

Effect of Urea in Sulfonamide Base on Healing of Clean Skin Wounds in Rabbits.*

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Urea has been used in the treatment of infected wounds since 1915. Since that time some 40 papers on the subject have appeared, but only one completed report (Chiariello¹) gives data on the effect of urea on relatively uninfected experimental wounds. A note claiming beneficial effects of urea in experimental wounds in animals appeared in 1940,² but no supporting data were included. An abstract by Greenbaum and Aye³ mentions some experimental work on effects of allantoin, allantoin-dipiperazine, 40% urea, and saline on wound healing in the fowl. Allantoin is claimed to exert the most beneficial effect, and urea the least. No data are given. All other papers have usually been concerned with uncontrolled infected clinical cases.

Low concentrations of urea are claimed by Robinson⁴ to stimulate proliferation in wounds, whereas Holder and MacKay,⁵ and many others claim that rather than acting as a stimulant to proliferation, only in strong concentrations does urea exert its beneficial effects through its cleansing solvent action in necrotic wounds, because of its bactericidal qualities, and because it removes inhibitors of topically applied sulfonamides.

Chiariello,¹ produced open skin defects in guinea pigs and rabbits by cautery and surgery, and measured the time to complete healing of the defects treated with 4% urea as compared with dry controls, or controls treated with tannic acid, saline, or olive oil. No effects were noted other than a subjectively observed slight decrease of the latent periods of the wounds produced by cautery.

Rabbits were used in the present study. Circular defects, 1-3 cm in diameter, were incised through the corium to the panniculus carnosus.

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¹ Chiariello, A. G., *Folia Med.*, 1939, **25**, 61.

² Annotations, *Lancet*, 1940, **238**, 1132.

³ Greenbaum, F. R., and Aye, C. E., *101st Proc. Am. Chem. Soc.*, 1941.

⁴ Robinson, W., *Am. J. Surg.*, 1936, **33**, 192.

⁵ Holder, H. G., and MacKay, E. M., *Ann. Surg.*, 1939, **110**, 94.

No aseptic precautions were taken. The wounds were protected by flanged celluloid collars with removable caps. The flanges were made fast to the shaved skin with flexible collodion. The wounds were treated with a water-soluble jelly consisting of a 4% gumkaraya glyceride, 1% boric acid as a fungicide and 0.5% sulfanilamide or 5% sulfathiazole. Three different concentrations of urea, 40%, 10%, and 2% were incorporated in the jelly base. One control and one experimental defect were incised in each animal symmetrically on either side of the dorsal midline at the shoulders. The wounds were redressed and traced on transparent cellophane every 48 hours for 12-14 days. The animals were hobbled and collared to prevent access to the wound caps. Anesthesia (pentothal-sodium[†]) was employed at each dressing, to facilitate more accurate tracings.

Blood agar plates with a 24-hour *Staphylococcus aureus* culture showed a marked inhibitory effect of the jelly base containing 5% sulfathiazole, and no effect of one containing 0.5% sulfanilamide. 40% urea did not enhance the inhibition by sulfathiazole.[‡] Sterile swabs taken from the surface of the urea-sulfathiazole treated wounds were cultured in Brewer's thioglycollate broth and on blood agar plates. Gamma type non-hemolytic streptococci were obtained,[§] but that they developed only after transfer from the bacteriostatic sulfathiazole medium of the wounds was shown by their absence in gram-Weigert stained sections of the tissues.

At the end of 12-14 days, the animals were sacrificed and paraffin sections of Bouin or formalin fixed tissue from the center of the wounds were stained by gram-Weigert's, Mallory's, and hematoxylin-eosin methods. The gram-Weigert's stain permitted an estimate of the presence or extent of bacterial contamination. This technic revealed a few relatively heavy contaminations of gram-positive cocci and bacilli in the defects treated with 40% urea and 0.5% sulfanilamide, and these were largely restricted to the surface regions. Very low-grade or no infections were found in those treated with the lower concentrations of urea and 5% sulfathiazole.

The slides of the cross-sectioned tissue were photographed and the rate of increase in area of the granulation tissue was estimated by planimetric means. Measurements from photographs and from the first cellophane tracings (made 48 hours after operating) also permitted calculation of the rate of epithelization.

[†] The pentothal-sodium was supplied by the Abbott Laboratories.

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The results of our preliminary experiments on 21 rabbits and 42 wounds are shown in Table I. It is seen that urea retards the rate of epithelization but stimulates the rate and extent of formation of granulation tissue in proportion to the concentration of urea. 40%, 10%, and 2% levels of urea retard the average rate of epithelization 108%, 16%, and 20%, respectively; whereas the 40% and 10%

TABLE I.
Effect of Urea on Healing of skin wounds in rabbits.

A. *Size of wounds* (Avg cm diam. between epith. boundaries at center of wound).

No.	% urea	No. of wounds	Days from 1st tracing* to autopsy	Diameter of wounds (cm)			
				Avg original diam. (calc'd from 1st tracings)		Final diam. (meas'd from photographs of sections†)	
				Controls without urea	Expts. with urea	Controls without urea	Expts. with urea
1‡	40	7	10	1.65	1.55	0.87	1.69
2	10	10	10.3	1.40	1.45	0.82	1.05
3	2	5	11	1.68	1.56	1.03	1.05

B. *Rate of epithelization* (mm per day mean rate of epithelial closure from 1st tracing, 48 hrs after op'n to autopsy. (Calc'd from diameters).

No.	% urea	No. of wounds	Days from 1st tracing to autopsy	Rate of epithelization (mm per day)				
				Controls without urea	Expts. with urea	% diff.	Student's "t" value ^{6,7}	Probability ^{6,7}
1	40	7	10	.78	— .14§	— 108	5.6	.0008
2	10	10	10.3	.51	.43	— 16	3.9	.0046
3	2	5	11	.61	.49	— 20	3.5	.0250

C. *Rate of granulation* (mm² per day mean rate of formation of granulation tissue, calc'd from planimetric meas. of photographs of cross sections).

No.	% urea	No. of wounds	Days from operation to autopsy	Rate of granulation (mm ² per day)				
				Controls without urea	Expts. with urea	% diff.	Student's "t" value	Probability
1	40	10	13	2.67	3.64	36	3.0	.0134
2	10	9	12.3	1.98	2.36	19	4.4	.0018
3	2	5	13.5	2.90	2.82	— 3	1.1	.3214

*48 hrs after operation.

†The final diameter cannot be accurately measured from cellophane tracings after more than 5 or 6 days from the time of operation, but must be more accurately determined from stained cross sections.

‡The jelly base in Group 1 contained 0.5% sulfanilamide, whereas those in Groups 2 and 3 contained 5% sulfathiazole. (See text for discussion.)

§A negative rate implies that the strong urea caused the wound to increase in size. (See Table I, part A.)

⁶ "Student," *Metron*, 1925, 5, 18 and 26.

⁷ Fisher, R. A., *ibid.*, 1925, 5, 3.

levels promote the rate and extent of granulation 36% and 19%, respectively. 2% urea had an insignificant effect on the rate of granulation tissue formation. 5% sulfathiazole was substituted for the 0.5% sulfanilamide first used in the experiments with 40% urea, since it was found to inhibit more efficiently bacterial growth. This substitution had no effect on the rate of healing, permitting comparisons to be drawn between the 3 experimental groups. Deletion of the cases which were shown to be significantly infected had no essential effect on the results.

Discussion. It is possible that these results may be explained by an inflammatory action of the urea. This might then result in marked production of granulation tissue from the wound-base, subsequent contraction of this tissue, resultant impaired vascular supply to the surface, and eventual retardation of epithelization as well as formation of overgrowths by fibroplastic stimulation.⁸ This concept is supported by Menkin's⁹ observations that strong urea is an inflammatory irritant in rabbit tissue. Maceration in our wounds was not apparent.

Because of the delicacy of rabbit tissue, caution must be exercised, as has been pointed out by MacKay,¹⁰ in the extension of these results to other species. Our results suggest that if strong urea is used in the chemical debridement of infected wounds, its use after clean granulation is initiated should be tempered with caution.

Summary. Urea retards the rate of epithelization of open skin wounds of rabbits, but stimulates the rate and extent of formation of granulation tissue; 40%, 10%, and 2% levels of urea in a glyceride jelly base containing sulfanilamide or sulfathiazole retard the average rate of epithelization 108%, 16%, and 20% respectively; 40% and 10% levels of urea promote the rate and extent of granulation 36% and 19% respectively, and 2% urea has little or no effect.

⁸ Arey, L. B., *Physiol. Rev.*, 1936, **16**, 327.

⁹ Menkin, V., *J. Exp. Med.*, 1932, **56**, 157.

¹⁰ MacKay, E. M., *Arch. Dermatol. Syphilol.*, 1940, **41**, 736.