teins are deficient in both the "X" and the "Y" factors, or whether one may occur without the other. A few preliminary tests have indicated that gelatine and egg proteins contain the "Y" and are deficient only in "X," but the results were not clean cut, and it is possible that other factors came in. Lack of time has prevented the extending of these observations.

Without more definite knowledge of the chemical nature of these two substances, speculation as to the manner in which they induce growth of the streptococci does not seem warranted. There is no means at present of knowing whether they act as "building-stones" in supplying some necessary grouping in the synthesis of the bacterial protein, or whether they simply initiate or accelerate some essential vital process. Perhaps, in the light of much recent work dealing with the effect of vitamines on bacterial and yeast growth, it is not unwarranted to believe that still other phases of animal metabolism may be cleared up in part through work on the metabolism of lower forms of life. In the case of the study of the streptococcus, there are at least three factors, still unidentified, which determine growth; namely, some substance in the charcoal-treated infusion, the "X" fraction, and the "Y" fraction. It is by no means felt that all or any of these factors if isolated, will prove to be new physiological compounds, but if such should be the case, one must believe, in order to explain their occurrence in meat, milk, etc., that they also play a part in animal metabolism.

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The supposed relation between alkalosis and tetany and similar conditions.

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Examination of the work of Wilson, Stearns and Thurlow[1] shows that their conception of an "alkalosis" as one of the consequences of parathyroidectomy rests essentially upon the sup-

[1] Wilson, D. W., Stearns, T., and Thurlow, M. de G., *Journal of Biological Chemistry*, 1915, xxiii, 89.

posed increases in the oxygen saturation of the blood at definite oxygen tensions. Wilson, Stearns and Thurlow used Barcroft's[2] formula

$$\frac{y}{100} = \frac{Kx^n}{1 - Kx^n},$$

in which y is the percentage saturation of the blood with oxygen, x is the oxygen tension in millimeters of mercury, and n is a constant to which Barcroft assigned the value 2.5 for human blood. Wilson, Stearns and Thurlow assumed the same value for dog blood although calculations from Barcroft's value for dog blood (Barcroft, page 50) show that, in this case, the value of n was not 2.5, but approximately 2.2. Barcroft worked at 40 mm. CO_2, Wilson, Stearns and Thurlow at 0 mm. CO_2. According to Barcroft, this difference does not affect the value of n in human blood. But calculating from the figures of Wilson, Stearns and Thurlow, which differ considerably from one another, the value of n is found to be not 2.5, nor 2.2, but approximately 1.5. Using this value of n in recalculating the value of K from the data of Wilson, Stearns and Thurlow it is found that this is not regularly greater in the blood of parathyroidectomized dogs than in the blood of normal dogs. The significance of such changes in percentage oxygen saturation as do occur is obscure. Many factors, other than change in reaction or in alkaline reserve may be responsible. Barcroft's figures (Barcroft, page 62) indicate a specific effect of phosphates in increasing the value of K and decreasing that of n. Moreover, any unrecognized decrease in n will increase the apparent value of K. A few calculations indicate that the retention of phosphate and acumulation thereof in the blood after parathyroidectomy may possibly be sufficient to account for any observed changes in percentage oxygen saturation without involving any change in reaction or in alkaline reserve. Hastings and Murray[3] have shown by their own work and by reference to the more recent literature that there is no direct evidence of either an increased alkalinity nor increased CO_2-capacity after parathyroidectomy.

[2] Barcroft, J., "The Respiratory Function of the Blood," Cambridge, 1914.

[3] Hastings, A. B. and Murray, H. A., Jr., *Journal of Biological Chemistry*, 1921, xlvi, 233.

An attempt was made to induce symptoms resembling tetany by the intravenous injection of $NaHCO_3$ into dogs, under cocaine anesthesia. Convulsions could be produced in this manner. There was, also, a decrease in the hydrogen ion concentration of the blood and plasma, as measured with the potentiometer or by calculation from the bicarbonate and total CO_2 content. The change in reaction was never great and was less in convulsions than before the neuromuscular symptoms had become so marked. Rapid injection produced apnea, with death from respiratory failure, and not convulsions (Table I). The bicarbonate content was enormously increased, the values observed ranging from 162 to 226 volumes per cent. CO_2. At the reactions observed, such bicarbonate concentrations require high tensions of CO_2 in the alveolar air, a value as high as 161 mm. being calculated in one case from the observed total and bicarbonate CO_2. The convulsions could not be relieved by the injection of HCl, although the bicarbonate content was thereby much reduced, to even below the normal level. One dog received distilled water and another a calcium chloride solution. The convulsions continued in both cases but the life of the latter animal seemed to be prolonged somewhat. One dog received a mixture of bicarbonates containing K, Ca and Mg in approximately 0.1 the concentration, relative to the Na, that obtains in dog plasma. Another dog received in one vein a mixture of sodium and potassium bicarbonates and, in another vein, a mixture of calcium and magnesium chlorides. The amounts injected were so adjusted as keep the relations between Na, K, Ca and Mg the same as those obtaining in dog plasma. Both dogs developed convulsions, but the *former* attained a higher concentration of sodium in the plasma than was secured in any other experiment.

Comparison with the results of similar experiments with other sodium salts[1] indicates that the concentration of sodium in the plasma required to produce convulsions is approximately the same for sodium bicarbonate, chloride, phosphate or sulfate (Table II). Attention is called to the experiments of Hougardy[2] who injected

[1] Greenwald, I., *Journal of Pharmacology and Experimental Therapeutics*, 1918, xi, 281.

[2] Hougardy, A., *Archives international de Physiologie*, 1904, i, 17.

sodium hydroxide and other alkalies into dogs and rabbits and to those of Scott[1] who injected sodium carbonate into decerbrate cats. Both obtained respiratory effects and the latter believed he obtained marked changes in the reaction of the blood but neither mention any sign of neuromuscular symptoms resembling tetany or convulsions. Hougardy found that the slightest excess of alkali killed his animals promptly.

Scott employed smaller doses and a slower rate than were used in the present experiments and obtained much lower values for total CO_2 content of the blood than are here reported for plasma. But the changes in hydrogen ion concentration he reports are at least as great as those in the present experiments. The validity of his conclusions as to the specific function of the bicarbonate ion as a respiratory hormone rests entirely upon the accuracy of his determinations of the hydrogen ion concentration. From *a priori* considerations and from the results here reported it would appear that the dialysis colorimetric method employed by Scott gives too high P_H values particularly under the high CO_2 tensions obtaining after the injection of sodium carbonate. The significance of the bicarbonate ion as a respiratory hormone is, therefore, highly questionable.

Within the last few years there have been a number of reported cases, and probably a much larger number of unreported cases, of tetany appearing in patients after the intravenous injection of sodium bicarbonate for therapeutic purposes. A high CO_2 combining capacity, 80 volumes per cent. or more, has been observed and the phrase "alkalosis" has been accepted as explaining the appearance of the symptoms. Palmer, Salvesen and Jackson[2] regard it as dangerous to administer sodium bicarbonate by mouth in amounts greater than those required to produce the first significant change in the reaction of the urine. Such caution would appear to be needless. Patients with gastric hyperacidity may take sodium bicarbonate in amounts sufficient to make the urine alkaline for days at a time without any sign of tetany. The appearance of an alkaline urine is not a danger sign. Rather is it an

[1] Scott, R. W., *American Journal of Physiology*, 1917, xliv, 196; 1918, xlvii, 43.

[2] Palmer, W. W., Salvesen, H., and Jackson, H., Jr., *Journal of Biological Chemistry*, 1920, xlv, 101.

indication that the kidneys are removing at least some of the excess alkali. A high CO_2-combining capacity, however, may be regarded as dangerous but not because of any change in reaction. A CO_2-capacity of 80 volumes per cent. would require an alveolar CO_2 tension of only about 60 mm., at $P_H = 7.4$. An adjustment of respiration to secure this would not seem to be beyond the body's powers. It appears more likely that the danger is that this high CO_2-capacity is due to a retention of sodium sufficiently great to disturb the normal kation equilibrium. As was indicated in a previous paper, the nature of the anion appears to be of significance as it affects the permeability of the tissue cells to the sodium. It may be that the sodium enters the cells and so poisons them or it may not enter and so bring about a change in the potential difference at the cell boundary.

The alkalosis of hyperpnea remains to be considered. Calculations from the figures of Collip and Backus[1] indicate that hyperpnœa increased the P_H of the plasma by from 0.1 to 0.4, with an average of from 0.2 to 0.25, depending upon the method of calculation. The 15 experiments give exceedingly concordant results. However, there is one scource of error that may apply to all and that would give too high P_H values after hyperpnœa. After forced respiration, the usual period of holding the breath may not be sufficient to bring the alveolar air into equilibrium with the blood and the value for the CO_2 tension may therefore be too low. It is unfortunate that no determinations were made of the hydrogen ion concentration nor of the CO_2 *content* of the plasma. However, there must have been some change in reaction for the character of the urine changed, becoming more alkaline in spite of the diminished excretion of ammonia and increased excretion of phosphates. But it by no means follows that this change in reaction was, *per se*, the cause of the tetany-like symptoms observed. The changes in the urine indicate a very decided disturbance in the equilibrium between the various ions within the body.

Forced respiration, like repeated gastric lavage or pyloric obstruction, is a very effective method of removing acid from the body. The organism is not so well adapted to caring for this

[1] Collip, J. B., and Backus, P. L., *American Journal of Physiology*, 1920, li, 568.

sort of disturbance as it is for the introduction of acid or alkali. Nevertheless, as Hastings, Murray and Murray[1] have shown, there is, at the most, an inconsiderable increase in the alkalinity of the plasma after pyloric obstruction. The CO_2 capacity is increased but not the alkalinity.

It is interesting to note that whereas in the paper dealing with the effects of forced respiration, Collip and Backus accept the view that tetany is due to alkalosis, even going so far as to suggest that muscle "cramp" and ether spasm may be due to alkalosis, in a later paper dealing with the effects of the sub-arachnoid and intra-arterial injection of sodium bicarbonate and other electrolytes, Collip[2] emphasizes the disturbance in the kation

TABLE I.

Exp	P_H Before[3]		P_H After $NaHCO_3$[3]		Plasma CO_2 after $NaHCO_3$.				Remarks.[7] $k = 3.04 \times 10^{-7}$ $\delta = 0.8$
	Blood	Plasma.	Blood	Plasma.	$NaHCO_3$ Per Cent.[4]	Total Per Cent.[5]	Tension (cal.) mm.[6]	Calcd P_H from $H = \frac{k\ CO_2}{\delta\ NaHCO_3}$	
4	7.06	7.40	7.06	7.15	162				No tremor or twitching. Death due to respiratory failure.
			7.43						Aërated blood.
5	7.02	7.37	7.37	7.45	183				Convulsions.
					162				Blood drawn after death in convulsions.
6		7.34	7.33	7.56	139				Convulsions.
			6.88		26				Blood drawn after death after injecting HCl.
7	7.01	7.33	7.50	7.51	177	191	122	7.51	Slight tremor.
			7.24	7.43	226	245	161	7.49	Convulsions.
					96	108	104	7.31	Blood drawn after death after injecting HCl.

[1] Hastings, A. B., Murray, C. D., and Murray, H. A., Jr., *Journal of Biological Chemistry*, 1921, xlvi, 223.

[2] Collip, J. B., *American Journal of Physiology*, 1920, lii, 483.

[3] More recent experiments indicate that the anticoagulant used lowered the P_H values in blood by about 0.33 and in plasma by about 0.15.

[4] Van Slyke, D. D., Stillman, E., and Cullen, G. E., *Journal of Biological Chemistry*, 1919, xxxviii, 167.

[5] Van Slyke, D. D., *Journal of Biological Chemistry*, 1917, xxx, 347.

[6] Assuming solubility of CO_2 in plasma to be 0.9 that in H_2O.

[7] Walker, J., and Cormack, W., *Journal of Chemical Society*, 1900, lxxvii, 5.
Michaelis, L., and Rona, P., *Biochemische Zeitschrift*, 1914, lxvii, 182.
Evans, C. L., *Journal of Physiology*, 1920, liv, 353.

equilibrium, though he also ascribes a specific stimulating effect to the bicarbonate ion. To the present author, it seems that this specific effect may be due entirely to the effect of the anion in determining the permeability of the tissues to the kation.

TABLE II.

Ex.	Salt.	Concn.	Time.	Sodium.			Remarks.
				Per Kilo Body Weight		Per 100 cc. Plasma.	
		N.	min	Injec. Gr'ms	Retained Gr'ms		
1	$NaHCO_3$	0.9	38	0.694	0.493	442	Twitching.
			44	0.831	0.631	447	Convulsions.
5	$NaHCO_3$	0.9	40	0.747	0.730	436	Twitching.
2	$NaHCO_3$	0.9	43	0.783	0.684	514	Twitching. Injected soln. contained 0.1 equivalent of K, Mg and Ca.
3	$NaHCO_3$	0.9	40	0.793	0.709	457	Convulsions. Injected soln. contained 1.0 equivalent of K, Mg and Ca.
4	$NaHCO_3$	0.47	68	1.140	0.87	420	No twitching or tremor. Death due to respiratory failure.
5	NaCl	0.60[1]	35	1.45	1.30	493	Convulsions.
6	Na_2HPO_4 NaH_2PO_4	0.38[1]	33	0.70	0.62	451	No twitching. Death due to respiratory failure.
8	Na_2SO_4	0.25[1]	111	1.29	0.82	480	Twitching.

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The peptolytic enzymes of hemolytic streptococci; methods.

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During the study of the virulence of hemolytic streptococci, it has been necessary to understand the action of the cocci on certain protein fractions. On account of the structure of these bacteria, the methods required to obtain the active proteolytic substance from the bacterial cell, and to accomplish the sterilization of the solution containing the enzyme were at first consuming and laborious. With the procedure outlined active

[1] These figures represent the average concentration. More dilute solutions were employed at first and more concentrated ones later. See protocols in Greenwald, *Journal of Pharmacology and Experimental Therapeutics*, 1918, xi, 281.