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.

ies were undertaken to examine the possibility that the failure of a homogenate to respond to ICSH may be attributed to an effect of this hormone upon the permeability of testicular cells to D-xylose and α-aminoisobutyric acid. In addition, the effect of FSH upon the permeability of testicular cells was studied.

Materials and methods. Slices of rabbit testis (500 mg wet weight) were prepared with a Stadie-Riggs microtome and incubated for periods of 5 minutes to 2 hours in 3 ml of Krebs-Ringer bicarbonate buffer to which D-xylose-1-C^{14} or α-aminoisobutyric acid-1-C^{14} was added in an atmosphere of 95% oxygen and 5% carbon dioxide with continuous shaking. One of each pair of flasks also contained ICSH or FSH dissolved in buffer. Slices of testis from each animal were also incubated with acetate-1-C^{14} (4.2 mg, 30 μc) and ICSH (10 μg) to establish the capacity of the testis to respond to ICSH *in vitro* in each case.

At the conclusion of incubation, the slices were decanted into a thick-walled pyrex ignition tube containing 10 ml of Krebs-Ringer bicarbonate buffer. The contents of the tube were gently mixed by inversion and then centrifuged at 0°C to 650 × g when the centrifuge was allowed to come to a stop. Five such washes were performed, the tissue being kept at 0°C throughout.

The slices were drained and dried in an oven at 120°C to constant weight. The tissue was then boiled in 3 ml water in a stoppered ignition tube for 3 minutes. The hot solution was vigorously stirred and allowed to cool. Aliquots of the cold solution and of each of the 5 washings and a suspension of the tissue residue after boiling were counted by liquid scintillation.

The radioactivity present in the boiled water fraction was regarded as a measure of the penetration of labeled substrate into testicular cells and was expressed as disintegrations per minute per 100 mg dry weight of testicular tissue.

Measurement of radioactivity in the buffer washings indicated that the fifth wash was almost devoid of radioactive material (less than 60 d.p.m.). The suspension of tissue residue left after boiling was also almost devoid of radioactivity (less than 30 d.p.m.). In each case the efficiency of counting was estimated by adding a sample of known radioactivity. The aqueous phase was counted by adding 0.2 ml to 3.0 ml of ethanol and 6.8 ml of scintillation fluid‖ (counting efficiency 30-40%) and tissue samples were counted in a suspension composed of 2 ml of ethanol and 8 ml of a 4% suspension of Cab-o-sil in scintillation fluid (counting efficiency 25-32%).

TABLE I. Effect of Gonadotropins on Penetration of D-Xylose-1-C^{14} into Testicular Tissue.

Addition		Radioactivity in boiled water fraction*		
ICSH μg/flask	FSH μg/flask	dpm/100 mg tissue (mean)	Standard error	No. of observations
—	—	8,244	± 415	12
10	—	8,470	± 267	6
25	—	8,663	± 301	6
—	5	8,433	± 243	6
—	10	7,924	± 216	6
—	25	6,463	± 283	6
—	100	10,080	± 222	6

* Incubation lasted 2 hrs. D-xylose-1-C^{14} 150 μg (0.63 μc) was added to each flask. Counting efficiency was 54%.

D-xylose-1-C^{14} was obtained from the National Bureau of Standards and α-aminoisobutyric acid-1-C^{14} from New England Nuclear Corp. Follicle-stimulating and interstitial cell-stimulating hormones were obtained from the Nat. Inst. of Health, Endocrine Study Section, Bethesda, Md.

Results. Table I shows the results of experiments with D-xylose-1-C^{14}. Neither ICSH nor FSH shows any appreciable influence on the radioactivity remaining after repeated washing of slices of testis.

Incubations were performed for 2, 20, 60 and 120 minutes. In no case was a significant difference observed between control flasks and those containing gonadotropic hormones. The concentration of added D-xylose-1-C^{14} was varied within the range of 5 to 100 μg per ml of medium without apparent effect in flasks to which gonadotropic hormones were added. In zero time control experiments, the tissue was frozen immediately after addition

‖ Scintillation fluid was prepared by dissolving *PPO* 4 g and *POPOP* 40 mg in 1 liter of toluene.

TABLE II. Effect of Gonadotropins on Penetration of α-Aminoisobutyric Acid into Testicular Cells.

Duration of incubation (min)	ICSH μg/flask	FSH μg/flask	dpm/100 mg tissue (mean of duplicate flasks)*
Zero	—	—	6,314
"	25	—	6,286
"	—	25	5,014
5	—	—	12,660
"	25	—	12,253
"	—	25	10,178
15	—	—	29,518
"	25	—	35,594
"	—	25	31,243
45	—	—	46,800
"	25	—	43,786
"	—	25	42,514

* Each flask contained α-aminoisobutyric acid-1-C^{14} 150 μg (4.2 μc). Counting efficiency was 56%.

to the medium and less than 400 d.p.m. per 100 mg tissue was observed in the boiled water extract. In control flasks ACTH was shown not to affect the penetration of D-xylose-1-C^{14} into slices of testis and ICSH was without effect on penetration of this compound into the cells of kidney slices.

Table II shows the results of similar experiments with α-aminoisobutyric acid-1-C^{14}. Again the 2 gonadotropins were without demonstrable effect on the penetration of this substance into testicular cells. In other experiments the influence of time and of concentration of α-aminoisobutyric acid-1-C^{14} were studied. In no case, however, was a significant increase in the penetration of α-aminoisobutyric acid into testicular cells observed. As in the case of D-xylose, ACTH was without effect on the penetration of α-aminoisobutyric acid-1-C^{14} into testicular cells and ICSH was without effect in the case of renal cells. In each rabbit incubation of slices in the presence of acetate-1-C^{14} with and without ICSH showed an increase in incorporation of acetate-1-C^{14} into testosterone in the presence of ICSH.

Discussion. Stimulation of the rate of incorporation of acetate-1-C^{14} into testosterone by slices of rabbit testis can be achieved in Krebs-Ringer bicarbonate buffer by ICSH without further addition of cofactors, glucose or amino acids to the medium. Addition of glucose to the medium does not enhance this response to ICSH. However, these observations do not exclude the possibility that damage to cells during the preparation of slices allows the release of glucose or amino acids into the medium and that ICSH promotes the transport of such substances into undamaged cells. Moreover, recent investigations have indicated the presence of free amino acids of testicular origin in bovine semen(2). The present studies failed to demonstrate an effect of gonadotropic hormones upon the penetration of D-xylose-1-C^{14} and α-aminoisobutyric acid-1-C^{14} into testicular cells *in vitro*.

The failure of ICSH to cause a measurable influence upon the penetration of the substances tested in the present system could be attributed to the fact that the hormone acts only upon Leydig cells and an effect could be obscured by the presence of the unresponsive germinal epithelium. Again, the exposure of cut cell surfaces and the damage associated with the preparation of slices may allow free access to many cells and in this way could mask the influence of gonadotropic hormones. However, since slices respond to ICSH *in vitro* by enhanced steroid biosynthesis in a medium without added sugar or amino acids, the response seen under these conditions would not seem to depend upon changes in cell permeability.

The failure of FSH to influence the transport of either test substance into testicular cells may also be explained by the small proportion of responsive cells, since the hormone may only affect one generation of the germinal cells and an effect on membrane transport could be masked by the presence of unresponsive tissue.

Golden and coworkers(3) have reported similar findings with the response of adrenal tissue to ACTH *in vitro*, although they were able to demonstrate an effect of ACTH on the permeability of adrenal cells to D-xylose-1-C^{14} *in vivo*(4). The present experiments do not exclude the possibility that ICSH may specifically affect the permeability of testicular cells to acetate or to a metal ion present in the buffer.

Summary. Experiments *in vitro* have failed to establish an influence of pituitary

gonadotropins upon the permeability of testicular cells to D-xylose-1-C^{14} and to α-aminoisobutyric acid-1-C^{14} under conditions which allow a clear-cut response with respect to the biosynthesis of steroids.

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Availability to Man of Strontium in Cow's Milk. (27451)

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It is generally accepted that calcium in cow's milk is more readily absorbed than from most other dietary constituents(1). Indeed, it has been shown that the uptake of calcium from milk is almost as high as from a stable inorganic salt(2). The same might be expected to be true for strontium in cow's milk but no direct measurements have been made. Although strontium in milk is only a trace element compared with calcium, the availability of strontium is important to the assessment of the health hazard from fallout, since, in Western diets milk has hitherto been responsible for rather more than half the total dietary intake of strontium-90(3).

We have reported that in experiments on a controlled diet, the relative availability of strontium in unfortified wheat flour and cow's milk was unity for 3 normal adults(4). The present communication describes a direct determination of the availability of strontium from milk compared with that from an aqueous solution of strontium chloride in 6 normal subjects from this Unit.

Method. A double tracer method was employed to measure the availability of strontium in milk. Strontium-87m, a short lived γ-emitting isotope of strontium ($T_{½}$ = 2.7 hours) as an aqueous solution of strontium chloride, was added to milk from a cow which a short time before had been given an intravenous injection of 250 μC of strontium-85, also a γ-emitting isotope of strontium ($T_{½}$ = 65 days). The milk-water mixture was taken by each subject whose urine was collected for some hours afterwards. The ratio of $\frac{\text{Strontium-85}}{\text{Strontium-87m}}$ in the urine divided by the ratio $\frac{\text{Strontium-85}}{\text{Strontium-87m}}$ ingested was taken as a measure of the availability of strontium in milk.

(a) *Test of experimental procedure.* To test the experimental procedure, 2 subjects each took orally 50 ml of an aqueous solution containing a mixture of 0.5 μC of strontium-85 and approximately 4 μC of strontium-87m. The pH of this solution was approximately 6. The strontium-85 was almost carrier-free but the strontium-87m contained about 1 mg of stable strontium as chloride. The strontium-87m was prepared by neutron-irradiation of a few milligrams of pure strontium carbonate in a nuclear reactor. The resulting strontium activity of the irradiated salt was separated from other induced radioactive contaminants by a method already described(5).

A known aliquot was removed from the aqueous solution before ingestion by each subject to serve as a radioactive standard. Urine was voided by each subject just before taking the radioactive dose and subsequently the urine was collected at the end of the 2nd, 3rd and 4th hours.