of embryo into slow- and fast-cleaving is based only on morphological criterion, *i.e.*, microscopical examination of litter mate embryos before transfer. (In the rabbit, the interval from one cleavage to the other varies from 6 to 16 hr.) In some cases the difference between the slow- and fast-cleaving embryos was one cleavage rate; in such cases the difference in number of blastomeres at 2 days *p.c.* may be due to some embryos being ready to cleave while others were completely cleaved. In other cases, however, the difference between slow- and fast-embryos was 3 or 4 cleavages.

In the rat, differences were found between embryos in rate of cleavage and there was a tendency for development of retarded embryos to be accelerated with time so that, by 120 hr *p.c.*, most of the viable ova had reached the blastula stage(8). Fetal size in the rabbit has been correlated with the concentration of glutathione in the fetus at birth, greater concentration being associated with higher growth rate(11,12,13). Further comparative studies are needed to evaluate the relationship between the number of blastomeres, at a given stage, in relation to subsequent rate of fetal development.

Summary. Two-day litter mate embryos were obtained from superovulated rabbits, divided into slow- and fast-cleaving according to the number of blastomeres per embryo, and stored in 1:1 serum:saline at 10°C for 48 hr. Slow- and fast-cleaving embryos obtained from one donor were transferred into the 2 Fallopian tubes of one recipient. The recipients were laparotomized at 8 days *p.c.* and autopsied at 29 days *p.c.* The viability of fast-cleaving embryos, as judged by per cent implants after storage, was higher than in slow-cleaving embryos. In most cases, 2-day embryos with more blastomeres had larger implants and heavier fetuses than embryos with less blastomeres; these differences were not statistically significant.

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Misleading Reductions of Serum Sodium and Chloride Associated with Hyperproteinemia in Patients with Multiple Myeloma. (27449)

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Conventional units which depict concentrations of electrolytes, clinically, in blood plasma or serum are milliequivalents per liter (meq/l), millimoles per liter (mM/l), milliosmoles per liter (mosm/l) and mg per 100 ml. Albrink *et al.*(1) have shown that under circumstances of extreme hyperlipemia (marked lactescence) spurious

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reduction of serum sodium and chloride is inferred unless the foregoing units are replaced by expressions corresponding to molality (or, more strictly, millimolality). Thus concentrations of sodium and chloride described as weight per volume of serum may be inappropriate in contrast to cogent descriptions in terms of weight of solute per unit weight of water of the serum. The present study was designed to determine whether or not interpretative advantages from the clinical viewpoint result from the use of millimolality instead of millimolarity when there is massive elevation in concentration of serum proteins.

Definitions. The terminology of Clark(2) is used in this paper: A millimol*ar* solution contains 0.001 mole of a substance per liter of solution whereas a millimol*al* solution contains 0.001 mole of a substance per kilogram of solvent. It is noteworthy that, in this paper, in accord with the custom of many investigators, molarity is used synonymously with millimolarity and molality with millimolality.

Materials and methods. Five patients with the characteristic clinical picture of multiple myeloma and concentrations of serum proteins exceeding 10 g per 100 ml comprised the primary experimental group. Controls consisted of 6 patients with various neoplastic diseases in whom serum total protein concentrations were either normal or slightly reduced. One of these 6 controls had genuine multiple myeloma with both typical bone marrow and electrophoretic pattern abnormalities. A seventh patient with multiple myeloma had a total serum protein intermediate between control and experimental groups.

Venous blood was obtained with degrees of stasis varying from none to pronounced. Serum was separated from the blood cells promptly. Heparinized plasma was used only occasionally. Sixteen blood samples were studied from the 5 patients in the experimental group and 8 bloods of the other 7 patients were similarly evaluated.

Water of serum was measured by difference between wet and dry weight of 1 ml kept in an oven at 105°C for 2 days until constant weight was attained. Sodium and potassium were measured using a Perkin-Elmer flame photometer with lithium as the internal standard. Chloride was measured by the method of Schales and Schales(3). Paper electrophoresis was performed on a Spinco Model R, and the pattern in all patients with myeloma was characterized by a gamma type of hyperglobulinemia. Serum and plasma total proteins were measured by the biuret method. All determinations were performed on single aliquots.

TABLE I. Total Protein and Water of Serum or Plasma.

	Water kg per liter	Protein g per 100 ml
Controls	.925–.942 (.935)	5.8– 7.4 (6.7)
Myeloma	.867–.908 (.890)	10.2–14.4 (12.1)

Reading and discussion. The average and ranges of total protein concentrations and water contents of serum and plasma of the hyperproteinemic and control groups are shown in Table I. The water content of myeloma sera with total proteins exceeding 10 g/100 ml averaged 4.7% lower than that of controls (range of 1.8 to 8.0). This substantial difference entails the corollary that concentrations of serum sodium, chloride, potassium (and indeed of all serum solutes) may display striking differences resulting from discrepancies between millimolarity (*e.g.*, mM of Na/l of serum) and millimolality (mM of Na/kg serum water).

A striking example of the magnitude of the disparity between millimolality and millimolarity is shown in Table II. The serum proteins were 14.4 g/100 ml and water was 0.867 kg/l of serum. The water content was 7.3% lower than the mean of the control sera thereby defining a corresponding difference in solute concentrations when the 2 units of expression are used. The clinical significance of this disparity (Table II) is self-evident. Differences between actual molarity and actual molality are pronounced. These differences are less pronounced but still impressive even if one takes into account the content of solids present on the average in the sera of controls. This is done in the column headed "hypothetic molality".

Serum sodium and chloride (hypothetic

TABLE II. Clinical Significance of Millimolality in a Patient with Intense Hyperproteinemia. (D.A. #69657 W)

Solute	Actual molarity	Actual molality	Hypothetic* molality
	mM/liter	mM/kg H_2O	mM/kg H_2O
Na	127	147	136
Cl	119	137	127
K	4.4	5.1	4.7

* Calculated with average water content of controls.

molality) differ from their true concentrations (actual molality) by 11 and 10 mM/kg of water respectively. The same percentual difference obtains for potassium although the absolute difference is insignificant because of the low concentrations involved in comparison to sodium and chloride. That similar differences (which may prove misleading clinically) among serum sodium and chloride were not unique is implicit in the fact (in 2 patients) 6 of the 16 hyperproteinemic sera are typified by the example cited in Table II.

The hyperchloremia illustrated in Table II is representative of our findings in 11 of the 16 serum or plasma samples in the experimental group. Its nature is obscure, since none of the usual clinical causes were apparent, but certain possibilities may be invoked: 1. The abnormal globulins behaved predominantly as cations rather than as anions. 2. Some other, unmeasured, cation accumulated. 3. Chloride may have leaked from erythrocytes in the course of separation of plasma or serum from cells. 4. The present method of determining chloride under circumstances of marked hyperproteinemia may yield misleading results.

The expression of concentrations of electrolytes and crystalloids in terms of millimolarity or analogues (mg/100 ml, meq/l, mosm/l) is convenient but on occasion misleading. Osmotic and other effects of ions are mediated by their distribution and magnitude in the water in which they are dissolved and are largely independent, save for possible binding to large molecules, of the increment of other serum solids. The utility of millimolarity as a reasonably accurate mode of expression of serum sodium and chloride concentrations within the purview of clinical biochemistry stems from the fact that serum water varies by only 1 or 2% under most circumstances. Nevertheless, extreme hyperlipemia or massive hyperproteinemia dictate measurement of serum water and use of millimolalities lest serious errors of interpretation be made.

Peters *et al.*(4) studied the relation of water to protein content of 94 blood sera of 73 subjects. Thirty were normal and 43 were patients with a wide variety of diseases. They calculated that the average volume displaced by 1 g of serum solids, presumably proteins predominantly, was 0.72 ml. This value of the partial specific volume of serum proteins in man agrees well with data derived from studies of purified proteins(5). It also contrasts with lower values for urea, glucose, and the salts of plasma, which displace less water. On the other hand, fats and fatty acids displace far more water than proteins. Peters' study included no subjects with serum proteins exceeding 10 g per 100 ml.

The relation between serum water and proteins in our data is plotted in Fig. 1. The distinct tendency for the water content to fall as protein content rises confirms the findings of Peters *et al.* Furthermore, the line of best fit for their data, when transposed upon our graph (Fig. 1), is a reasonable approximation of the line of best fit (not shown) for our data. Thus the partial specific volume of the serum proteins in our series coincides with that found by Peters *et al.* It is about 0.70 to 0.75 ml per gram despite the grossly abnormal character of the proteins present in our patients with multiple myeloma.

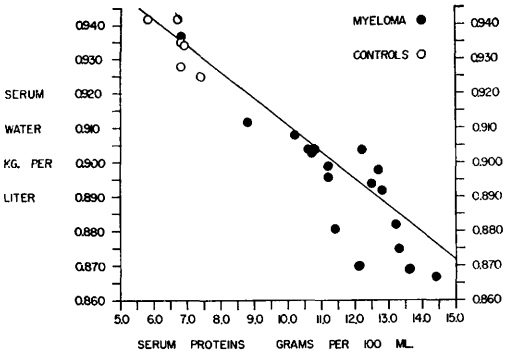

FIG. 1. Relation of water content of serum to concentration of serum total proteins. The line is transposed from data of Eisenman *et al.*(4).

The question may be raised as to whether abnormal binding of electrolytes to protein may occur in the sera of myeloma patients. Preliminary studies of this possibility have been undertaken using a centrifugal (about 700 g) method(6) of ultrafiltration. Seven sera of 4 patients with myeloma (proteins 10 g/100 ml) were ultrafiltered and compared to 4 controls.

Distribution ratios of the millimolalities of sodium and potassium between ultrafiltrates and sera in both experimental and control groups were, on the average, not significantly different from ratios previously measured in our laboratory. Present mean ratios were, for potassium and sodium in the hyperproteinemic group, 0.97 and 0.90 respectively and did not differ significantly from similar ratios of 0.92 and 0.91 in controls. More intensive studies of these parameters in normal subjects and in patients with flagrant disorders of electrolyte metabolism(7) coupled with assessment of ultrafiltrability of electrolytes shortly after death(8) disclosed distribution ratios indistinguishable from those at hand. These distribution ratios, moreover, agree with predictions based upon considerations of Gibbs-Donnan equilibrium.

Summary and conclusions. Does extreme elevation in concentration of human blood serum proteins undermine the validity of conventional units of expression of concentrations of sodium and chloride in clinical biochemistry? The foregoing study has yielded an affirmative answer and emphasizes the validity of millimolality (*e.g.*, mM Na per kg serum water) in contrast to the misleading interpretations which may derive from use of millimolarity (mM of Na per liter of serum). The ultrafiltrability of sodium and potassium, in preliminary studies, is consistent with predictions based upon Gibbs-Donnan equilibrium.

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Interstitial Cell-Stimulating Hormone and Penetration of D-Xylose-1-C^{14} and α-Aminoisobutyric Acid-1-C^{14} into Slices of Testis.*† (27450)

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Slices of rabbit testis have been shown to respond to ICSH§ *in vitro* by increasing the incorporation of acetate-1-C^{14} into testosterone(1). On the other hand, it has not been possible to demonstrate stimulation of a testicular homogenate by ICSH *in vitro*. Evidence has been offered to show that insulin acts by increasing the transport of glucose into muscle cells by using D-xylose-1-C^{14} since this sugar is not metabolized by mammalian tissues. Similarly, the metabolically inert α-aminoisobutyric acid-1-C^{14} has been used as a model amino acid to study the influence of various hormones on the transport of amino acids into cells. The present stud-

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§ The following abbreviations are used: FSH, follicle-stimulating hormone, ICSH, interstitial cell-stimulating hormone.