Red cells from uremic patients with the exception of those in the extreme terminal stage were found to hemolyze more slowly in saponin systems than normal cells, which is in agreement with the previous study.[4] In this investigation certain quantities of indican, urea and phenol were added individually and collectively to a suspension of known amounts of washed normal red cells in saline and were found to have no hemolytic activity whatsoever in their proportion found in the plasma. Furthermore, as can be seen from Fig. 1, urea, phenol and indican, added to systems of red cells in plasma, definitely inhibited saponin hemolysis and reproduced curves which could be compared to those obtained by Herrald and Pijoan for nephritic blood.

In light of these test tube experiments we are led to the belief that in the anemia of nephritis, which develops with the manifestation of renal insufficiency, there is no increased hemolysis of the red cells by urea, indican and phenol, and that these substances inhibit the action of a standard hemolysin.

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## Effect of Urea Upon Growth of Fibroblasts from Cardiac Explants in Tissue Culture.

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It has been found recently that allantoin, a purine derivative occurring in maggots' secretions, stimulated the healing process in deep purulent wounds.[1] Another derivative, urea, was discovered to be more effective in bringing about the same results.[2] It was thought that, since urea may be obtained from allantoin by hydrolysis, the growth promoting effect of allantoin might be due to the urea and not to allantoin itself.

Fibroblasts from cardiac explants in tissue culture grew more abundantly in the presence of 0.5% allantoin than those in control cultures; although the growth increase was not sufficiently great to be of real significance.[3] It was deemed of interest to discover

1 Robinson, William, *J. Bone and Joint Surg.*, 1935, **17**, 267.

2 Robinson, William, *Am. J. Surg.*, 1936, **33**, 192.

3 Shipp, Mary E., and Hetherington, Duncan C., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 180.

TABLE I.

| Days Growth | Urea 0.5% Series: Mean Total Area, sq.mm. | Probable Error of Mean | Standard Deviation | Total Growth, % | Total Death, % | D—Difference of Means | $\sigma_D$—Standard Error of Difference of Means | *Significant difference of Growth, $D>3\sigma_D$ | Control Series: Mean Total Area, sq.mm. | Probable Error of Mean | Standard Deviation | Total Growth, % | Total Death, % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.89 | ±.02 | ± .36 | 0 | 4 | 0 | .050 | 0< .15 | 0.89 | ±.04 | ± .37 | 0 | 1 |
| 1 | 1.13 | ±.02 | ± .33 | 27 | 4 | .04 | .052 | .04< .16 | 1.17 | ±.03 | ± .41 | 34 | 1 |
| 2 | 2.39 | ±.05 | ± .85 | 168 | 6 | .30 | .121 | .30< .36 | 2.09 | ±.05 | ± .88 | 123 | 2 |
| 3 | 4.26 | ±.13 | ±1.97 | 379 | 8 | .62 | .273 | .62< .81 | 3.64 | ±.13 | ±1.92 | 309 | 6 |
| 4 | 5.16 | ±.20 | ±2.82 | 468 | 9 | .20 | .449 | .20<1.35 | 4.96 | ±.19 | ±2.97 | 343 | 11 |
| 5 | 6.49 | ±.22 | ±3.37 | 629 | 19 | .21 | .469 | .21<1.41 | 6.28 | ±.22 | ±3.36 | 550 | 14 |
| 6 | 8.08 | ±.29 | ±4.52 | 808 | 21 | .11 | .640 | .11<1.92 | 7.97 | ±.32 | ±4.83 | 795 | 17 |
| 7 | 9.66 | ±.33 | ±5.11 | 985 | 25 | .41 | .900 | .41<2.70 | 10.07 | ±.42 | ±6.36 | 1031 | 21 |
| 8 | 11.29 | ±.43 | ±6.63 | 1169 | 26 | .90 | .895 | .90<2.68 | 12.09 | ±.51 | ±7.63 | 1253 | 22 |
| 9 | 11.85 | ±.47 | ±7.21 | 1231 | 32 | .71 | 1.044 | .71<3.13 | 12.56 | ±.53 | ±7.92 | 1311 | 33 |
| 10 | 11.93 | ±.47 | ±7.11 | 1241 | 37 | .83 | 1.030 | .83<3.09 | 12.75 | ±.53 | ±7.77 | 1332 | 35 |

*Significant difference of growth was determined for any given day from the following formulæ:

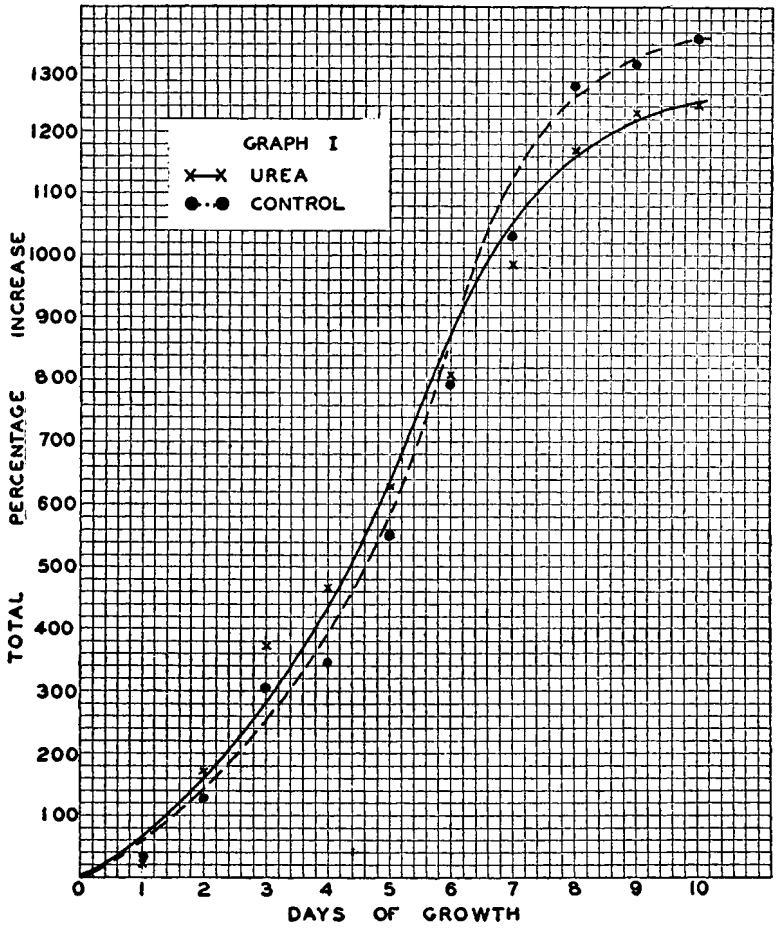
$$\sigma_D = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}} \text{ and } D>3\sigma_D$$

Where for any day $\sigma_1$ and $\sigma_2$ are the standard deviations of the control and urea series respectively and $N_1$ and $N_2$ the number of cultures; "D" represents the difference of the means and $\sigma_D$ is the standard error of the difference.[4]

[4] Mills, *Statistical Methods*, H. Holt and Co., 1930, p. 558.

whether the simpler product, urea, would increase the growth of fibroblasts to a greater extent than did the allantoin and to this end the present experiment was devised and conducted.

All cultures were made by the cover-slip-hanging-drop method with the observance of aseptic precautions. All water was triply distilled in an all-glass pyrex apparatus. The plasma was obtained by centrifuging blood drawn, without the use of an anti-coagulant, from the wing veins of young hens. The embryo juice was made by extracting 7-day chick embryos in Tyrode solution (pH 7.5) containing 0.25% dextrose. For the test series, urea, in quantities sufficient for a final dilution of 0.5%, was added to an aliquot part of an adjusted Tyrode solution before the extraction of the embryos. This concentration of urea was chosen because it was the same as that of



allantoin used in a previous experiment[3] and further because the 2% solution, as used in clinical treatment of deep wounds[2] was too toxic for the cultures. Heart tissue of 7-day chick embryos was planted in a mixture of equal parts of plasma and of embryo juice, the latter containing the urea. An equal number of controls using the same plasma, stock embryo juice and embryo hearts was planted at the same time as each series of urea cultures. All cultures were incubated at 37°C. and a daily record was kept on each culture until death. With the use of a delineascope and planimeter the area of the original explant and the areas of the daily outgrowths of each of the 335 cultures were measured. One hundred of the control cultures and one hundred of the experimental cultures lived for 10 days or more and were used for statistical study of the daily growth, because it was found that the majority had reached their maximum growth before the tenth day and also because the results could be based upon and compiled from the same hundred cultures for each day. (Table I.)

Neither the control cultures nor the experimental series showed any significant differences in the behavior or structure of the cells. The difference in death rate for the 2 series of cultures (Table I) indicates that the presence of urea had no detrimental effect in these experiments. Graph I was constructed from statistical data of Table I and it may be seen that the growth curve of the experimental series indicates a wholly insignificant increase over that of the control from the second through the sixth day. Thereafter the control curve rises above the experimental one.

It has been reported that urea in suitable dilutions produces proliferation of capillaries by sprouting.[5] Since this compound does not bring about a significant stimulation of the growth of fibroblasts from cardiac explants in tissue culture, it is suggested that the hastened healing observed in deep, infected wounds following irrigation with urea solutions may be attributed to changes in the capillary bed and the associated effects therefrom upon the granulation tissue.

[5] Abel, Richard, Abstract, *Anat. Rec.*, Supplement No. 3, 1937, **67**, 1.