marked increase in primitive cells, myeloblasts and lymphocytes: Myeloblasts 22.0%; Myelocytes 7.0%; Jung Kernige 1.0%; Stab Kernige 1.0%; II Segment Neutrophiles 3.0%; Monocytes and Reticulo-endothelial elements 4.0%; Lymphocytes 42.0%; Megaloblasts 2.0%; Normoblasts 1.0%; Primitive cells 17.0%.

It is obvious that the method of sternal puncture secures a specimen from only one small part of the extensive marrow system. Hence it is not surprising that variations seen by examining sections from many portions of the marrow may not be reflected in the sternal specimen. However, when the marrow is altered throughout its extent, alterations in the sternal marrow are to be expected.

The sternal puncture method is excellent for comparison of peripheral blood and marrow cell morphology. For clinical diagnosis it will only occasionally be useful.

6907

Effect of Urea upon Activity of Bacteriophage.*

J. BRONFENBRENNER AND D. M. HETLER.

From the Department of Bacteriology, Washington University Medical School.

In attempting to minimize denaturation of phage by heat we employed a number of substances which various investigators have found to prevent denaturation of proteins.[1] While some of these substances delayed denaturation of phage (saccharose, glycerine and to lesser extent $CaCl_2$ or glucose) other substances (sodium salicylate, Bayer 205, or urea) exhibited no gross protective effect. During these experiments it was noted, however, that when mixtures containing phage and urea were plated with homologous bacteria at intervals during the experiment (before the inactivation of phage has occurred) the plaques of lysis appeared to be larger and noticeably clearer than on control plates containing the phage without the addition of urea. This apparent intensification of lysis in the presence of urea suggested the possibility that addition of urea may perhaps bring out the lytic activity of phage under circumstances where lysis is ordinarily inhibited, as for instance on 4%

* This work was supported from a grant by the Rockefeller Foundation to Washington University for research in science.

[1] Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 802.

agar. As we have shown, under these circumstances bacteria failed to undergo lysis, although the phage effect is not entirely abolished, as evidenced by the increase in the rate of multiplication of bacteria in contact with it.[2] We were interested in determining whether the addition of urea to the medium would promote lysis under these circumstances.

To measured amounts of a suspension of bacteria (growth from 10 agar slants of an 18 hour culture suspended in 40 cc. of broth) were added different amounts of a sterile (filtered) 50% solution of urea as shown in the protocol; 1.5 cc. of the respective mixtures were added each to 10 cc. of molten agar of different concentrations, and after thorough mixing poured into Petri plates, each mixture was made in duplicate. After a complete solidification of agar (15-30 minutes), a series of small drops (about 2 mm. in diameter) of bacteriophage (titer 10^{-6} cc.) was deposited on the surface of each plate by means of a fine capillary. As a control, similar drops of plain broth were also placed on each plate and incubated overnight at 37° C.

Next morning the plates were examined to determine the extent of lysis of bacteria in contact with droplets of phage. Findings illustrated in the protocol represent such an experiment in which *B. coli* and a corresponding bacteriophage were used. It will be noted that:

1. By itself, presence of urea in the medium did not cause any marked change in the growth of *B. coli* until the concentration of urea reached 6%, when it inhibited the growth. (columns 9 to 13).

2. In the absence of urea (columns 1 and 2) the intensity of lysis decreased as the concentration of agar increased. In the plates with agar concentration of 4% and 5%, the lysis was completely absent; moreover, the growth of bacteria on the spots where phage was deposited was much heavier than on the surrounding medium,[2] (E. G. = excess growth) while the spots on which broth was placed (control) showed an even growth throughout. (0 = no change.)

3. Addition of urea to the medium intensified the lysis (columns 3-8) in proportion to the concentration of urea present. This was indicated on 2% and 3% agar by the greater size and clearness of lysed areas as compared with controls (columns 1 and 2); while on 4% or 5% agar a fair degree of lysis was secured in the presence

[2] Bronfenbrenner, J., and Hetler, D. M., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 480, and 1932, **29**, 806.

TABLE I.
Effect of Urea upon Lysis of *B. Coli*.

Column No.	1	2	3	4	5	6	7	8	9	10	11	12	13
50% urea added in cc.	—	—	.6	.6	.8	.8	1.0	1.0	—	.6	.8	1.0	1.2
H_2O added in cc.	1.0	1.0	.4	.4	.2	.2	—	—	1.0	.4	.2	—	—
B. coli suspension*	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5
Final concentration of urea, %	—	—	3	3	4	4	5	5	—	3	4	5	6

		Resulting mixtures added each to 10 cc. of molten agar of different concentration and poured into Petri plates												
		Drops of phage** and of broth respectively deposited on the surface of agar								Inhibition of growth by concentrated agar and by urea (control)				
1% agar	phage	++++	++++	++++	++++	++++	++++	++++	++++	heavy	heavy	heavy	good	poor
	broth	0	0	0	0	0	0	0	0	growth	growth	growth	growth	growth
2 "	phage	++	++	+++	+++	+++	++++	++++	++++	heavy	heavy	good	good	poor
	broth	0	0	0	0	0	0	0	0	growth	growth	growth	growth	growth
3 "	phage	+	EG	+	+	++	++	++	+++	heavy	good	good	good	poor
	broth	0	0	0	0	0	0	0	0	growth	growth	growth	growth	growth
4 "	phage	EG	EG	0	+	+	+	++	++	good	fair	fair	fair	poor
	broth	0	0	0	0	0	0	0	0	growth	growth	growth	growth	growth
5 "	phage	EG	EG	EG	EG	EG	0	++	++	good	fair	fair	fair	poor
	broth	0	0	0	0	0	0	0	0	growth	growth	growth	growth	growth

* Bacterial growth from 10 slants suspended in 40 cc. of broth.
** Phage titer 10^{-6} cc.
++++ = very marked lysis.
+++ = marked lysis.
++ = fair lysis
+ = weak "
EG = excess growth of bacteria under the drop of phage.
0 = no change.

of 5% urea (columns 7 and 8), in corresponding plates in the absence of (columns 1 and 2) or with lower concentrations of urea (columns 3 to 6) only trace or no lysis occurred.

4. Stained contact prints made by depositing glass cover slips directly upon the spots where phage was placed previously showed that wherever lysis took place, surviving organisms located in the immediate proximity of lysed areas were greatly swollen. On the contrary, contact prints from the spots where deposition of drops of phage failed to elicit lysis as well as from the spots where drops of plain broth were deposited (control) showed the organisms of normal size exclusively. For example, organisms were found to be normal in size under the drops of phage on 5% agar containing 4% urea (columns 5 and 6) while on 5% agar containing 5% of urea (columns 7 and 8) they were greatly swollen.

Experiments analogous to the one demonstrated here were repeated a number of times with various combinations of phage and bacteria. In general it was found possible to reproduce this phenomenon regularly with various Gram-negative bacteria. When Gram-positive bacteria were used, clear-cut results were obtained only with *B. megatherium.* With *B. diphtheriae* and with Staphylococcus these experiments were not successful on account of the inhibition of growth by urea even in the lowest concentration used. (3%).

In the earlier experiments we came to the conclusion that failure of lysis to take place on media containing high concentration of agar or gelatin is not due to the change in the susceptibility of bacteria to lysis nor to the inactivation of phage through the adsorption by the colloids of the medium, but to the competition for water between the medium and the bacteria.[3]

If this conclusion is valid, the present experiments suggest that addition of suitable concentrations of urea to the medium (5% agar) prevents the binding of water by the agar and thus permits the swelling and subsequent bursting of bacteria to take place.

[3] Bronfenbrenner, J., Monograph on "Filterable Viruses." Edited by T. Rivers (Williams and Wilkins, 1927).