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Hyaluronidase Content of Normal and Inflamed Guinea Pig Skin.

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In the presence of hyaluronidase the epidermal challenge reaction in guinea pigs sensitized to paraphenylenediamine is markedly increased as compared to control animals (Mayer and Kull[1]). This observation suggested that hyaluronidase plays a role not only in the spread of invasive organisms and the ensuing bacterial inflammation (Duran-Reynals[2]), but also in certain non-bacterial inflammations of the skin.

In order to obtain further evidence of this hypothesis it was desirable to determine whether the hyaluronidase content of the guinea pig skin was increased during allergic and non-allergic inflammations.

Methods. Guinea pigs were sensitized to paraphenylenediamine in the manner described by one of us.[3] After sensitization had been established, the animals were challenged with 10% paraphenylenediamine in petrolatum applied to the left flank, and sacrificed 24 hours later. 3 x 4 inch strips of skin were removed from challenged and unchallenged sites.

Physical inflammations were produced by application of heat (water at 70°C) for 1 and 2 minutes respectively on the shaven skin and by ultraviolet irradiation (Hanovia lamp, distance 12 inches) for various exposure periods. Skin samples were obtained from both inflamed and normal sites.

Identical weights (based on dry weight) of inflamed and non-inflamed skin were successively subjected to freezing and thawing and then ground with sterile sand. The resulting tissue-brei was extracted with 10 ml of 0.1 M acetate buffer, pH 6.0, containing 0.15 M NaCl, and centrifuged. The supernatant fluid was assayed viscosimetrically according to the method of McClean and Hale,[4]

TABLE I.

Treatment	Guinea pig No.	Intensity of inflammation	Hyaluronidase content skin-viscosity reduction units per g dry wt*	
			Normal skin	Inflamed skin
Paraphenylene diamine dermatitis	1	++++	.015	.456
	2	++++	.043	.185
	3	++++	.157	.925
	4	++++	.022	.856
	5	++++	.045	1.110
Heat-induced inflammation	6	++++	.00	.391
	7	+++++	.034	.556
Ultraviolet erythema	8 (2'†)	+	.040	.0061
	9 (5')	+	.00	.054
	10 (20')	++	.0126	.034
	11 (30')	++	.045	.0

* The skin viscosity reduction unit was obtained by applying the formula of McClean and Hale[4] with the modification of 60' being equivalent to one unit. The values are extrapolated by applying $\sqrt{}$ time vs. viscosity.

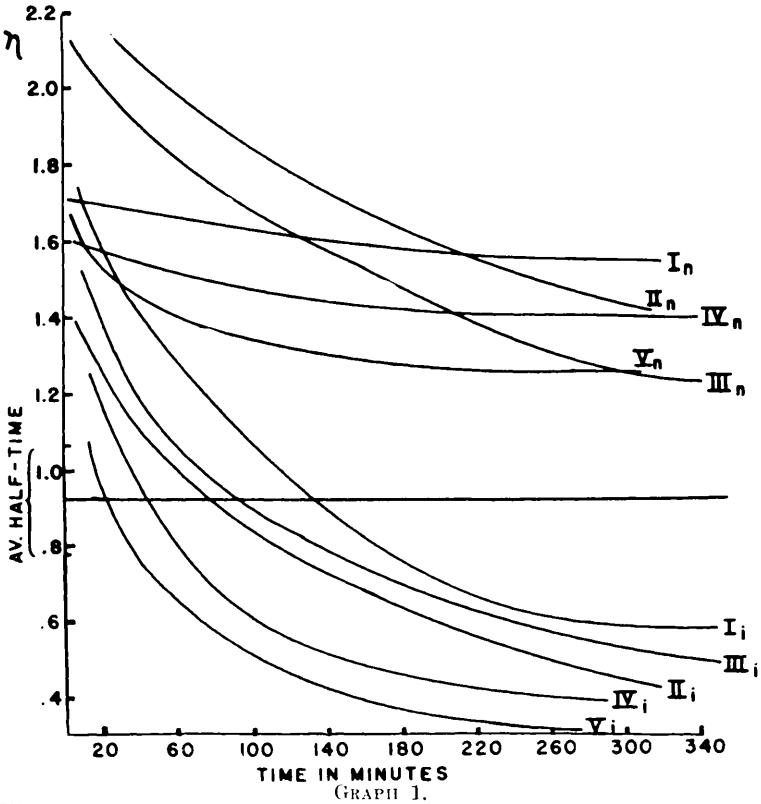
† Minutes of exposure to ultraviolet. One minute equal to one guinea pig skin erythema dose.

[1] Mayer, R. L., and Kull, F. C., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 392.

[2] Duran-Reynals, F., *Bact. Rev.*, 1942, **6**, 197.

[3] Mayer, R. L., *Arch. f. Dermat. u. Syph.*, 1931, **163**, 223.

[4] McClean, D., and Hale, C. W., *Biochem. J.*, 1941, **35**, 159.



GRAPH 1.

Viscosity Reduction with Normal and Inflamed Skin from Guinea Pigs Allergic to Paraphenylenediamine.

In, IIn, IIIn, IVn, and Vn are the viscosity reduction curves obtained from normal skin; Ii, IIi, IIIi, IVi, and Vi are the viscosity reduction curves obtained from inflamed skin.

using 0.4 cc of the above supernate and 0.6 ml of 0.417% hyaluronate (prepared from umbilical cords according to Kass and Seastone[5]) dissolved in the acetate buffer.

Results. The results of the experiments on 11 animals shown in Table I indicate that the free hyaluronidase content in allergic or heat-inflamed skin is up to 40 times as high as that of the corresponding normal skin.

Unlike the inflammations induced by the allergic challenge or by heat, the 24-hour ultraviolet reaction after the administration of 2 to 60 guinea pig erythema doses did not result in a consistent increase of free hyaluronidase. No increases were observed in 2 skins which had been irradiated for 2- and 30-minute periods respectively, while 2 other skins, irradiated for 5- and 20-minute periods respectively showed a slight but not significant increase.

Negative results were also obtained with 2 guinea pigs sensitized to horse serum upon subjection to local Arthus reactions.

Graph 1 shows the course of viscosity reduction of the tests in animals presenting allergic inflammation (animals 1-5).

Discussion. While hyaluronidase in bound form is known to exist in the normal skin (Meyer[6]), our experiments have shown that normal guinea pig skin also contains very small amounts of free hyaluronidase. During inflammation produced by a challenging application of paraphenylenediamine on sensitized skin or after treatment of the skin with hot water, the amounts of polysaccharide-depolymerizing enzymes (probably chiefly hyaluronidase) are markedly increased—up

[5] Kass, E. H., and Seastone, C. V., *J. Exp. Med.*, 1944, **79**, 319.

[6] Meyer, K. F., *Physiol. Rev.*, 1947, **27**, 335.

to 40 times.

Ultraviolet irradiation, producing definite erythema in guinea pigs, results in an occasional but very slight increase of these enzymes, contrary to the Arthus phenomenon, which in 2 rabbits did not result in increased amounts. It is noteworthy that the ultraviolet treatment and the Arthus phenomenon primarily concern the cutis, whereas the heat-induced inflammation and the epidermal allergic challenge reaction concern mainly the epidermal tissue.

Although the previous experiments on the influence of antihistaminics upon the hyaluronidase reaction[1] suggest a relationship between skin inflammation and the liberation of hyaluronidase, further experiments are necessary to determine whether hyaluronidase plays an active role during these inflammations or is only a concomitant factor subsequent to an inflammatory process. Another unsolved problem is the source of the liberated enzyme.

Conclusion. The content of polysaccharide-depolymerizing substances (probably chiefly hyaluronidase) during allergic dermatitis (paraphenylenediamine) and heat-induced inflammation on guinea pig skin is markedly increased.

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Effect of Intravenous Cytochrome C on Capacity for Effort Without Pain in Angina of Effort.

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Within the past few years, reports have appeared on the effect of Cytochrome C* on tissue anoxia. From the clinical point of view, Proger and his co-workers have presented evidence that myocardial anoxia can be relieved by intravenous injection of Cytochrome C, as demonstrated by the prevention or reversal of the electrocardiographic changes induced by breathing 10% oxygen.[1] It is their contention that, under such circumstances, Cytochrome C enhances the uptake of oxygen by the myocardium.

Another method of testing the effect of a drug on myocardial anoxia is to determine the capacity for effort without pain in patients who have angina pectoris brought on by effort. Since this method depends on a subjective end-point, it is necessary to use careful controls and a completely "blind" technique, as described by Gold and his co-workers, in order to eliminate variable psychosomatic effects.[2] This method has been used by us in an evaluation of the effect of intravenous aminophylline on angina of effort, and it proved to be a satisfactory investigative technique.[3]

The subjects of the study were all selected from the active attendance of the Cardiac Clinic. All of them had either arteriosclerotic or hypertensive heart disease or a combination of both, and all presented the symptom of chest pain on effort. No patients were included in this study who had spontaneous chest pain unrelated to effort, excitement, heavy meals, or very cold weather. Also excluded were patients who showed any clinical evidence of congestive failure.

The method consisted of determining the subject's capacity for effort without pain by having him walk back and forth over a

* Cytochrome C used in this study was furnished by Wyeth, Inc., Philadelphia, Pa.

1 Proger, S., and Dekaneas, D., *J. Pediat.*, 1946, **29**, 729.

2 Gold, H., Kwit, N. T., and Otto, H., *J. A. M. A.*, 1937, **108**, 2175.

3 Bakst, H., Kissin, M., Leibowitz, S., and Rinzler, S., *Am. Heart J.*, in press.