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.

The authors are grateful to Dr. Hans Rilling for valuable technical advice and to the National Institutes of Health, Endocrine Study Section, for gifts of ICSH and FSH.

1. Hall, P. F., The Mechanism of Action of Interstitial Cell-stimulating Hormone. Ph.D. Thesis, Univ. of Utah, 1961.

2. Hopwood, M. L., Gassner, F. S., *Fertility and Sterility,* 1962, in press.

3. Golden, M., Scully, E., Eichhorn, J., Hechter, O., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 354.

4. Eichhorn, J., Halkerston, I. D. K., Feinstein, M., Hechter, O., *ibid.,* 1960, v103, 515.

Received March 19, 1962. P.S.E.B.M., 1962, v110.

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.

TABLE I. Comparison of Urinary Excretion of Strontium-85 and Strontium-87m Taken by Mouth Simultaneously as an Aqueous Mixture.

Subject	Age & sex	Period of collection hr after dose	% of dose in urine		Ratio strontium-85 to strontium-87m
			Strontium-85	Strontium-87m	
J.B.	39	0–2	.152	.140	1.08
	♀	2–3	.297	.286	1.04
		3–4	.149	.142	1.05
W.H.A.R.	60	0–2	.225	.229	.98
	♂	2–3	.206	.201	1.02
		3–4	.237	.226	1.05

Mean 1.037 ± .012

About 20 mg of strontium carrier as chloride were added to each urine and the insoluble carbonates, including strontium, were precipitated by addition of excess sodium carbonate. After the temperature had been raised to the boiling point for a few minutes, the precipitate was separated by centrifuging at 1200 *g* for 5 minutes. The supernatant was discarded and the residue dissolved in 2N hydrochloric acid. In this way the volume of liquid to be assayed for radioactivity was reduced to 10 ml and the relatively small activity due to potassium-40 and to radioactive caesium from fallout was removed.

The strontium activity in each extract was assayed 4 times at approximately hourly intervals and at 24 to 48 hours later. The aliquot removed from the radioactive mixture ingested by each subject was also assayed at the same times. The strontium-85 content of each mixture was determined from the last measurement (1 to 2 days after the dose) when the strontium-87m was negligible. A semilogarithmic plot of the activity against time served to give the strontium-87m component as the difference between the total activity and that due to strontium-85. All count-rates were corrected for radioactive decay after correction for the counter-background and the paralysis time of the counting equipment. It should be mentioned that the observed radioactive half-life of the strontium-87m prepared for experimental use was 2.7 ± 0.05 hours and that due to the strontium-85 was 65 ± 1 days.

(b) *Main investigation using radioactive milk.* After voiding urine, 6 subjects (4 male and 2 female) each drank a radioactive milk mixture at about 10 a.m. usually in a post-absorptive condition. The mixture was composed of 100 ml of milk (containing 0.64 μC of strontium-85 from a cow which had been injected with strontium-85) to which was added about 10 ml of an aqueous solution of strontium-87m with an activity of about 4.5 μC. The volume was made up to 200 ml by addition of inactive milk and 10 ml were removed to serve as a radioactive standard of the dose.

The procedure for collection of the urine as well as the subsequent chemical treatment and the radioactive assays were exactly as already described.

Results. The results of the preliminary experiment are given in Table I.

The ratio of $\frac{\text{Strontium-85}}{\text{Strontium-87m}}$ in the urine divided by the ratio of $\frac{\text{Strontium-85}}{\text{Strontium-87m}}$ ingested was 1.037. The result to be expected for this ratio would, of course, be unity. However, the overall experimental error was assessed as not less than 2% and it was concluded from the results obtained that the experimental method was satisfactory.

The results of the main investigation are given in Table II whence it will be seen that the overall mean of 18 observations of the ratio of percentage of strontium-85 to percentage of strontium-87m in urine was 0.99 ± 0.014. Since the strontium-85 had been metabolically incorporated in the milk while the strontium-87m was a dilute aqueous solution of strontium chloride mixed with milk and therefore to be regarded as fully ionic, it is concluded that the availability of strontium in cow's milk is unity.

Discussion. It has already been noted that

TABLE II. Comparison of Urinary Excretion of Strontium-85 in Cow's Milk with that of Strontium-87m as Aqueous Solution Taken Simultaneously by Mouth.

Subject	Age & sex	Period of collection hr after dose	% of dose in urine		Ratio strontium-85 to strontium-87m
			Strontium-85	Strontium-87m	
B.P.	30	0–2	.201	.215	.93
	♂	2–3	.188	.200	.94
		3–4	.319	.313	1.02
J.G.	30	0–2	.156	.164	.95
	♂	2–3	.170	.163	1.04
		3–4	.120	.125	.95
H.S.*	21	0–2	.104	.119	.88
	♀	2–3	.190	.98	.96
		3–4	.292	.285	1.03
S.W.	25	0–2	.159	.162	.98
	♀	2–3	.289	.276	1.05
		3–4	.201	.191	1.05
S.B.	33	0–2	.474	.440	1.07
	♂	2–3	.468	.472	.99
		3–4	.344	.368	.93
P.A.	27	0–2	.095	.096	.91
	♂	2–3	.089	.0855	1.04
		3–4	.175	.164	1.07

* Fasting. Mean .990 ± .014

the avilability to man of strontium in milk and whole flour has been compared in this laboratory(4). Thus, for 3 adults, on a special experimental diet in which strontium-85 was metabolically incorporated into cow's milk and strontium-90 similarly incorporated into wheat grain, the relative availability as measured by the ratio

$$\frac{\left(\frac{\text{strontium-90}}{\text{strontium-85}}\right)\text{urine}}{\left(\frac{\text{strontium-90}}{\text{strontium-85}}\right)\text{diet}}$$

was 0.90 ± 0.05. From the present results therefore, it is to be concluded that strontium in wheat flour is also approximately fully available to man. Such a full availability of strontium-90 when metabolically incorporated into grass has been shown to hold in rabbits (6).

During the last decade, the contamination of food products with strontium-90, as a result of fallout, has resulted in a wide range in the specific activity of various dietary components. Thus, the specific activity of milk is relatively high while that of chalk (creta praeparata) which is usually added to flour in Britain, is quite small. In creta praeparata as well as cow's milk, wheat flour and drinking water, the strontium can be regarded as fully available. Moreover, these components account for some 80% of the strontium in an average British diet. If we assume that the remaining 20% of the dietary strontium, chiefly from meat, vegetables and fruit, is also fully available, then the specific activity of the strontium ingested will be equal to the specific activity of the absorbed strontium. It is of interest therefore to compare the average specific activity of the diet at a given time with the mean specific activity of the plasma.

In 1958, the specific activity of strontium in an average British diet was 4.5 $\mu\mu$C strontium-90/mg strontium(3). A result obtained in this laboratory for the strontium-90 concentration in bulked plasma from British subjects for the same year was 1.5 $\mu\mu$C/g calcium(7). No measurement was made of the calcium or strontium concentration in this plasma but if it is assumed to be 400 μg/g Ca(8), the specific activity of strontium in this plasma would be approximately 3.8 $\mu\mu$C/mg. This assessment can only be an approximation, but it is compatible with the conclusion that, under equilibrium conditions, the specific activity of strontium-90 in plasma is approximately equal to that in the diet.

Summary. A double tracer method has been used to measure the availability of stron-

tium in cow's milk. Thus, strontium-87m as a dilute aqueous solution of strontium chloride was added to milk from a cow which had received strontium-85 intravenously. Urines were collected from 6 adult volunteers after ingestion of this milk mixture. It is shown that the availability of strontium in the milk is equal to that of strontium as chloride in dilute aqueous solution. The consequences of the result upon the specific activity of strontium-90 from fallout in certain body pools are discussed.

The authors are indebted to Dr. J. F. Loutit for his active interest and helpful criticism. Our thanks are also due to Drs. J. F. Loutit and R. M. Mole for help in preparation of the script.

1. Davidson, Sir Stanley, Heiklejohn, A. P., Passmore, R., *Human Nutrition and Dietetics,* E. & S. Livingstone Ltd., Edinburgh and London, 1959.
2. Patton, H. P., *J. Nutr.,* 1955, v55, 519.
3. Agricultural Research Council Radiobiological Lab. Report 3, 1960.
4. Carr, T. E. F., Harrison, G. E., Sutton, Alice, *Nature,* 1962, in press.
5. Harrison, G. E., Raymond, W. H. A., *J. Nucl. Energy,* 1955, v1, 290.
6. Carr, T. E. F., Wall, J., *Brit. J. Nutr.,* 1961, v15. 165.
7. Harrison, G. E., Sutton, Alice, Maycock, W. D. A., *Nature,* 1961, v189, 324.
8. Harrison, G. E., Raymond, W. H. A., Thretheway, H., *Clin. Sci.,* 1955, v14, 681.

Received March 22, 1962. P.S.E.B.M., 1962, v110.

Measles Vaccination. I. Serologic Responses to Vaccination with Inactivated Vaccine. (27452)

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That measles has created a problem in homes for the mentally retarded may be one reason why programs of prophylaxis and studies of attenuated measles vaccine have been carried out in such populations(1,2). However, the same reasons for desiring to protect an unusually susceptible group of patients with involvement of the central nervous system enjoin caution in employing a living vaccine that may produce fever and some of the symptoms of the natural disease. To protect susceptibles in our vulnerable population and minimize side-effects, our interests seemed better served by use of an inactivated measles virus vaccine than by use of a living vaccine.

The following studies were directed toward determining (a) incidence of serologically-susceptible patients in a closed population, (b) antibody responses of serologically-immune and susceptible individuals to vaccination with an inactivated-measles virus-vaccine, (c) persistence of antibody titers induced by vaccination, and (d) protective value of the serologic evidences of immunity (natural or induced) if challenged by naturally occurring measles.

Materials and methods. Neutralizing antibody (NA) titers for determination of serological susceptibility or immunity were carried out in cultures of transformed human amnion cells with the Philadelphia 6 strain (3) of measles virus. Six-tenths ml of undiluted, heated (56°C, 30 minutes) serum was mixed with 0.3 ml of virus (30-50 TCD_{50} per 0.1 ml) in Hanks' balanced salt solution and incubated for 1 hour at room temperature. Each mixture in an amount of 0.2 ml was then added to each of 5 cell cultures and the stationary cultures incubated for 21 days at 36.5°C, during which time they were frequently examined for presence or absence of measles virus-induced cytologic changes. NA titers following vaccination were similarly determined after a 21-day period except that 0.3 ml of serum and 100 TCD_{50} per 0.1 ml of virus were employed. Sub-passages of cells and fluids from cultures in which the indicator virus was neutralized failed to show pres-

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