Letter to the Editor

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H. Skovbjerg (1) reported the isolation by immunoprecipitation of the sucrase-isomaltase complex of the human fetus as a high molecular weight precursor with a molecular weight of 260,000. We used another methodology to study the structure of this complex. Adult small intestines were obtained from kidney donors. Three fetal intestines, 16–24 wk gestational age, were obtained after therapeutic abortion. Brush-border membrane fractions were prepared according to Schmitz *et al.* (2) and separated on 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis in the buffer system of Laemmli (3). The proteins were then transferred on nitrocellulose sheets by the technique of Burnette (4) and incubated with antisucrase-isomaltase antibodies and radioiodinated protein A. Antihuman sucrase-isomaltase antibodies were obtained in the rabbit, pure human sucrase isomaltase was purified after serial chromatographic steps as briefly described in reference 5.

The results obtained are shown in Figure 1. In adult jejunum we can observe two forms of the enzyme: the high molecular weight precursor with apparent molecular weight of 280,000 and a broad band corresponding to isomaltase and sucrase two subunits of apparent molecular weight 140–160,000. The two subunits cannot be clearly separated in this system. In human fetuses the enzyme exists only as the uncleaved high molecular weight precursor, but this form migrates clearly more distally than the adult precursor form. The difference could be evaluated at a molecular weight of approximately 10–20,000. The specific activities in the homogenate and the brush-border fraction are the same for adult and fetal small intestines. These results clearly show that human adult and fetal prosucrase-isomaltase are different. This difference may be due either to modification of the glycosylation process as suggested by Auricchio *et al.* (6), or to the expression of an embryonic form of sucrase-isomaltase related to a fetal gene as described for instance for globin gene (7) or myosin gene (8). We are presently testing the first hypothesis with lectin-binding studies (9).

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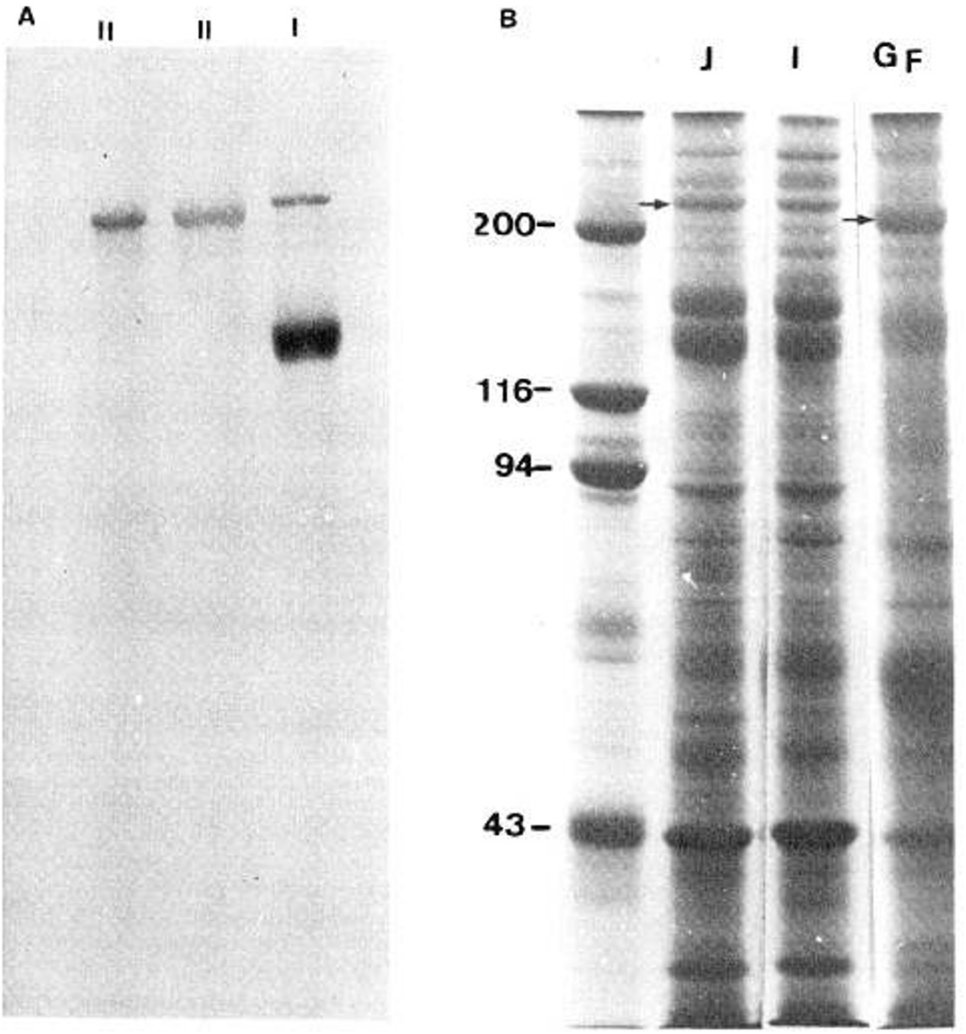

Fig. 1. (*A*) Autoradiographic detection of sucrase-isomaltase complex on nitrocellulose sheet with specific antihuman sucrase-isomaltase antibodies. I. Adults small intestine (33 mU sucrase) and II. fetal small intestine (30 mU sucrase). (*B*) Polyacrylamide gel electrophoresis of adult and fetal small intestinal brush-border membrane. Coomassie blue staining. Abbreviations: J, adult jejunum; I, adult ileum; and GF, fetal small intestine. The *arrows* indicated the position of prosucrase-isomaltase.

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Letter to the Editor

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The volume of distribution of bromide is often used to estimate the volume of extracellular water in the body. The "corrected" bromide space (CBS) is assumed to equal the volume of extracellular water. To calculate the CBS, the dose of bromide (Br dose) is divided by the equilibrium concentration of bromide in extracellular fluid ($[Br]_e$), with a correction for the amount that is assumed to be distributed in non-extracellular sites (principally erythrocytes):

$$CBS = \frac{\text{Br dose} \times 0.90}{[Br]_e}$$

Because $[Br]_e$ cannot be measured directly, it is calculated from the concentration of bromide in serum ($[Br]_s$) by applying two additional correction factors: the Donnan equilibrium factor 0.95 (8, 11, 12), and the concentration of water in serum (7, 8, 12), which is approximately 94% (12). Thus,

$$[Br]_e = \frac{[Br]_s}{0.95 \times 0.94}$$

and, therefore

$$CBS = \frac{\text{Br dose}}{[Br]_s} \times 0.90 \times 0.95 \times 0.94$$

These corrections are not needed when one uses bromide to estimate total body chloride (10) because the Donnan factors for these two elements are the same, and both are measured in the same serum sample.

Several authors (1, 4, 7, 9, 13, 14) have applied one or two of these three factors incorrectly, multiplying rather than dividing $[Br]_s$ by the Donnan factor and the serum water correction. The result in most cases has been a large, systematic overestimation of extracellular water. When both the Donnan factor and the serum water correction are used incorrectly (1, 4, 9, 13, 14) the resulting errors are additive; extracellular water is then systematically overestimated by 25–31%, depending on the factor used for the serum water correction (0.92–0.94). It should be noted, parenthetically, that even with correct application of these factors there is a slight overestimation of the bromide space if no correction is made for bromide lost in urine before the serum sample is obtained.

Table 1 shows the corrected bromide space results for infants as reported (1, 4, 9, 13, 14) and the values that would have been obtained with correct application of the Donnan and serum water factors. The mean extracellular water volume calculated by Brans *et al.* (1) was 335 ml/kg, higher than the "expected" volume of 286 ml/kg (ref. 1, Table 3). They concluded that "infants with congenital heart disease appear to have . . . slightly expanded volumes of extracellular water" (1). This conclusion seems to have been drawn erroneously as a result of the aforementioned errors in correcting the bromide space (unless the "expected" volume was also calculated incorrectly).

Table 1. *Corrected bromide space (CBS) of infants*

Authors	Subjects	Mean CBS as reported (ml/kg)	Correctly calculated* mean CBS (ml/kg)
Cassady (4)	TermAGA†	376	296
	Premature AGA†	424	334
Brans *et al.* (1)	Infants with CHD‡	335	264
Thornton *et al.* (13)	Term	504	397
	Premature	640	504
Thornton *et al.* (14)	Normocythemic	510	402
	Polycythemic	520	409
Cheek *et al.* (9)	TermV§	358	273
	TermC ‖	354	270

* Using same Donnan factor and serum water correction used by original authors: Donnan factor 0.95 (1, 4, 9, 13, 14); serum water factor 0.934 (1, 4, 13, 14) or 0.92 (9).

† AGA, appropriate birth weight for gestational age, less than 24-h-old.

‡ CHD, congenital heart disease.

§ V, vaginally delivered, 24-h-old.

‖ C, delivered by cesarean section, 24-h-old.

The extent of these errors is not known because many authors report only that corrections were made for the Donnan equilibrium and serum water, without giving details of the calculations. It must be presumed, however, that other papers (2, 3, 5, 6) citing the method of Cassady (4) or Thornton *et al.* (14) also contain bromide space results that were incorrectly calculated. In some cases extracellular water was incorrectly estimated from bromide space and then subtracted from total body water to yield intracellular water (1, 2, 5, 6, 9, 14). This, of course, led to underestimation of intracellular water by the same amount. For example, the mean intracellular water of 328 ml/kg reported by Brans *et al.* (1) should have been 399 ml/kg.

As far as we know, the most recent experimental paper to correctly use and describe the three corrections for bromide space was published in 1954 (8). Most of our information regarding the extracellular water of infants is based on bromide space measurements. Many of these data are demonstrably wrong (1, 7, 9, 13, 14); others cannot be judged because the details of bromide space correction were not given. We conclude that infants contain less extracellular water than was previously thought.

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