Bruised Tissue IV. Effect of Streptokinase and Trypsin on Healing.* (24177)

M. K. HAMDY, M. S. RHEINS, F. E. DEATHERAGE
(Introduced by George Y. Shinowara)
Departments of Animal Science, Agric. Biochemistry and Bacteriology, Ohio Agric. Exper. Station, Ohio State University, Columbus, O.

It has been shown that each of 3 successive bruises in animals healed more rapidly than did the preceding one(1). The factor or factors responsible for this accelerated healing are, at least in part, present in whole blood from a previously bruised animal (rabbit), and may be passively transferred to a normal animal by transfusion. No single substance has been isolated which would completely account for accelerated healing of a secondary wound. Engley, *et al.*,(2) have suggested that an unidentified humoral factor may be involved while Zamencik, *et al.*,(3) established the existence of an enzyme (aminoexopeptidase) in lymph and serum of dogs, and observed that levels of this enzyme rise abruptly in lymph draining an area of burned or traumatized tissue. Others(4-6) have reported the presence of proteolytic enzymes in damaged tissue. Sherry and Alkjaersig(7) described the beneficial effect of streptokinase for dissolution of intravascular thrombi and extravascular fibrinous exudates in dogs, while Gordon and Ablondi(8) reported that intravenous injection of this enzyme reduced the severity of inflammation experimentally produced in rabbits. Trypsin also has been shown effective in reducing experimentally induced edema(9). These enzymes have proven successful as anti-inflammatory agents by other investigators(10-15). The purpose of this study was to determine the influence of trypsin and a mixture of streptokinase and streptodornase on the biochemistry of healing of experimentally induced bruises in rabbits and cattle.

Materials and methods. Bruising and sampling. Rabbits (6-8 lb) and cattle (700-850 lb) were employed. Areas to be bruised and symmetrically located control areas were denuded. Procedures for inflicting bruises and for procuring tissue samples were previously described(16). Morphologic examination of bruised tissue, detection of tissue bilirubin as a reflection of hemoglobin degradation and conductivity measurements(1,17) served as criteria for the effectiveness of test enzymes in promoting the healing process. *Bilirubin.* Presence of bilirubin in bruised tissues(16) was detected using Fouché reagent, forming no color (−) in its absence and a light blue color in presence of 0.5-1.0 mg bilirubin/100 g tissues (indicated by +). This color turns to dark blue (++) and (+++) at a concentration of 1-2.5 mg bilirubin and to green (++++) above this concentration(17). This blue green color (probably biliverdin) is a reaction product of oxidation of bilirubin by ferric chloride in the trichloro-acetic acid solution (Fouché reagent). *Enzymes.* Trypsin† and streptokinase-streptodornase varidase‡ (SK-SD) enzyme preparations were used. Unless otherwise stated, these enzymes were introduced into the experimental animals 12 hours after bruising.

Results. Effect of injecting trypsin and SK-SD on bruised tissue. Working levels of test enzymes were established by injecting normal rabbits either intramuscularly or subcutaneously in graded amounts. The results indicated that a single injection of trypsin to 10 mg did not result in necrosis, while daily injections of 6 mg for 15 days resulted later in erythematous areas at injection sites. A single injection of SK-SD to 15,000 units

* Published with approval of Associate Director, Ohio Agri. Exp. Station. Journal Article No. 45-58.

† Paraenzyme, a sterile suspension of trypsin in sesame oil (Generously provided by National Drug Co., Phila., Pa.).

‡ A purified and concentrated combination of enzymes produced by a strain of streptococcus (Lederle Lab. Div. of American Cyanamid Co., Pearl River, N. Y.).

TABLE I. Effect of Trypsin and Streptokinase-Streptodornase (SK-SD) on Healing Time, Bilirubin Detection and Conductivity in Rabbit Bruises.

No. of animals	Agent used	Healing time (days)	Bilirubin detection (hr)	Conductivity $\times 10^{-2}$ (ohm^{-1} cm^{-1}) After 2 days	After 5 days
10	2 mg trypsin	6.5 ± .2	52 ± 2.5	6.1 ± .3	2.95 ± .3
11	10,000 units (SK-SD)	5.8 ± .2	48 ± 3.8	5.8 ± .3	2.9 ± .4
7	1 ml saline (control)	8.0 ± .3	71 ± 2.5	5.9 ± .5	4.1 ± .3
5	1 ml sesame oil "	8.2 ± .4	72 ± 3.0	5.4 ± .4	4.3 ± .3
7	No treatment "	7.9 ± .3	70 ± 3.0	5.7 ± .3	4.3 ± .4

failed to elicit any discernible effect. Twenty thousand units as a single injection, or daily administration of 10,000 units for 15 days, produced inflammatory manifestations.

Five groups of rabbits were bruised according to a standard procedure(16). Twelve hours later, animals in Group I were injected intramuscularly with 2 mg of trypsin at the site of the bruise; Group II animals received 10,000 units of SK-SD by the same route; rabbits in Group III were inoculated with 1 ml of physiologic saline; Group IV, 1 ml of sesame oil; and Group V animals served as untreated controls. Observations of the bruised areas continued for 9 days. At intervals animals were sacrificed; bruised and control areas were compared grossly; and the average resistances offered by these tissues were measured and conductivities calculated using the cell constant, as previously described(17). Samples of bruised and control tissues were immediately excised, scored and tested for the presence of bilirubin. The latter substance was apparently bound to protein, thus confirming the report by Stenhagen and Rideal(18).

As shown in Table I, injections of trypsin or SK-SD accelerates the healing process of bruised tissues as determined by gross examination. Average healing occurred in 6.5 days in rabbits which received trypsin, 5.8 days in animals treated with SK-SD, while controls (untreated, saline, or sesame oil) required approximately 8 days for complete repair. The first detection of bilirubin (an early indication of reparative processes) in each group paralleled the above pattern. Conductivity measurements after 2 days were of approximately the same magnitude, but after 5 days, values for enzyme-treated tissues approached those previously established for normal tissues. This experiment was repeated in part in cattle. In these animals concentrations of enzymes employed for treatment were increased to 10 mg of trypsin and 45,000 units of SK-SD. The results were similar to those described above.

Enzyme injections at sites remote from bruises. A number of rabbits, bruised singly over the gluteus muscle, were divided into 5 groups. Each animal in the first group was injected daily in the bruised muscle with 2 mg of trypsin; animals in the second group were treated in the same manner, except that daily injections of enzyme were introduced into a site remote from the bruise. The third and fourth groups received 10,000 units of SK-SD daily at the locus of the bruise and in a distant tissue, respectively. The fifth group of animals were untreated. Animals from each group were sacrificed after 52, 88, and 106 hours, and the same measurements were conducted as before (Table II). Bilirubin was detected in bruised tissue after 52 hours, in animals receiving the enzyme preparation at site of bruise but not in the control (untreated animals or animals receiving the enzyme at a site remote from bruise). After 88 and 106 hours, very little difference was noted among the various groups, either in quantity of bilirubin detected or in conductivity values. After 106 hours, evidence of more rapid repair was obtained from those animals treated with both enzyme preparations at sites of the bruises (conductivity values). Rate of healing in those rabbits injected in other than damaged tissues resembled that noted for untreated controls.

Influence of ascorbic acid in healing. Rabbits, bruised over the gluteus muscle, were

TABLE II. Effect of Site of Injecting Trypsin or SK-SD on Healing of Bruised Tissues in Rabbits. Three animals bruised per series.

Treatment received (daily)	Bilirubin detection after			Conductivity × 10^{-2} (ohm^{-1} cm^{-1})		
	52 hr	88 hr	106 hr	52 hr	88 hr	106 hr
2 mg trypsin (at the site*)	+	++++	+++	6.0 ± .3	4.8 ± .3	3.3 ± .3
Idem (at remote site†)	—	++	++++	5.9 ± .2	5.0 ± .3	4.5 ± .3
10,000 units SK-SD (at the site)	+	+++	+++	6.0 ± .3	4.9 ± .2	3.6 ± .3
Idem (at remote site)	—	++	++++	6.1 ± .3	5.2 ± .3	4.2 ± .3
No treatment (control)	—	++	++++	5.9 ± .2	5.1 ± .3	4.6 ± .3

* At the site of bruised tissue. † At site remote from bruised tissue.

TABLE III. Effect of Injecting Ascorbic Acid and Trypsin on Healing of Rabbit Bruises. Three animals bruised per series.

Treatment received (daily)	Bilirubin detection		Conductivity × 10^{-2} (ohm^{-1} cm^{-1})	
	After 52 hr	After 106 hr	After 52 hr	After 106 hr
2 mg trypsin + .17 mg ascorbic acid	++	+	6.2 ± .3	2.8 ± .2
2 mg trypsin	+	+++	6.0 ± .3	3.3 ± .3
0.17 mg ascorbic acid	+	+++	6.0 ± .3	5.0 ± .3
No treatment (control)	—	++++	5.9 ± .2	4.6 ± .3

grouped according to treatment to be rendered. Animals of the first group were injected daily with 2 mg of trypsin at the bruise site, as before. Each animal in the second group received the same quantity of trypsin as animals of first group, and, in addition, 0.17 mg of ascorbic acid contained in 0.5 ml of 1/15 M phosphate buffer (pH 7.4).§ Animals in the third group were injected with the ascorbic acid only, while the fourth group was retained as untreated controls. Animals from each group were sacrificed after 52 and 106 hours.

The results (Table III) indicate that bruised tissues of the group treated daily with the mixture of enzyme and ascorbic acid healed at a significantly more rapid rate than did tissue of animals in the remaining groups.

Discussion. The enhanced healing observed in bruised animals following treatment with trypsin or SK-SD may confirm the opinions of others regarding the mode of action of these agents(7-15). Most likely trypsin functions directly to accelerate proteolysis of damaged tissue, while the SK-SD activates plasminogen. Thus the normal body defense mechanisms are enhanced to function more efficiently.

Activation of proteolytic enzymes either artificially, as reported here, or as a natural phenomenon associated with tissue repair, may account for accelerated healing of successive bruises, provided critical levels of these enzymes exist at the time of bruising. Sherry and Alkjaersing(7) reported that trypsin and streptokinase are able to activate blood plasminogen to form plasmin, which is capable of splitting arginine and lysine esters as established by Troll and Sherry(19).

Some bruises did not respond to trypsin or streptokinase treatment and accordingly healed at a much slower rate. This may be due to the presence of a high level of antitrypsin or antistreptokinase. It is also possible that animals which did not respond to streptokinase had or have had a recent streptococcal infection and hence had a high titer of antibodies specific against the inoculated enzyme.

Müllertz(15) reported that failure of some animals to respond to the streptokinase treatment may be attributed to absence of plasminogen or to high level of antistreptokinase, a specific immune antibody developed during streptococcal infection or to a high level of antiprotease that occurs in rather high con-

§ The ascorbic acid solution was prepared immediately prior to administration to minimize oxidation.

centration in animal sera, or to a strict species specificity to streptokinase.

The precise role of ascorbic acid in repair processes is not known. The possibility exists that Vit. C may be utilized by injured tissues for synthesis of collagen(20). Lemberg, *et al.*,(21) reported that coupled oxidation may occur between hemoglobin and ascorbic acid for the formation of choleglobin. Bourne (22) established that the tensile strength of a healing wound in guinea-pig suffering from partial deficiency of ascorbic acid is considerably less than in normal animals.

Summary. 1. Rabbits previously bruised healed more rapidly following injections of trypsin and SK-SD than did their untreated counterparts. 2. Injections of enzyme preparations into tissue remote from the bruised areas did not result in accelerated healing. 3. Combination of Vit. C and trypsin was superior to trypsin alone in promoting tissue repair.

1. Hamdy, M. K., Kunkle, L. E., Rheins, M. S., Deatherage, F. E., *J. Animal Sci.*, 1957, v16, 496.

2. Engley, F. B., Jr., Allgöwer, M., Snyder, C. D., *Ann. N. Y. Acad. Sci.*, 1955, v59, 326.

3. Zamencnik, P. C., Stephenson, L. M., Cope, O., *J. Biol. Chem.*, 1945, v158, 135.

4. Peters, R. A., *Brit. Med. Bull.*, 1945, v3, 81.

5. Ungar, G., Damgaard, E., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 378.

6. Neville-Jones, D., Peters, R. A., *Biochem. J.*, 1948, v43, 303.

7. Sherry, S., Alkjaersig, N., *Ann. N. Y. Acad. Sci.*, 1957, v68, 52.

8. Gordon, S., Ablondi, F. B., *ibid.*, 1957, v68, 89.

9. Martin, G. J., *ibid.*, 1957, v68, 70.

10. Tillett, W. S., *ibid.*, 1957, v68, 151.

11. Raker, C. W., *ibid.*, 1957, v68, 144.

12. Innerfield, I., *ibid.*, 1957, v68, 167.

13. Miller, M. J., *ibid.*, 1957, v68, 185.

14. Lichtman, A. L., *ibid.*, 1957, v68, 196.

15. Müllertz, S., *ibid.*, 1957, v68, 38.

16. Hamdy, M. K., Deatherage, F. E., Shinowara, G. Y., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 255.

17. Hamdy, M. K., Kunkle, L. E., Deatherage, F. E., *J. Animal Sci.*, 1957, v16, 490.

18. Stenhagen, E., Rideal, E. K., *Biochem. J.*, 1939, v33, 1591.

19. Troll, W., Sherry, S., *J. Biol. Chem.*, 1955, v213, 881.

20. Hunt, A. H., *Brit. J. Surg.*, 1941, v28, 436.

21. Lemberg, R., Legge, J. W., Lockwood, W. H., *Biochem. J.*, 1941, v35, 339.

22. Bourne, G. H., *Lancet*, 1944, v246, 688.

Received June 6, 1958. P.S.E.B.M., 1958, v98.

Effect of 6-Dimethylaminopurine-3-Amino-D-Ribose on Adenosine Triphosphate Formation in Yeast.* (24178)

DAVID M. KESSNER, BENJAMIN A. BOROWSKY AND LILLIAN RECANT
(Introduced by Robert E. Shank)
Nutrition Research Laboratory, Department of Preventive Medicine, Washington University School of Medicine, St. Louis, Mo.

It has been demonstrated that 6-dimethylamino-purine-3-amino-d-ribose (Aminonucleoside) induces the nephrotic syndrome in rats (1,2). In an effort to obtain evidence of the mechanism of production of this experimental nephrosis, the effect of this compound on adenosine metabolism has been investigated. This study was prompted by reports which indicate that Aminonucleoside may interfere with some phase of purine metabolism. Agosin and Von Brand(3) and Hewitt, Gumble, Wallace and Williams(4,5) demonstrated that the trypanosomacidal action of the drug in infected mice could be blocked by simultaneous administration of various substituted purines. These observations, and similarity in chemical configuration of adenosine and Aminonucleoside, suggested the possibility of competitive inhibitory effect of Aminonucleoside upon adenosine metabolism. To test this hypothesis, an *in vitro* system known to metabolize adenosine was sought. Crude yeast fractions

* Supported by research grant from St. Louis Heart Assn.