so that it should have affected the level of the free threonine pool but the percentage of administered threonine which was oxidized was not changed. The rapid appearance of radioactivity in the picric acid-precipitable proteins points to the possibility, at least in the case of threonine, of some metabolic route from administered amino acids to bound amino acids to the oxidative pathways possibly without participation in free amino acid pools.

Summary. When different amounts of uniformly labeled L-threonine, L-valine, L-histidine, or glucose were administered intraperitoneally to young chicks previously fed complete purified diets or diets lacking different essential amino acids, the total percentage of administered activity recovered as $C^{14}O_2$ during 14 hour experimental periods averaged 10% for threonine, 25% for histidine, 40% for valine, and 50% for glucose. There were large differences in the fraction of each amino acid incorporated into picric acid-precipitable protein. Peak $C^{14}O_2$ production occurred about one hour after administration of glucose, threonine, or valine; 2 hours after administration of histidine. Neither previous diet (complete or lacking the amino acid injected) nor 10-fold variations in amounts of amino acids administered appeared to affect fractions oxidized or synthesized into proteins.

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Comparative Passage of Calcium and Strontium from Plasma into Cerebrospinal Fluid in Man.* (25222)

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Comparative passage of strontium and calcium through various membranes is of importance in determining distribution of Sr^{90} in the body. *In vitro* ultrafiltration experiments with serum using Sr^{85} and Ca^{45} as tracers have shown that filtration of strontium is greater than that of calcium and that strontium is less completely bound to plasma proteins than calcium(1). Since passage of these ions through various membranes *in vivo* may differ from that *in vitro*, the relative transfer rates from plasma into different body fluids were studied. This report deals with passage of Sr^{85} and Ca^{45} from plasma into cerebrospinal fluid in man. The question whether cerebrospinal fluid is formed by ultrafiltration or secretion has been much argued(2). It was hoped that these tracer studies might also help clarify this point.

Materials and methods. Tracer doses of Sr^{85} (approximately 0.2 μc/kg body weight) or of Sr^{85} and Ca^{45} (approximately 0.2 μc/kg body weight of each) were administered as chlorides in single intravenous injections to 17 patients in whom lumbar puncture was to be performed for medical reasons. The intervals between injection of the tracers and lumbar puncture ranged from 5½ to 47 hours, at

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TABLE I. Ratios of Sr^{85}, Calcium, and Phosphorus in Cerebrospinal Fluid Samples Obtained Approximately 24 Hours after Intravenous Administration of Sr^{85}.

Patient	Diagnosis	Protein CSF,* mg %	Protein Plasma, g %	Hr	CSF:* Serum ratios† Sr^{85}	Ca	P
1	Cancer of lung	25	5.8	23	.46	.52	.33
2	*Idem*	32	8.2	24	.33	.56	.37
3	Diabetes mellitus	32	6.1	"	.35	.56	.45
4	Cancer of cervix	75†	6.7	"	.54†	.54	.34
5	breast		6.8	"	.29	.51	.48
6	uterus		6.1	26	.18	.53	.48
7	"	78	5.8	"	.40	.52	.44
8	Hypernephroma	44	6.9	28	.40	.50	.38

* CSF = Cerebrospinal fluid.
† Previous paravertebral inj. of alcohol may have rendered spinal dura more permeable.

which time samples of cerebrospinal fluid and plasma were obtained. Concentrations of Sr^{85}, Ca^{45}, calcium, phosphorus, and total protein were determined in plasma and cerebrospinal fluid. Stable calcium could be determined only on those samples of cerebrospinal fluid not utilized for Ca^{45} analysis. Blood urea nitrogen was determined on the plasma of each patient and plasma proteins were determined by paper electrophoresis on most patients. Calcium was determined by the method of Kramer and Tisdall as modified by Clark and Collip(3), inorganic phosphorus by the method of Fiske and SubbaRow(4), total serum protein by the Kjeldahl method, a small correction being made for non-protein nitrogen, and cerebrospinal fluid protein by the biuret method. Sr^{85} was counted in a well scintillation counter and Ca^{45} in a gas flow counter following precipitation with carrier calcium as the oxalate, both methods having been previously described(5). Concentrations of Sr^{85} and Ca^{45} were calculated as percent of administered dose per liter and were expressed as ratios of cerebrospinal fluid to plasma.

Results. Table I shows cerebrospinal fluid to plasma ratios of Sr^{85} approximately 24 hours after intravenous administration of the tracer. The ratio of stable calcium in cerebrospinal fluid to that in plasma is close to the average value of 0.53. Values for Sr^{85}, on the other hand, are consistently lower, except for Patient 4, in whom the spinal dura might have been rendered more permeable by paravertebral injection of alcohol 3 hours prior to lumbar puncture.

Table II shows relative values of Sr^{85} and Ca^{45} in cerebrospinal fluid and plasma at different time intervals following simultaneous intravenous injection of the tracers. No appreciable difference in passage of the 2 radioisotopes was noted except in Patient 17, in whom a greater percentage of Ca^{45} than of Sr^{85} was found in cerebrospinal fluid after 47 hours.

Table III shows plasma levels of Sr^{85} and Ca^{45} following intravenous injections of the tracers. The data are in agreement with those obtained on a large number of patients studied with both tracers at this institution. Plasma levels of the 2 radioisotopes are practically identical during the 2 days following intravenous injection, so that comparison of cerebrospinal fluid/plasma ratios is meaningful despite considerable time lag in passage of the 2 radioisotopes into cerebrospinal fluid.

Calculations of the approximate rate of cerebrospinal fluid formation were made on the assumption that maximum concentration of calcium in cerebrospinal fluid was half that

TABLE II. Comparative Passage of Sr^{85} and Ca^{45} from Plasma into Cerebrospinal Fluid at Different Time Intervals following Intravenous Administration of the Tracers.

Patient	Diagnosis	Hr	CSF*: Serum ratios Sr^{85}	Ca^{45}
9	Syphilis	5½	.10	.10
10	Multiple sclerosis	12	.22	.25
11	Cancer of lung	21	.42	.39
12	Polyarthritis	22	.37	.40
13	Peripheral neuropathy	22	.44	.43
14	Cancer of stomach	22	.34	.33
15	" " esophagus	24	.42	.36
16	Diabetes mellitus	42	.45	.42
17	Cancer of lung	47	.34	.51

* CSF = Cerebrospinal fluid.

TABLE III. Plasma Levels of Sr^{85} and Ca^{45} at Varying Time Intervals following Intravenous Injection of the Tracers.

Patient	Time after inj. (hr)	% of dose/1000 ml plasma Sr^{85}	% of dose/1000 ml plasma Ca^{45}
9	5½	2.77	2.55
10	½	4.13	4.30
	12	2.79	2.83
16	½	4.59	4.17
	15½	2.14	2.12
	42	1.37	1.49
17	½	6.09	5.67
	23	2.04	1.94
	40	1.38	1.43

of the plasma from which it was formed. This assumption is borne out by the data on stable calcium listed in Table I. It was also assumed that average Ca^{45} plasma concentration, from the time of injection of tracer, was twice the Ca^{45} concentration at n hours. Total volume of cerebrospinal fluid varies from 80-100 ml in different individuals(6). An assumed total volume of 100 ml was used in the calculations. Time of formation of the total amount of cerebrospinal fluid was calculated as equal to $\frac{n}{Ca^{45} \text{ in CSF}/Ca^{45} \text{ in plasma}}$, where Ca^{45} concentrations in cerebrospinal fluid and plasma were determined after n hours. Rate of formation of cerebrospinal fluid was then $\frac{100}{\text{time of formation}}$.

Using the 5½ hour value of Patient 9 in Table II, time of formation of cerebrospinal fluid was $\frac{5\frac{1}{2}}{0.10}$, or 55 hours, so that rate of formation was 100/55, approximately 2 ml/hour, or of the order of 0.03 to 0.04 ml/hour/kg body weight. Similar results were obtained by using the data of the other patients listed in Table II.

Discussion. Cerebrospinal fluid/plasma ratios of Sr^{85} and Ca^{45} were practically the same except in one patient in whom the ratio was greater for Ca^{45}. As Sr^{85} is less completely bound to plasma proteins than Ca^{45}, and ratio of Sr^{85} to Ca^{45} in ultrafiltration experiments *in vitro* is 3:2 or 5:4, it may be concluded that there is a preferential passage of Ca^{45} through the membranes involved in formation of cerebrospinal fluid. It therefore appears that cerebrospinal fluid in man is formed by secretion rather than by ultrafiltration.

The low content of inorganic phosphorus in cerebrospinal fluid compared to that in plasma has been previously reported(7), and in agreement with the data reported here, also indicates that cerebrospinal fluid may be formed by secretion. It has been argued that these low ratios are due to ultrafiltration followed by modification of the cerebrospinal fluid(2), possibly by reabsorption of some of the inorganic phosphorus. However, the data of Table II do not readily admit of this interpretation. Because of the low values of Sr^{85} and Ca^{45} in the cerebrospinal fluids of Patients 9 and 10, reabsorption of these ions should have had only a negligible influence. Nevertheless, Sr^{85} and Ca^{45} ratios were the same in both cases. If these ratios were due to ultrafiltration followed by considerable reabsorption, one would not expect an increase in the ratios of both radioisotopes with time (Table II).

No correlation was evident between rate of passage of Sr^{85} into cerebrospinal fluid and plasma or cerebrospinal fluid proteins. The factors which determine passage of the ions across membranes into the spinal canal varied considerably from patient to patient.

The rate of cerebrospinal fluid formation in dogs has been reported to be 0.2 ml/hr/kg(8), appreciably greater than the value here calculated for man. Rate of formation for dogs, however, was determined after complete drainage of cerebrospinal fluid, a highly unphysiological condition, so that the values obtained cannot be related to those in normal animals nor to human beings.

Studies carried out in man have indicated a rapid passage of deuterium into the subarachnoid space, the deuterium concentration in the spinal fluid equalling that of plasma in about 80 minutes(9). However, deuterium was probably involved mostly in exchange at the surfaces of brain, spinal cord, and cerebral ventricles, rather than in formation of new cerebrospinal fluid. Studies with Na^{24}, Rb^{86}, and P^{32}, as well as other radioisotopes, have revealed considerably lower equilibration rates

than obtained with deuterium(8-10). The different rates of passage of various elements into cerebrospinal fluid point to the process of secretion rather than ultrafiltration.

Summary. Comparative rates of passage of strontium and calcium from plasma into cerebrospinal fluid have been studied in man following intravenous injection of Sr^{85} and Ca^{45}. Cerebrospinal fluid to plasma ratios of Sr^{85} and Ca^{45} were approximately the same except in one patient who showed a greater ratio for Ca^{45}. As a greater proportion of Sr^{85} than of Ca^{45} is unbound to protein, differential passage into the cerebrospinal fluid in favor of Ca^{45} appears to take place. The data indicate that cerebrospinal fluid may be formed by secretion rather than ultrafiltration. Rate of formation of fluid has been calculated to be approximately 0.03 to 0.04 ml/hr/kg of body weight.

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Effects of Antibiotics on Multiplication of L Cells in Suspension Culture. (25223)

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A number of agents known to have an antitumor effect in animals or man were recently shown by Eagle and Foley(1,2) to be highly toxic against a number of cultures of human and animal cells. They suggested that determination of cytotoxicity in cell cultures might prove to be of value as a primary screen for detection of new carcinolytic agents. Their technic depends on the cytotoxic agent affecting ability of the cells to adhere to glass and to multiply under these conditions. During the past few years the method of Owens *et al.* (3) for growing mammalian cells in suspension culture has been reproduced (with modifications) in several laboratories(4,5), and it seemed to us that cells growing in suspension would be more suitable for study of the effects of cytotoxic agents than cells growing on glass surfaces. We have examined the effects of many substances on the L cell line of mouse fibroblasts growing in suspension culture and wish to report here on the effects of certain antibiotics on this cell line.

Methods. Stock cultures of the L cell line were grown in suspension in the modification of Eagle's medium(6) summarized in Table I. Approximately 200 ml of sterile medium and 20 million cells (taken from suspension cultures) were added to a sterile 500 ml flat-bottomed centrifuge bottle containing a sterile teflon-covered 2-inch magnet and capped with a black rubber stopper. The bottle was then placed on a magnetic induction stirrer located in a 37° incubator and the power adjusted so that the magnetized bar turned at 225 rpm. At the end of a 3-or 4 day incubation period