TABLE 1

Type of rat	No. of rats	Average wt		Daily vol of bile (ml)	Bile cholesterol	
		Initial (g)	Final (g)		Concentration (mg/100 ml)	Daily output (mg)
			Series 1			
Control	6	—	181	11.1	27 (22–36)*	2.9 (1.9 – 3.8)
Hypothyroid	6	174	190	9.8	13 (12–14)	1.2 (1.1 – 1.3)
Hyperthyroid	10	150	158	10.0	45 (27–62)	4.4 (2.7 – 6.2)
			Series 2			
Control	10	249	310	14.5	17 (13–20)	2.4 (1.7 – 3.4)
Hypothyroid	11	232	266	13.2	8 (4–13)	1.1 (0.35– 2.0)
Hyperthyroid	7	252	308	19.4	38 (21–61)	7.4 (3.9 –11.5)

* Figures in parentheses represent range.

ond group of the same series was given stock diet plus powdered thiouracil (constituting 0.25% of the diet) for 33 days, and the third group received stock diet plus powdered thyroid substance (constituting 0.12% of the diet) for 18 days. The second series was maintained for 42 days, controls receiving stock diet, the second group receiving thiouracil in addition as 0.3% of the diet, and those given thyroid substance receiving this material as 0.3% of the diet. Dietary supplements administered as above have been shown (*6*) to induce hypothyroidism and hyperthyroidism, respectively, in rats. The general condition, weight changes, and behavior of our groups of rats confirmed this observation.

At the termination of the feeding periods, the bile duct of each rat was catheterized (*3*), and bile was collected for 24 hr. Bile cholesterol was extracted from each individual 24-hr sample according to the method of Foldes (*7*) and analyzed according to an adaptation of the method of Saifer and Kammerer (*8*), as described previously (*3–5*).

As Table 1 demonstrates, the hyperthyroid rat in both series was found to excrete far more cholesterol in his bile than the normal animal. Thus, in the first series, both the concentration of cholesterol in the bile and the daily biliary excretion of cholesterol were almost twice that found in the control rat. In the second series, more than twice the control amount of biliary cholesterol was excreted by the hyperthyroid rat. Conversely, the biliary concentration and daily output of cholesterol in the hypothyroid rat was about half that found in the normal control rat. It should be mentioned that the variations observed between the first and second series were quantitative, not qualitative, and very possibly were attributable to differences in age and weight of the animals in the two series (*9*).

This disturbance in biliary concentration and output of cholesterol in thyroid derangement represents a second abnormality in the metabolism of this steroid in thyroid dysfunction. The previously known abnormality—namely, the alteration in plasma concentration of cholesterol—is a change, the opposite in direction to the present biliary findings. The mechanism responsible for both the biliary and the plasma changes of cholesterol concentration in thyroid derangement is now under investigation.

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Notes on the Strontium Content of Sea Water, Celestite Radiolaria, and Strontianite Snail Shells[1]

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I. The strontium value of sea water has been in doubt because of the wide range in reported values. The values of strontium in mg/l for a salinity of 35 parts/mille as reported by different workers with different methods are as follows: Desgrez and Meunier (*1*), 8.7; Thomas and Thompson (*2*), 13.2; Ramage (*3*), 40.–50.; Noll (*4*), 7.0–7.9; Miyake (*5*), 14.4; Vinogradov (*6*), 8.0; and Vinogradov (*7*), 10.0.

In the present study 235 determinations of Sr/Ca ratio have been made on 160 samples from diverse parts of the Atlantic, including samples in all seasons from Long Island Sound, deep samples from the middle Atlantic and opposite Gibraltar, and samples from the Gulf Stream.[2] Arc and flame spectrophotometric methods were used on single and double oxalate precipitations. The preferred value of atomic Sr/Ca ratio from these analyses is 9.23 atoms/1,000 atoms

[1] From a dissertation on The Biogeochemistry of Strontium, presented to the faculty of Yale University in partial fulfillment of requirements for the Ph.D. degree. The stimulating direction of G. E. Hutchinson is gratefully acknowledged.

[2] Collected by Bingham Oceanographic Laboratory and Woods Hole Oceanographic Laboratory and made available by G. A. Riley, E. F. Thompson, and Dean Bumpus.

Ca, which corresponds to 8.10 mg/l strontium in salinity of 35 parts/mille.

These analyses, when treated by analysis of variance, indicate that strontium is a conservative element varying with salinity. The total variation of the Sr/Ca ratio after analytical error has been removed is less than 8.4% for 95% of a group of analyses. The means of Ca/Cl and Mg/Cl ratios quoted by Thompson and Wright (*8*) have about the same variation, with 4.15% and 6.9% deviation at the 5% probability level. Thus a trace element such as strontium is not necessarily nonconservative in the ocean.

TABLE 1

ANALYSIS OF *Acanthometra*
(Percentage of ash)

	Ca and Sr	SO_4	R_2O_3	SiO_2	Sr*	Ca*	Loss
	17.3	—	1.3	6.0	—	—	—
	18.5	—	—	6.4†	—	—	—
	16.5	—	.5	27.8†	—	—	—
	25.1	—	1.5	13.6	—	—	—
	—	38.7	—	5.8	—	—	—
	—	41.2	—	.4	—	—	—
	—	> 31.	—	1.2	—	—	—
	—	—	—	—	28.1	.8	—
Mean:	19.4	36.9	1.1	8.7	—	—	33.9
Gravimetric analyses by Scheviakov (*17*):							
	22.5	—	17.6	21.6	—	—	29.6

* Spectrophotometric analysis.
† Silica determined with HF.

The analyses of this study roughly confirm the strontium values reported by Desgrez and Meunier, Noll, and Vinogradov as being close to the correct value. Since Miyake's method was a gravimetric separation method and therefore less reliable (*9*), and since Thomas and Thompson have never published any details or data, the overwhelming weight of all evidence places the strontium content of sea water at about 8.1 mg/l. This is considerably lower than the value of 13 mg/l being used in some reference books (*10–12*).

II. Although celestite was suggested by Müller (*13*) in 1858 as the substance of crystals found in *Collosphaera,* a radiolarian, Bütschli's (*14*) investigations in 1906 first demonstrated the celestite nature of the skeletons of radiolaria in the suborder Acantharia. Although this finding was supported by further evidence (Popofsky [*15*], Schmidt [*16*]), that the skeleton was celestite was never completely certain because the chemical analyses made of *Acanthometra pellucidum* by Scheviakov (*17*) were held to indicate that the skeleton was composed of calcium aluminum silicate. The issue is clearly stated by Hutchinson (*18*).

In the present study a plankton sample of *A. pellucidum* (sp. ?) from the Atlantic[3] was analyzed gravimetrically, spectrophotometrically, and with x-ray powder pattern methods.[4] The diffraction pattern was identical with that of celestite. The results of the chemical analyses are shown in Table 1, with the analyses reported by Scheviakov (*17*). The results are very rough because of the small amounts of material that were available for manipulations. They support the conclusion from the x-ray patterns that the main mineralogical crystal form present is celestite. What Scheviakov's figures mean is still uncertain. Also obscure is the physiology of the deposition of celestite.

Incidentally, a curious error has crept into some standard references (*10–12*). The radiolaria were described as having a skeleton of strontium *carbonate,* although apparently no original worker has really maintained this.

III. In the course of experiments in which snails (*Physa*) were raised in culture bottles with varying strontium concentrations, snails were raised with shells of more than half strontianite. The Sr/Ca ratio in the shells was about one third the Sr/Ca ratio in the aquatic culture medium. The visible appearance was similar to that of normal aragonite shells.

X-ray diffraction demonstrated that the shells with high strontium contained tiny crystals of both aragonite and strontianite, instead of an isomorphous intermediate of a substitution series. A diffraction pattern of an artificially mixed powder of strontianite and aragonite was nearly identical with the diffraction pattern of the shells. There were lines of both crystal species present.

The inducement of high strontium in the calcareous skeletons of organisms described above is not without precedent. Papillon (*19*), König (*20*), Stoeltzner (*21*), and Kinney and McCollum (*22*) produced up to 10% strontium content in bones of rats fed on high strontium. Wheeler (*23*) produced eggshells high in strontium by feeding chickens high strontium food. Robison and Rosenheim (*24*) produced deposition of bone salts in bone tissue culture experiments by introducing a medium high in strontium.

[3] Collected by the Bingham Oceanographic Laboratory. Species identification is not completely certain.

[4] Grateful appreciation is expressed to Horace Winchell, of the Brush Mineralogical Laboratory, Yale University, for use of x-ray facilities, and to James Clark for the first determination.

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Vitamin B_{12} in Activated Sewage Sludge[1]

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During studies of the biological oxidation of dairy wastes, it occurred to us that the mixed microflora might contain significant quantities of vitamin B_{12}. Further investigation confirmed this idea, and led us to test the vitamin B_{12} content of aerator sludge from a municipal sewage treatment plant. The apparent vitamin B_{12} content shown by direct assay with *Lactobacillus leichmannii* ATCC 4797 was determined essentially by the method of Skeggs, *et al.* (*1*). Since such activated sludge could be expected to contain almost any product of plant or animal metabolism, it was tested further by alkaline destruction of vitamin B_{12} (*2*) and chromatographic separation on paper from other materials which stimulate the growth of the test organism (*3*).

The amount of vitamin B_{12} indicated by the alkaline destruction procedure (Table 1, col. 4) was about that shown by chromatographic separation (not tabulated). Materials stimulating the growth of *L. leichmannii* which had R_F values of about 0.4, presumably thymidine, and 0.8 made up the major part of the components which move more rapidly than vitamin B_{12} complex. Essentially the total activity shown by the direct assay was given by the sum of the various components separated chromatographically. These data indicate that the alkaline destruction method is a satisfactory measure of the vitamin B_{12} activity of these preparations for *L. leichmannii*.

TABLE 1

VITAMIN B_{12} CONTENT OF ACTIVATED SLUDGE
(All Data Calculated on Moisture-Free Basis)

1	2	3	4
Sample	Apparent B_{12} (μg/g)	Alkali-stable growth factors (as B_{12}) (μg/g)	Vitamin B_{12} (by diff) (μg/g)
Dairy waste sludge			
Wet	8.8	1.5	7.3
Municipal sewage sludge			
Wet	9.7	0.4	9.3
Lyophilized	6.7	.4	6.3
Dried for 24 hr at 105° C	4.0	.4	3.6
Dried for 24 hr at 70° C in circulated air	7.0	.4	6.6
Dried, under vacuum, on steam drum	2.7	.3	2.4
Commercial product			
Sample A	3.2	0.4	2.8
Sample B	6.4	2.0	4.4

A vitamin B_{12} content of 1.5 mg/lb (3.3 μg/g) has been proposed by the Association of American Feed Control Officials as a required level for a commercial feed supplement. If the minimum values are considered (Table 1, col. 4), the amount of vitamin B_{12} in the samples dried under mild conditions was well above that required. A commercially available dried sludge contains similar significant amounts of vitamin B_{12}.

The existence of such a large potential supply of vitamin B_{12} in activated sludge is important, for dried sludge has heretofore been of value only as a fertilizer. Chick feeding tests are now being conducted at Beltsville, Maryland, by Herbert R. Bird, Bureau of Animal Industry, U. S. Department of Agriculture.

[1] Report of a study made under the Research and Marketing Act of 1946.

[2] We wish to acknowledge the cooperation of J. C. Lewis, Western Regional Research Laboratory, in making the initial tests, and of Robert P. Bolenius, superintendent of the Abington Township (Pa.) Sewage Treatment Plant, for collecting experimental material.

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Gordon E. McCallum has succeeded **Robert H. Flinn** as chief of Health Emergency Planning, USPHS. Mr. Flinn is on assignment to the Civil Defense Administration. Mr. McCallum holds the rank of sanitary engineer director in the Public Health Service.

Austin Smith, editor of the *Journal of the American Medical Association,* has accepted appointment as a member of the surgeon general's committee of consultants for the study of the indexes to medical literature, published by the Army Medical Library.