Presence of a Necrosin-like Fraction in the Extracts of Injured Tissue.* (18194)

VALY MENKIN

From the Agnes Barr Chase Foundation for Cancer Research, Temple University School of Medicine, Philadelphia.

The pattern of injury in inflammation has been found to be referable to the euglobulin fraction as recovered particularly from acid exudates(1-3). This pattern can be modified by the chemistry of the irritating agent and by the anatomic location of the lesion. Nevertheless, the basic signs of injury seem to be due to the liberation by injured cells of this toxic euglobulin. These studies have received confirmation in the hands of Tanturi and of his collaborators(4). This material has been termed by the writer, necrosin(1,3). Recently it has been shown that a similar substance can be recovered from the injured tissues of the soft sea-shell clams, *Mya arenaria*(6). In the case of these invertebrates, the toxic material was also found in the euglobulin fraction of the crushed tissues, and similarly to the necrosin of vertebrate exudate, it was thermolabile. Thus, it would seem that necrosin is a substance released by injured cells throughout the animal kingdom. To verify this point further, various visceral organs of dogs were crushed. The extracts obtained were fractionated in an endeavor to determine whether the euglobulin fraction likewise contained a toxic substance not found either in the pseudoglobulin or albumin fractions of these extracts. Such findings would permit greater latitude of generalization on the liberation of necrosin whenever cells are injured. Smith and Smith have confirmed the presence of necrosin in canine exudates(5). In addition, they have pointed out the presence of a (closely) similar toxic material in menstrual blood. This is not wholly surprising for such material contains disintegrated cellular products from the endometrium combined with elements from the blood. In this connection the writer has expressed the point of view in an earlier communication that menstrual blood is a sort of modified physiologic exudative material(7).

Several dogs were killed and one of the kidneys, a sample of liver tissue, and of spleen were removed. The tissues were weighted. A volume of sterile saline equal to about 5 times the weight of the sample of tissue to be studied was added. The tissue was then thoroughly ground in a mortar to which sterile sand was also added. The saline extract was then centrifuged. The supernatant was treated with $(NH_4)_2SO_4$ at 1/3 saturation to obtain the euglobulin precipitate fraction. The euglobulin precipitate was separated from the pseudoglobulin-albumin fractions by centrifugation. These 2 fractions were then dialyzed in cellophane tubes against running tap water. After about 24 hours, the fractions were found free of sulfate ions. The respective fractions were then injected in amounts of 0.5 cc into the skin of normal rabbits. The final readings were made 18 to 22 hours later. The severity of the induced lesions was recorded. The data are assembled in Table I.

It is clear from this table that in each case the euglobulin fraction of kidney, liver, and spleen caused a pronounced local inflammatory reaction. The pseudoglobulin-albumin fraction, on the other hand, produced negligible effects. The specificity of the canine proteins hardly enters into the picture, for when the pseudoglobulin-albumin fraction of dog tissues were used on rabbits the reactions were minimal. Necrosin has been found to

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TABLE I. Presence of an Injury Factor in Euglobulin Fraction of Various Organ Extracts.*

Rabbit No.	Euglobulin of kidney	Euglobulin of liver	Euglobulin of spleen	Pseudoglobulin-albumin of kidney	Pseudoglobulin-albumin of liver	Pseudoglobulin-albumin of spleen
4-83	2+	2+	2+	—	—	—
4-94	2+ to 3+	2+ to 3+	2+ to 3+	t to +	t to +	t to +
5-51	3+	4+	3+	+	t	t to +
1-51	4+	2+	4+	t	t	t
1-52	4+	4+	5+	t	t	t
1-54	3+	3+	3+	t	t	t
			Effect of heating the extracts from 80°C to boiling			
R-155	t	t	t	—	—	—
R-160	t	0	+	—	—	—

* The number of plus signs indicates the severity of the induced cutaneous lesion by the extract.

t = trace.

To prevent bacterial contamination, the various chemical fractions were prepared in sterile glassware and injections were made under sterile precautions. [Furthermore, both euglobulin and pseudoglobulin-albumin fractions were prepared under precisely similar conditions and at the same time, thus rendering bacterial contamination of one fraction more than that of another somewhat unlikely.]

be thermolabile(1,6). In a similar fashion the euglobulin fraction of kidney, liver, and spleen were inactivated by heating between 80°C to almost boiling. This heating procedure caused coagulation of the proteins present in the extract, leaving a watery-like supernatant phase which in all cases was utilized for the injection purpose. The gross picture of the cutaneous lesion induced by the euglobulin fraction revealed severe central necrosis surrounded by an erythematous zone. The pseudoglobulin-albumin fractions manifested a mere papule with mild redness at the point of inoculation. The microscopic sec-

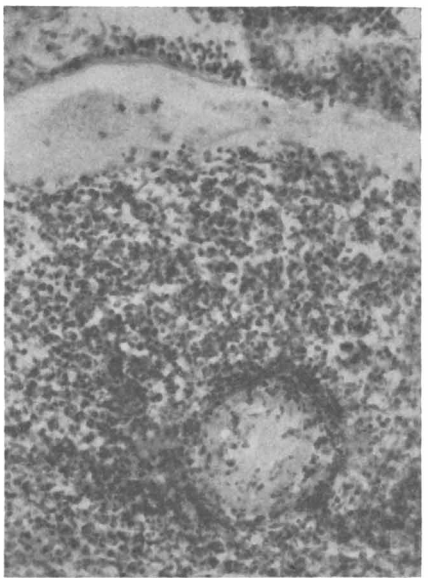

FIG. 1.
Euglobulin of kidney—22 hr and a half lesion—Abscess-like lesion with tremendous leukocytic infiltration in the skin tissue of the abdomen of a rabbit. Note the fibrinous strands in a venule and the endoarteritis of an arteriole. ×210.

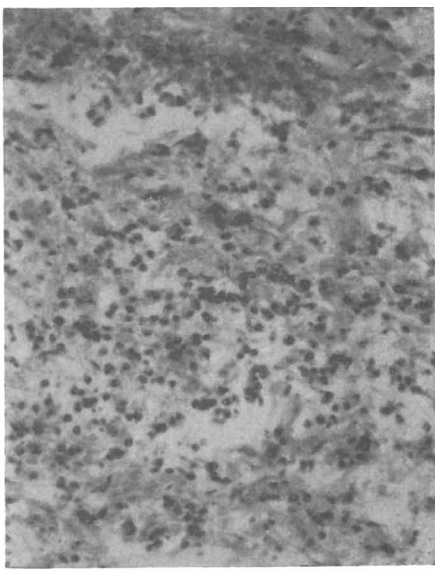

FIG. 2.
Pseudoglobulin-albumin fraction of above kidney extract. 0.5 cc inj. into dermis of abdomen of a rabbit induced a relatively mild reaction with some leukocytic infiltration. Contrast this picture with the dense leukocytic infiltration caused by the euglobulin fractions (*cf.* Fig. 1). ×210.

tions substantiated the gross picture (Fig. 1). This photograph shows the dense leukocytic infiltration by the euglobulin fraction of the extracts. In some instances thrombosed lymphatics and swollen bundles of collagen were observed. In contrast, the pseudoglobulin-albumin fractions induced on microscopic study a relatively mild reaction (Fig. 2). The thermolability and the severely injurious property of the euglobulin fraction of the several organ extracts studied suggest strongly that here again we are dealing with a substance similar to the necrosin recovered from inflammatory exudate. Inflammation is a manifestation of severe cell injury. Crushing tissues mechanically performs the same operation as an irritant. This yields a similar substance from cells that have been severely injured.

Conclusions. The crushing and mashing of cells from dog kidney, liver, and spleen yields by $(NH_4)_2SO_4$ fractionation a necrotizing substance located in the euglobulin fraction of the saline extracts. The pseudoglobulin-albumin fraction of the tissue extracts do not contain this toxic substance. This substance is thermolabile. It does not seem to differ in its biological property of inducing severe cell injury from necrosin as obtained especially from acid inflammatory canine or human exudates(1,2).

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Pantothenic Acid Deficiency and Conjugation Reactions. III. Inhibitory Effect of ω-Methylpantothenic Acid.* (18195)

MAURICE E. SHILS. (Introduced by C. G. King)
(With the technical assistance of Sarah A. Chester)
From the School of Public Health, Columbia University, N. Y.

The decreased acetylation of aromatic amines by the pantothenic acid deficient rat was reported(1,2) following the discovery that pantothenic acid is a constituent of coenzyme A(3). As part of our studies of factors influencing the acetylating ability of deficient rats, we report here on some of the effects of N(α,γ-dihydroxy-β,β-dimethylvaleryl)-β-alanine, termed ω-methylpantothenic acid† by Drell and Dunn(4). Omega methylpantothenic acid was first tested by Nease (5) and reported to show no growth-promoting activity for *Lactobacillus casei.* Drell and Dunn(4,6) noted that it inhibited the growth of a number of lactic acid bacteria. More recently, Drell and Dunn(7) have demonstrated reversible inhibition of growth with mice using this analog. Inhibition of growth of rats by the analog was prevented by simultaneous administration of pantothenic acid (8). Before the latter reports were available we decided to test this compound for its possible effects on mammals with particular reference to its influence on the acetylation of sulfanilamide by pantothenic acid deficient

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† We are indebted to Dr. Max S. Dunn, Dept. of Chemistry, University of California at Los Angeles for the sodium salt of ω-methylpantothenic acid. According to Dr. Dunn (personal communication) sample 1 contained 3% of impurities and sample 2 contained 7%, presumably the lactone portion.

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