrhea) are common in certain adult populations. We believe that this lactase deficiency in adults is genetically determined.

Seventy percent of 20 healthy Negroes in Baltimore were lactose-intolerant and had low lactase levels (*1*), and 72 percent of 40 African Negroes in Uganda had low levels of intestinal lactase (*2*). A similar prevalence may exist in American Indians (*3*), Greek Cypriots (*4*), and Australian aboriginal children (*5*). By contrast, in adult Caucasian Americans the prevalence of lactase deficiency and intolerance to milk is probably no greater than 5 to 10 percent (*1*, *6*).

The milk-intolerant adults usually had been able to drink milk as infants, and milk-induced symptoms first occurred in adolescence or the early 20's. Incidence of lactose-induced symptoms in Negroes increases with advancing age, suggesting a gradual decrease in lactase activity after weaning (*7*).

Knowing that milk intolerance is common in adults in Formosa and that powdered milk is being exported there and to other Asian countries, we surveyed a group of healthy Orientals for prevalence of milk and lactose intolerance. We selected at random 20 healthy physicians and medical personnel (14 men and 6 women, aged from 23 to 38 years; mean, 31 years) for study. None were related or had gastrointestinal disease (one had had a duodenal ulcer); three had asthma, hay fever, or known drug allergies; seven, natives of China (Formosa), had been in the United States 4 years (mean); three, born in the continental United States of Chinese parents, had never visited Asia; ten, natives of the Philippines, had lived in the United States 4 years (mean). The control group, 20 healthy Caucasians, aged 18 to 54 years (mean, 32.6) (*1*), were born in the continental United States.

To test lactose tolerance we administered 50 g of lactose in 300 to 400 ml of water after a fast of at least 4 hours. Venous blood samples were obtained at 0, 15, 30, 60, 90, and 120 minutes, and total reducing substances (blood sugar) were determined by a ferricyanide

method adapted to the autoanalyzer. Normally, the blood sugar increases as the monosaccharide products of lactose digestion are absorbed, and no symptoms are produced. The control subjects received 50 g of lactose per square meter of body surface (maximum of 100 g). Increases of blood sugar with either 50- or 100-g tests are of similar magnitude in patients with "normal" lactase levels (*8*).

Fourteen of the 20 Orientals stated that usually 1 to 4 hours after ingestion of one or two glasses of milk or of one portion of ice cream they experienced flatulence, abdominal bloating, abdominal cramping pain, or diarrhea; five had such violent symptoms that they avoided all milk products; the others could tolerate small amounts of milk, as in cereal or coffee; all 20 had consumed milk without ill effect during infancy, and most began to notice milk intolerance in adolescence or in their early 20's; five, who later became milk-intolerant, remember drinking milk quite frequently during childhood; 13 had a parent or sibling with similar intolerance to milk.

Lactose-tolerance tests produced symptoms in 30 minutes to 4 hours in 19 of the 20 Orientals: 19 had abdominal bloating, flatulence, and diarrhea, varying from one to ten liquid bowel movements; 17 had abdominal pain and cramping. The symptoms result mainly from hypermotility and large amounts of fluid secreted into the bowel to maintain isotonicity by diluting the undigested and unabsorbed lactose. The peak in blood sugar rises after the lactose loads are less than 26 mg per 100 ml of blood in all 20 Orientals (Fig. 1) (mean rise, 14.5 mg per 100 ml), demonstrating that the lactose was not adequately hydrolyzed to glucose and galactose.

The results in the healthy Caucasian American control group were quite different. Two were milk-intolerant and had lactose-induced symptoms. Blood sugar rose less than 26 mg per 100 ml in only 2 of the 20 (Fig. 1). The mean rise, excluding two diabetics, was 37.6 mg per 100 ml. Another subject gave a family history of milk intolerance.

From our study of a randomly selected population sample, we conclude that (i) milk and lactose intolerance are common in persons of Oriental extraction; and (ii) an inherited lactase deficiency presumably causes this milk intolerance. Possibly, there are two intestinal lactases, an "infantile" enzyme allowing most subjects to drink milk as infants, and a second or "adult" lactase, perhaps not developed in persons who later became milk-intolerant.

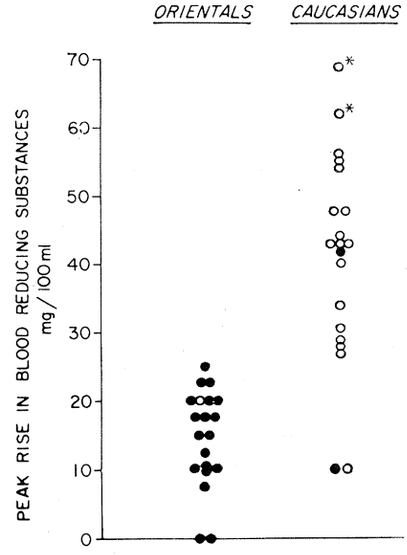

Fig. 1. Results of lactose-tolerance tests; values are maximum rises in blood-reducing substances; 19 Orientals developed abdominal cramps or diarrhea. Solid circle, symptoms with lactose load; open circle, no symptoms; asterisks, diabetic.

Etiology, other than genetic causes, of the milk and lactose intolerance seems unlikely. Adaptation to a lack of enzyme substrate (lactose) was considered since adults drink less milk than children do; and the enzyme lactase is a β-galactosidase—an inducible enzyme system in bacteria. But 5 of the 19 lactose-intolerant subjects studied had consumed milk in large quantities until after adolescence, and most consumed some milk, as a beverage, in cereal, or in coffee; nine had unsuccessfully tried to improve tolerance to milk by slowly increasing intake over several years.

Generalized disaccharidase deficiencies can result from damage to the enzyme sites in the intestinal epithelium, which does not adequately explain the symptoms of these patients without history of acute gastroenteritis, celiac disease, tropical sprue, ulcerative colitis, or other gastrointestinal illnesses associated with lactase deficiency. Although jejunal biopsies of persons living in Asiatic countries such as Pakistan and Thailand show mild abnormalities (*9*), similar nonspecific abnormalities in Yemenites tended to disappear after prolonged exposure to Western environment in Israel (*10*). Three of our subjects were born of Oriental parents in the continental United States and had never been in Asia; 16 of the other 17 had been in the United States for over 1 year. Although we did not obtain final proof by intestinal biopsy, it seems unlikely that mucosal damage caused the high incidence of milk and lactose intolerance.

Environmental and selective factors in Asia may possibly affect maintenance of low lactase levels as a genetic trait (genetic polymorphism) (*11*). Since milk production is low in most of Asia, drinking milk after weaning is unusual in Formosa and the Philippines.

Many Asian nations still receive much powdered milk (containing 38 g of lactose per 100 g) from various private, religious, and governmental organizations. Not knowing the frequency of milk-induced symptoms in the recipients, we should reevaluate the importance of milk as a source of nutrition and protein during adult life when planning diets for Asians, Africans, and probably other non-Caucasian population groups. Milk intolerance should be recognized in those groups who might otherwise be advised to consume large amounts of milk, as in pregnancy or for the treatment of peptic ulcer disease.

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12. *Note added in proof*: Similar results for Chinese and Indians were recently reported [A. E. Davis and T. Bolin, *Nature*, **216**, 1244 (1967).
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