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Effect of Certain Amino Acids on Healing of Experimental Wounds of the Cornea.

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As a non-vascularized tissue, the cornea is unique from a biological point of view. It receives its nourishment by diffusion of fluids through the entire corneal substance. In trauma this diffusion may be disturbed leading to vascularization and loss of transparency.

Earlier many attempts were made to nourish the elements of tissue cultures and to increase their rate of proliferation *in vitro*.¹ The attempt to influence the regeneration of corneal defects by embryonic tissue extracts encountered many difficulties. Fischer² found that in tissue cultures *in vitro* the embryonic extracts could be replaced by an artificial medium in which amino acids furnished the building stones for the synthesis of protoplasm. Cystine, which seemed to function both as an energy-furnishing ingredient and as a growth catalyst, apparently played a major role in this medium. This special function of cystine is probably connected with its sulfur content, as free sulfhydryl groups often activate proteolytic systems in the process of growth.

Encouraged by Fischer's results the writer has made an attempt to influence the regeneration of experimental wounds of the cornea by administration of amino acids to this tissue *in vivo*. For the first experiment 36 guinea pigs were used. The technic employed was a modification of the method of Gundesen and Liebman.³ Superficial vertical incisions were made across the cornea into the epithelium of both eyes by means of a Castroviejo double knife with blades 2 mm apart. The cornea was immediately stained with fluorescein and the epithelium between

the two lines evenly abraded with a spatula. The corneae were periodically stained and the fluorescein-positive areas registered on a chart drawn on logarithm paper. The chart consisted of a ring symbolizing the limbus with a vertical double line in the center. By counting the number of stained and unstained squares of the paper, a quantitative expression of the healing process could be obtained.

In 18 of these animals (1,a) the amino acid solution was dropped into the right eye every hour while the left control eye received a corresponding amount of physiological saline. In the remaining 18 animals (1,b) an ointment containing the amino acids was applied twice a day to the right eye together with a 5% boric acid ophthalmic ointment which was applied to both eyes.

The same experiment was repeated on 6 other animals (2) but instead of a stripe, a round erosion, 3.5 mm in diameter, was made with a trephine in a similar manner. The amino acid solution was made in 6% NaCl and contained 2 mg cystine, 5 mg proline, 6 mg asparagine and 14 mg glutamine per ml, and was adjusted to pH 7.2. The ointment was prepared by emulsifying 70 g aquafor with 30 ml amino acid solution which contained the aforementioned amino acids in 10-fold concentration.*

A complete regeneration of the corneal defect in the eyes treated with amino acids was achieved within 12 to 42 hours (in less than 30 hours in an average), while the healing process in the control eyes required 55 to 120 hours.

In a third series of experiments the effect of amino acids on healing of deep non-per-

¹ Biseoglie, V., and Schaeffer, A. J., *Die Gewebezuchtungen in vitro*, Springer, Berlin, 1928.

² Fischer, A., *Acta Physiol. Scandinav.*, 1940, **2**, 143.

³ Gundesen, T., and Liebman, S. D., *Arch. Ophth.*, 1944, **31**, 29.

* I am indebted to Dr. Ernest Geiger for suggestions and for the material used in these experiments.

TABLE I.

Summary Table Showing Type of Corneal Injury and the Average Healing Time in Control and Amino Acid-treated Animals.						
Expt. No.	Experimental injury	Treatment		No. of Animals	Healing time*	
		Control	Amino acid treated		Control eye	Treated eye
Guinea pigs						
1,a	Vertical 2 mm. erosion	Physiol. saline hourly	Amino acid soln. hourly	18	64(55-108)	22(15-32)
1,b		5% boric acid ointment twice daily	5% boric acid ointment containing amino acids twice daily	18	78(66-120)	26(12-42)
2	Round erosion. 3.5 mm	physiol. saline hourly	Amino acid soln. hourly	6	62(50-112)	21(14-38)
Rabbits						
3,a	Deep non-perforating trephine wound	" "	" " "	2	95(86-104)	35(28-42)
3,b		5% boric acid ointment twice daily	5% boric acid ointment containing amino acids twice daily	10	108(96-120)	40(32-56)

* The first figure gives the average and the values in parentheses the minimum and maximum values of the individual experiments.

forating trephine wounds of the cornea was examined. In 12 rabbits a central defect 2.5 mm in diameter and 0.4 mm deep was made into the substance of the cornea of both eyes. The right eye was treated in 2 animals (3,a) with hourly administration of drops containing amino acids while the left eye received saline solution. The other animals received ointment applications as in the first experiment (3,b).

In the eyes treated with amino acids, the corneal defect was covered with epithelium within 32 to 56 hours and in the control eyes between the 4th and 5th day. The biomicro-

scopic parallel with the histological examination showed that in the amino acid-treated eyes the same phase of the healing process is reached after 2 days which the control eyes reached only after the 6th day of experiment.

The results of treatment of over 100 clinical cases in man with the same ointment will be published elsewhere. Although it is difficult to evaluate these data quantitatively since the ointment was never applied alone, it would appear that the application of amino acids accelerates the healing of corneal wounds particularly when ulcerative processes are involved.

15261

Accidental Air Embolism and Fibrin Formation in the Heart of Rabbit.

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During operations on the spinal column, cord and skull, a number of rabbits died more or less acutely and their death was at first attributed to that diffuse symptom-complex "vascular shock." Autopsies, however, soon

revealed cleaner factors that played an undoubted rôle in the fatal outcome; gas bubbles were seen in the pulmonary artery associated at times with fibrin formation in the heart, especially in the right ventricle.