Lusk in 1913[4] showed that sleeping dogs could produce as low as 750 calories, while Benedict and Talbot in 1915[5] found the average heat production per unit of surface of 105 new-born infants was 612 calories.

In 1921 Benedict and Talbot[6] constructed a curve of the metabolism from birth to puberty from studies based on a new series of 256 infants and children, see following chart. This shows a rapid increase from 612 calories in new-born infants to 1,170 calories in boys of about one year of age, weighing 11 kilograms.

During the past year we have studied, with the assistance of Miss Margaret Moriarty and Mrs. A. J. Dalrymple, the heat production of 22 premature infants. Since premature infants have proportionally more body surface per unit of weight than larger subjects, they should produce more heat per unit of surface. On the contrary, our studies show quite the reverse. The average heat production per unit of surface of the 22 premature infants studied was 597.3 calories in 24 hours. We have included in this group premature infants of 3 days to 3 months of age. A critical analysis will be made of these cases later, but it seems probable that the few older infants of this series will fall in the category of malnutrition, in which the metabolism is usually high.

Six of these 22 infants were studied during the first 11 days of life and in these instances the metabolism was extraordinarily low. They are represented in the lower part of the chart by dots inside the light line.

This data is presented as further evidence that Rubner's law of the constant relationship between heat production and body surface is not a physiological law, and that it does not hold true in infants.

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Studies on salt action. IV. The mutual influence of acidity and salt concentration upon bacteria.

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Four years ago[1] we presented certain preliminary results of

[4] Lusk, *Jour. Biol. Chem.*, 1913, xiii, 450.

[5] Benedict and Talbot, Carnegie Institution of Washington Publication No. 233.

[6] Benedict and Talbot, Carnegie Institution of Washington Publication No. 302.

[1] Winslow and Falk, PROC. SOC. EXP. BIOL., 1918, xv, 67.

studies on the toxic influence of calcium and sodium salts upon *Bact. communis*; and two years ago one of us[2] called attention to the possible influence upon such salt effects of the reaction of the solution.

In our earlier experiments the reaction of the distilled water and salt solutions to which the bacteria were exposed was not controlled though the media were in general alkaline (P_H 8.0–9.0). Under these conditions we obtained the following results with various concentrations of NaCl and $CaCl_2$.

PER CENT. OF BACTERIA SURVIVING AFTER 9 HOURS.

	Tonicity of Salt.								
	0.	0.1	0.2	0.5	1.0	3.0	5.0	7.0	10.0
NaCl.....	89	115			83	64	58	20	< 1
$CaCl_2$.....	89	70	92	45	22	< 1	< 1	< 1	< 1

Later experiments, in which the hydrogen-ion content was maintained at various desired levels by repeated readjustment, gave quite different results, as indicated below.

PER CENT. OF BACTERIA SURVIVING AFTER 9 HOURS.

	PH.					
	5.0	5.5	6.0	7.0	7.5	8.0
Water.....	82		106	54		12
5.0 isotonic NaCl.....	27	20	87	76	8	9
1.0 isotonic $CaCl_2$.....	134		128	106		

These results (which are all based on averages of a sufficient number of tests to eliminate chance variations) were at first highly puzzling. In the unadjusted alkaline solution 5.0 isotonic NaCl was moderately toxic and 1.0 isotonic $CaCl_2$ highly toxic. At adjusted P_H values on the other hand 5.0 isotonic NaCl remained at practically all reactions slightly more toxic than water but 1.0 isotonic $CaCl_2$ entirely lost its toxicity and became actually stimulating, at least at P_H values between 5.0 and 7.0. It appeared therefore that neither 5.0 isotonic NaCl nor 1.0 isotonic $CaCl_2$ is in itself toxic at adjusted P_H values between 6.0 and 7.0; and that at an adjusted P_H much over 7.0 both salts, as well as distilled

[2] Falk, PROC. SOC. EXP. BIOL., 1920, xvii, 210.

water, are definitely toxic. What conditions could have existed in the earlier work, at uncontrolled P_H, to make 1.0 isotonic $CaCl_2$ toxic and 5.0 isotonic NaCl nearly non-toxic?

The key to this riddle, first suggested by the experiments reported here two years ago, has been found in the influence of salt content upon the power of a bacterial suspension to regulate its reaction. We find, as shown below, that in an unadjusted alkaline water solution containing bacteria the reaction quickly reverts toward the neutral point, while in a similar isotonic $CaCl_2$ solution the reversion is much slower. In slightly acid solutions (P_H 6.0) this difference in behavior is not apparent.

	Distilled Water.			Isotonic $CaCl_2$		
	P_H Initial.	9 Hrs.	Per Cent. Bacteria Surviving After 9 Hrs.	P_H Initial.	9 Hrs.	Per Cent. Bacteria Surviving After 9 Hrs.
Unadjusted.	9.2	8.0	91	9.2	8.9	< 1
Adjusted...	6.0	6.1	76	6.0	6.5	90

These last experiments indicate again that at a P_H controlled between 6.0 and 7.0 neither water nor 5.0 isotonic $CaCl_2$ has toxic action; that in water with an initial P_H over 9.0 the reaction falls to about 8.0 in 9 hours and no toxic effect is manifest; and finally that in an isotonic $CaCl_2$ solution of initial P_H over 9.0 the reaction changes but slightly and the bacteria show the high mortality to be expected in such an alkaline solution. The only anomaly that remains is the fact that the alkalinity of the unadjusted water solution although it falls considerably still remains rather high. In these last experiments a P_H of 8.0 after 9 hours is non-toxic while as previously indicated a P_H maintained at 8.0 throughout an experiment is toxic. We may explain this on the probable assumption that in a solution whose reaction is being brought toward neutral by the regulating action of contained bacteria there are zones immediately surrounding the bacterial cells which have an alkalinity lower than the average for the suspension as a whole. Thus our P_H readings in such a suspension do not represent the actual concentration of hydrogen ions about the cell as they do in a suspension of artificially controlled reaction.

It appears then that the toxic effect of $CaCl_2$ reported in our first experiments is an indirect one and is exerted only in an alkaline solution in which it interferes with the regulative action exerted by bacterial cells upon the reaction of a water or NaCl solution.

142 (1889)

Studies on salt action. V. The influence of various salts upon bacterial growth.

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Previous studies on salt action conducted in this laboratory have dealt with the effect of certain mineral salts upon the death rate of bacteria in water suspension.[1] The present investigation relates to the influence of various salts upon growth in a one per cent. pepton solution. The pepton used contained about 4 per cent. ash and the solution had a reaction of P_H 6.8–7.0. The salts studied were added in the form of chlorides in varying concentration. The solutions were inoculated with *Bact. communis* and incubated at 37° C., the rate of growth being determined by comparing the turbidity produced with standard suspensions of dead bacterial cells. Check determinations by the plate method indicated the substantial accuracy of this procedure.

Twenty-three salts in all were studied and the limiting toxicity determined as indicated below.

The results in general confirm those reported by Matthews[2] for *Fundulus*, and Eisenberg[3] for bacteria; and it is evident, as the former author pointed out, that there is a rough general relationship between toxicity and solution tension. We are making a further analysis of the relation between the toxic action of these salts and their other physico-chemical properties.

The new point brought out in our studies is the general occurrence of a definitely stimulating action, exerted by concentrations of salts below the inhibitive level. In the case of 15 out of the

[1] Winslow and Falk, PROC. SOC. EXP. BIOL., 1922, xix, 311.

[2] *Am. Jour. Physiol.*, 1904, x, 290.

[3] *Centr. f. Bakt.*, Abth. I, 1918, lxxxii, 69.