

such a diet corroborate our former statement and do not support the conclusion of Stehle.

Similar experiments were undertaken with these dogs on a diet enriched in lime by the addition of dried milk. With one animal a negative calcium balance was appreciably increased. It seems unlikely, however, that this outcome is a neutralization phenomenon. It has repeatedly been shown that when acid is introduced into the organism the increased production of ammonia is sufficient to neutralize it.

When sodium chloride instead of hydrochloric acid was added to the diet rich in lime the urinary calcium output was increased and the calcium balance was favorably affected.

The magnesium balance and the partition of this element between the different paths of excretion was not significantly altered in any of the experiments just described.

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Studies on salt action. I. Effect of calcium and sodium salts upon the viability of the colon bacillus in water.

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The experiments here reported are preliminary observations designed to test the suitability of a certain bacterial characteristic, the curve of viability in water, for use in more extensive studies of salt action planned for the future.

The organisms used were typical colon bacilli of the *B. communis* (sucrose negative), type, isolated from a polluted stream in the autumn of 1916. These bacteria were grown on standard nutrient agar slants at 37° C. for 16–18 hours. The growth from the surface was washed off in pure water, shaken for 5 minutes to break up clumps and added in 1 c.c. portions to the bottles of sterilized pure water or salt solution in which the viability was to be tested. These solutions had been warmed to 37° before seeding. Plates were made on agar one minute after seeding and the bottles were then replaced in the 37° incubator and kept there for 24–52 hours, the number of surviving organisms being determined

at stated intervals. Counts were made on standard agar after incubation for 24 hours at 37° C.

The "pure water" used was twice redistilled in glass vessels and again redistilled in a block tin condenser. It gave a negative test for ammonia with Nessler's reagent. The sodium chloride was purified by treatment with calcium hydroxide (to remove magnesium), and with sodium carbonate (to remove calcium and barium), and was then recrystallized from pure hydrochloric acid and from pure water. The calcium chloride was twice recrystallized and the crystals dried in a current of air and in the drying oven.

TABLE I.
EFFECT OF CALCIUM ALONE.

Sol.	Grams CaCl ₂ in 100 C.c. Water.	Theoretical Tonicity.	Bacteria per C.c. Time after Seeding.				
			1 Min.	3 Hrs.	6 Hrs.	9 Hrs.	24 Hrs.
A ..	0.000		24,400,000	36,700,000	34,900,000	34,200,000	17,200,000
B ..	0.161	0.1 iso	21,100,000	13,100,000	4,900,000	4,800,000	2,900,000
C ..	0.805	0.5 "	27,900,000	2,100,000	230,000	140,000	44,000
D ..	1.610	1.0 "	18,200,000	2,800,000	160,000	53,000	170
E ..	8.050	5.0 "	11,400,000	14,500	0	0	0

TABLE II.
EFFECT OF SODIUM ALONE.

Sol.	Grams NaCl in 100 C.c. Water.	Theoretical Tonicity.	Bacteria per C.c. Time after Seeding.				
			1 Min.	2 Hrs.	6 Hrs.	25 Hrs.	52 Hrs.
A ..			25,130,000	25,030,000	23,650,000	23,500,000	20,000,000
B ..	0.085	0.1 iso	40,230,000	39,250,000	46,080,000	42,500,000	40,000,000
C ..	0.425	0.5 "	40,650,000	36,530,000	48,600,000	25,500,000	25,100,000
D ..	0.850	1.0 "	34,350,000	43,300,000	41,430,000	38,000,000	16,850,000
E ..	4.250	5.0 "	2,420,000	163,000	5,000	900	750
F ..	8.500	10.0 "	4,540,000	42,500	0	0	0

TABLE III.
EFFECT OF CALCIUM AND SODIUM TOGETHER

Sol.	In 100 C.c. Water, Grams.		Theoretical Tonicity.	Bacteria per C.c. Time after Seeding.				
	NaCl.	CaCl ₂ .		1 Min.	3 Hrs.	6 Hrs.	9 Hrs.	24 Hrs.
A ..	.355	.134	0.5 iso	1,940,000	2,150,000	2,800,000	5,200,000	7,600,000
B ..	.710	.268	1.0 "	1,050,000	3,850,000	2,500,000	4,900,000	4,800,000
C ..	1.420	.536	2.0 "	910,000	3,200,000	4,300,000	4,400,000	4,600,000
D ..	3.550	1.340	5.0 "	1,020,000	1,275,000	1,200,000	2,200,000	1,260,000
E ..	7.100	2.680	10.0 "	890,000	98,000	34,000	45,000	8,000

Ratio of Na \longleftrightarrow Ca is the same in all solutions (5 \longleftrightarrow 1).

The solutions to which the bacteria were exposed contained respectively, calcium chloride alone, sodium chloride alone, and a mixture of the two salts in the ratio of five molecules of sodium to one molecule of calcium. The tonicity varied from nothing to a ten times isotonic condition.

It is evident that the calcium salt alone exerts a marked lethal action. Even a 0.1 isotonic solution shows a distinct reduction in bacterial numbers and increasing proportions grow more deadly till a 5.0 times isotonic solution produces sterility in 6 hours. The sodium salt is less harmful. Tonicities of 0.5 and 1.0 show a slight effect, and a tonicity of 5.0 a marked effect, but the latter concentration does not completely sterilize even after 52 hours. A mixture of the two salts in proportion of 5 parts of sodium to one of calcium is much more favorable than either salt alone. Up to and including a tonicity of 5.0 the bacteria actually increase in numbers. Line *D* of Table III shows that 3.5 per cent. sodium chloride plus 1.3 per cent. calcium chloride had no harmful influence, while in Table II .4 per cent. of sodium chloride alone, and in Table I less than .2 per cent. of calcium chloride alone exerted a demonstrable lethal effect.

The toxic effect of calcium and sodium salts and their antagonistic influence upon each other seem to be much the same among the bacteria as in the higher forms of life; and the viability curve in water would seem well suited for the study of such phenomena.

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Protein sparing by glucose in experimental diabetes.

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In diabetes there are abnormalities not only in carbohydrate but also in protein and fat metabolism as well. There is frequently a marked loss of protein in this disease, which may be either direct or the result of a failure on the part of carbohydrate to spare protein. Our experiments deal with one phase of protein metabolism in experimental diabetes, the influence of the removal of the major portion of the pancreas on the characteristic protein sparing action of glucose. For this purpose several female dogs