

material. A further study of the properties of this extract is now under way.

	Wt. of samples	Total nitrogen	Animo nitrogen	Animo nitrogen after hydrolysis	Phosphorus
	mg.	per cent	per cent	per cent	per cent
Tubercle Bacillus	25.32	2.83	.19	1.91	
	22.18	4.50	.31	1.43	
	21.96	1.96	.035	1.53	
Pneumococcus Type I.	18.17	{ 6.23	{ 0.57	{ 2.06	1.24
		{ 6.07	{ 0.53	{ 2.10	
	14.2	4.45	.34	1.82	—
	28.36	1.45	.16	.47	—
	18.08	3.81	.12	.69	—
	20.8	3.28	.32	1.10	—
	20.7	4.20	—	—	—
Meningococcus	18.86	{ 3.69	{ 0.41	{ 3.38	2.2
		{ 3.76	{ 0.40	{ 3.49	
	25.1	5.21	—	—	—
	18.06*	2.44	.16	.19	—
	14.88*	2.25	—	—	—
	16.8 *	1.64	.16	.47	—

*Represent successive stages of an attempt to purify a single preparation.

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The preserving effect of alkali on the blood cells of *Limulus*.

By KENNETH C. BLANCHARD (by invitation).

[From the Marine Biological Laboratory, Woods Hole, and the Department of Comparative Pathology, Washington University, St. Louis, Mo.]

1. According to Leo Loeb (1910) alkali may under certain conditions have a preserving effect on the blood cells of *Limulus*.¹ If we add a drop of *Limulus* blood to an excess of an isotonic sodium chloride solution, those amebocytes which come into direct contact with the solution soon send out sharp pseudopodia and lose their granules and become hyaline. Similarly in a watery solution of very weak alkali the cells soon lose their granules and are dissolved; but if both substances are combined, a small

¹ Loeb, L., *Pflüger's Arch.*, 1910, cxxxi, 465.

amount of alkali being added to the isotonic sodium chloride solution, the cells are preserved much better and their granules may be protected against destruction for a considerable period of time. Alkali may have the same preserving effect even if added to isotonic solutions of anelectrolytes like glucose.

2. This effect of alkali on ameboid cells observed by this method is apparently contrary to the effect of alkali which Loeb and the writer observed by means of the tissue culture method.² If the latter procedure is used, alkali causes in the main a more rapid extension and solution of the cells, although even here a preserving effect is noticeable under certain conditions.

Considering this apparent contradiction in the effects of alkali observed, it was necessary to repeat and if possible to extend those earlier experiments, especially in view of the fact that so far they have apparently not been repeated by other investigators.

3. In carrying out such experiments at Woods Hole, last summer, we could confirm the preserving effect of the addition of alkali to isotonic solutions of sodium chloride on the amebocytes of *Limulus*. We found furthermore that alkali has likewise a preserving effect if added to isotonic solutions of glucose, to which a small amount of sodium chloride has been added. Even in combination with an isotonic solution of urea to which a certain amount of isotonic sodium chloride or potassium chloride has been added, alkali may prolong the time during which the cells, and especially the cell granules, are preserved.

4. Addition of alkali does not exert this effect through a direct osmotic action; the amounts of alkali which are thus effective are too small for this purpose, inasmuch as the preserving action is obtained in a solution of 0.01 to 0.005 N NaOH. As to the mode of action through which alkali exerts this influence, Loeb suggested that it might be due to an inhibiting effect of alkali on certain cell activities, which somehow may favor the solution of the granules. We noticed that alkali under the conditions of this experiment diminishes or prevents the ameboid activity of the cells, and also the extension of the blood cells which usually occurs when in contact with the glass; and it is especially the latter process which favors the solution of the granules. Alkali seems not only to restrict or inhibit the

² Loeb, L., and Blanchard, K. C., *Am. J. Physiol.*, 1922, lx, 277.

formation of the typical tongue pseudopodia, but also that of the balloon pseudopodia and the circus movements which were produced in mixtures of urea and potassium chloride.

The fact that other agencies which inhibit these processes (KCl, NH₄Cl for instance) likewise exert a certain preserving effect on the blood cells seems to support this interpretation. However, further experiments must decide whether in addition other factors may not contribute to this preserving effect of alkali.

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The effect of acid on the amebocyte tissue of *Limulus* in tissue cultures.

By LEO LOEB, JESSIE M. BIERMAN and ELIZABETH GILMAN.

[From the Department of Comparative Pathology, Washington University, St. Louis, Mo., and the Marine Biological Laboratory, Woods Hole, Mass.]

In previous publications¹ we have shown, (1) that a small amount of acid added to an isotonic sodium chloride solution exerts a certain preserving effect on the amebocytes in a drop of *Limulus* blood surrounded by an excess of the solution. (2) That in tissue cultures of amebocyte tissue there occurs not only an active migration of the amebocytes into a sodium chloride solution, which has been made slightly acid, but that under certain conditions the addition of acid may increase the duration and intensity of this migration, which is followed by, or associated with, a formation of tissue-like structures not unlike those produced by the mesenchyme of vertebrates.² (3) That this effect of acid is due to its action on the cells, which consists in an increase in contraction and consistency of the protoplasm. (4) Inasmuch as we had previously shown that there exists a parallelism between the effect of various agencies on the state of con-

¹ Loeb, L., *Folia Haematologica*, 1907, iv, 313. *Pflüger's Arch.*, 1910, cxxxi, 465. *Washington Univ. Studies*, 1920, v, 8. *Scient. Series 3. Am. J. Physiol.*, 1921, lvi, 140. *Science*, 1922, lvi, 237. Loeb, L., and Blanchard, K. C., *Am. J. Physiol.*, 1922, ix, 277.

² We use the term "outgrowth" to designate these effects.