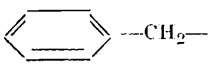
TABLE II.
Penicillin in Cerebrospinal Fluid Two Hours After Initial Intravenous Injection of 500,000 Units.

Patient	Age, yrs	Color	Sex	Penicillin G*	Penicillin BT†
BC	57	B	F	.045	.0
MW	35	W	F	.0	.0
RR	35	B	M	.031	.0
JS	43	B	M	.045	.0
JO	52	W	M	.0	.045
McL	38	W	M	.0	.0
IJ	35	B	M	.023	.022
JM	44	W	M	.031	.0
HZ	63	W	M	.045	.0
MK	41	W	F	.090	.022
NW	61	W	M	.045	.022
			Avg	.032	.010

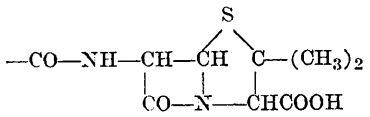
* Sodium penicillin G in aqueous solution.
† n-butylthiomethyl penicillin in aqueous solution.
‡ Oxford units of penicillin per cc of cerebrospinal fluid.
§ Any penicillin concentration less than 0.022 unit per cc has been regarded as zero.

Formula of R



Benzyl or G

Penicillin Nucleus



$CH_3—CH_2—CH_2—CH_2—S—CH_2—$
n-Butylthiomethyl or BT

Structural comparison of the two penicillins employed in this study.

intravenous injection of 500,000 units in 11 patients. From the work here reported it appears that BT penicillin does not diffuse into the cerebrospinal fluid as readily as benzyl penicillin and plasma concentrations resulting from its use are essentially the same as those following the use of benzyl penicillin.

The authors wish to express their indebtedness to Doctors Harvey Bartle, Robert Bookhammer, Raphael Durante, Herbert Freed, and Anthony Frignito for their kindness in allowing us to study patients on their psychiatric services at the Philadelphia General Hospital; to Miss Barbara V. Prey for penicillin assays here reported, and to Mr. Joseph L. Ciminera for the statistical analysis of data.

16756

Effect of Necrosin on Spontaneous Tumors in Mice.

VALY MENKIN.

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The pattern of injury with inflammation has recently been found by the writer to be referable to the liberation of a toxic substance by injured cells.[1] This substance is located in or at least it is associated with the euglobulin fraction of exudates of dogs and of man. It has been termed necrosin. Recent as yet

* Aided (in part) by a grant from the National Advisory Cancer Council.

[1] Menkin, Valy, *Arch. Path.*, 1943, **36**, 269.

unpublished studies have demonstrated a similar substance in injured tissues of invertebrates. The presence of necrosin in canine exudates has been confirmed by Smith and Smith[2] and by Tanturi.[3] Ludford has shown that trypan blue tends to accumulate in some of the cells of the stroma of tumors.[4] Duran-Reynals has demonstrated that the dye T-1824 and other poorly diffusable dyes localize from the circulating blood in spontaneous tumors of mice, rabbits, and of chickens.[5] He also showed that the sera of various animals localize from the blood into transplantable and spontaneous tumors of mice.[5] Duran-Reynals interpreted his findings in the light of a greater permeability on the part of the capillaries of a tumor than under normal circumstances.[5]

In view of these findings it was thought it would be of interest to see whether necrosin, being associated with a protein fraction, would also localize, when injected subcutaneously and at a distance from the tumor, into the tumor substance itself. The effect of the localization of such a toxic substance into experimental tumors would doubtless also yield valuable information. The experiments to be presently reported represent the first of a series of such observations.

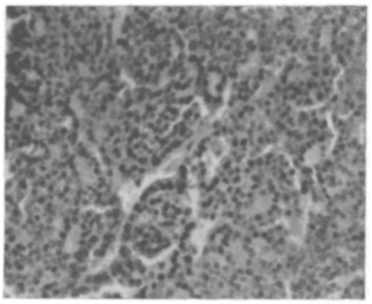

FIG. 1.
An area of the spontaneous tumor which occurs in a strain of Swiss mice. Note the general cellular anaplasia with few areas of adenomatous tendency. ×225.

Methods and materials. In a strain of Swiss mice a type of spontaneous tumors, presumably mammary in character, occurs in females.[6] The tumors may appear almost

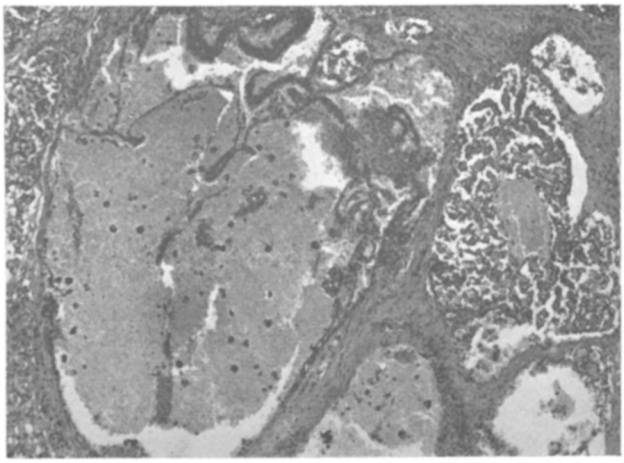

FIG. 2.
In such tumor one frequently encounters large vascular sinusoids with marked congestion. These spaces, however, are discrete and appear unruptured. ×100.

2 Smith, O. W., and Smith, G. V., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 116.

3 Tanturi, C. A., Canefa, J. F., and Banfi, R. F., *Revista "Medicina,"* 1945, **6**, 143.

4 Ludford, R. J., *Proc. Roy. Soc.*, ser. B., 1929, **104**, 493.

5 Duran-Reynals, F., *Am. J. Cancer*, 1939, **35**, 98.

6 Dunn, Thelma B., Mammary Tumors in Mice by Members of the Staff of the National Cancer Institute, American Association for the Advancement of Science, Smithsonian Institution Building, Washington 25, D.C., 1945, pp. 13-38.

TABLE I.
Effect of Necrosin on Spontaneous Tumor in Swiss Mice (3 mice were C_3H; 3 mice belonged to dba strain).

No. of mice	No. of mice with frank hemorrhagic necrosis in tumor or in lung metastasis, or necrotic material in tumor	% of mice with frank necrosis in tumor, in metastases, or in both
73 mice inj. with necrosin (1 to 8 inj. subcut.; 0.2 μg to 1748 μg per inj.)	67 (including the C_3H and dba mice)	91.8
31 non-inj. mice*	7	22.6

Chemical analysis of one sample of necrosin utilized yielded the following data:

	%
Carbon	41.24
Hydrogen	6.32
Nitrogen	11.58
Sulfur	1.56

* At times very old non-injected mice with very vascular spontaneous tumors display at necropsy variable amounts of necrosis in the tumor, with occasional necrosis likewise occurring in some of the metastases in the lungs. This suggests that canine necrosin administration hastens the time of necrosis to occur in the neoplasms studied.

anywhere in the subcutaneous tissue, but they are more prevalent in the ventral surface of the animal. The tumors are occasionally found in the axilla, in the shoulder, and even in the dorsal surface of the neck. Sometimes these tumors appear in the lower portion of the pelvis. The tumor may show areas of anaplasia interspersed with other areas of adenomatous tendency (Fig. 1). The tumors are characterized by their great vascularity. The presence of large vascular sinusoids is frequently seen (Fig. 2). The tumor is essentially carcinomatous in appearance.

Swiss mice with such tumors, varying in their size, were injected subcutaneously, at a distance from the tumor, with a colloidal solution of necrosin. The amount of necrosin varied from the equivalent of 1748 μg by dry weight to 0.2 μg. The injections, however, were always done in the fluid state in amounts ranging from .05 cc to 0.2 cc. The injections were repeated at intervals of 24 hours or longer. A study of the effect on the tumors was made at 1 hour, 4 hours, 6 hours and 7 hours after the injection of necrosin, and also at the death of the animal. Death occurred usually, though not always, several days after the last injection of necrosin.

Results. The results on over 100 mice are assembled in Table I.† In 73 mice injected with necrosin 91.8% manifested various degrees of necrosis in the tumor substance. Frequently the necrosis was hemorrhagic in character (Fig. 3). The tumor substance became in large part replaced by the free oozing of dark bloody-like material. Only here and there tufts of the original neoplastic tissue remained. Sometimes, although perhaps not as frequently, a mucoid-like yellowish fluid was found instead of the usually relatively firm tumor substance. This was taken to be a type of necrosis. Rarely a caseous-like central necrosis was also apparent in the tumor substance.

In all this work the effect on the metastases was of real interest and perhaps even of greater significance than the effect on the tumor proper. The tumor most frequently metastasizes to lung where the metastases appear, in the gross, as translucent-like foci. Microscopically these foci appear as discrete carcinomatous areas (Fig. 4). The injection of necrosin is accompanied not only by necrosis in the tumor proper, but also by hemorrhagic necrosis in many of the metastatic foci in lung tissue (Fig. 5). The gross appearance of the tumor following necrosin administration is seen in Fig. 6. Microscopically the appearance of the tumor in a treated mouse is, as stated above, exemplified in Fig.

† Since the completion of this manuscript further studies on this subject have been continued. This now involves well over 150 mice in 20 independent series of experiments.

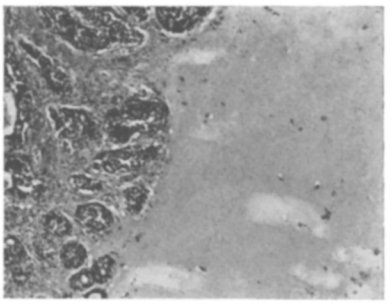

FIG. 3.
This animal received 3 subcutaneous injections of 0.5 cc of necrosin diluted with saline 1:5. Over 2 weeks later the animal died. A large amount of hemorrhagic fluid replaced in large part the tumor substance. ×75.

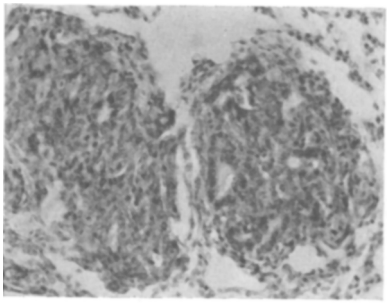

FIG. 4.
The spontaneous tumor of Swiss mice frequently metastasizes to lung. The illustration represents such metastasis. They appear as discrete carcinomatous areas. ×150.

3. The appearance, in the gross, of the tumor in a mouse treated subcutaneously with another globulin such as the leukocytosis-promoting factor of exudates of dogs[7] appears in Fig. 7. In brief, the leukocytosis-promoting factor of exudates seems to have no significant effect on the character of the tumor.

Necrosin is found to induce some necrotizing effect on the tumor as early as one hour or so following the subcutaneous injection of the material, in turn administered at a distance from the tumor. An effect can be elicited by the injection of as little as 0.2 μg of necrosin. The euglobulin fraction of normal canine blood serum usually fails to induce any effect on the tumor or on its metastases. Since necrosin contains a slight amount of proteolytic enzyme,[8] an attempt was made to determine the effect of papain. The injection of this enzyme seems to induce no significant visible effect. These studies, however, are being continued with other proteolytic enzymes.‡ Digestion of necrosin for several hours with either crystalline trypsin or papain does not seem to affect significantly the end result on the tumor. Necrosin administered to Swiss mice with spontaneous tumors does not appear to influence the longevity of the treated animal when compared with untreated mice. There may be several factors involved for this lack of effect on the life span. These are at present under investigation. One definite fact is a concomitant severe injury to the liver. This is illustrated in Fig. 8. The injury may be in the form of severe hepatic vacuolation. Sometimes varying degree of cellular infiltration is encountered. The kidneys at times reveal evident injury. A similar effect of necrosin was described in an earlier communication to occur in dogs.[1] Methods of obviating injury to the liver by necrosin are also being studied.

Some degree of necrosis is found in only 22.6% of the tumors of untreated mice (Table I). In such animals, however, the metastatic lesions of the lungs are seldom involved. It is for this reason that a study of the effect of necrosin on the metastatic lesions in the lung may perhaps yield more significant information than its effect on the tumor proper. Only several mice were available which belonged to different strains. These

[7] Menkin, Valy, *The Lancet*, May 17, 1947, pp. 660-662.

[8] Menkin, Valy, *Am. J. Physiol.*, 1946, **147**, 379.

‡ Since the completion of this manuscript, preliminary studies by the repeated subcutaneous injections of 0.5 mg of crystalline trypsin (Armour) in saline into Swiss mice with spontaneous tumors have yielded hemorrhagic necrosis in only some of the metastatic foci of the lungs. This would perhaps suggest a possible explanation of the action of necrosin, *i.e.*, through its slight degree of proteolytic property. This phase of the work is being studied further.

§ These studies are also being done on what has been found to be Strain A mice with apparently the same effect. This will, however, form the subject of a future communication.

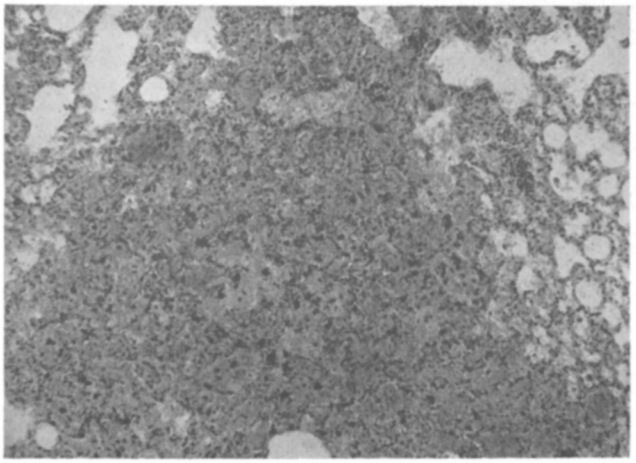

FIG. 5.
A metastatic focus in the lung of a Swiss mouse having received 3 subcutaneous injections of 0.5 cc of necrosin diluted with saline 1:5. Over 2 weeks later the animal succumbed. The metastatic area manifests considerable hemorrhagic necrosis. ×85.

FIG. 6.
Gross appearance of a spontaneous tumor in a Swiss mouse in turn injected subcutaneously twice with .05 cc of necrosin diluted with saline (1:5). The amount of necrosin was equivalent to 10 mg per injection. The animal was sacrificed one day following the second injection. Note the marked degree of hemorrhagic necrosis.

FIG. 7.
Gross appearance of a spontaneous tumor in a Swiss mouse following the subcutaneous injection of 0.1 cc of canine leukocytosis-promoting factor (α 1 and α 2 globulins of exudates). The mouse died 5 days after administration of the globulin material. There is no evidence of any necrosis in the tumor.

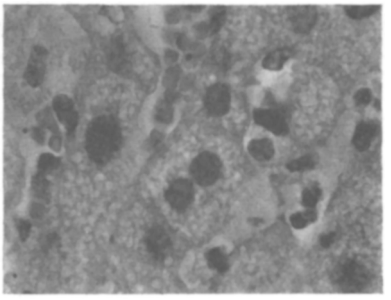

FIG. 8.
Appearance of the liver in a mouse injected subcutaneously twice with .05 cc of necrosin. The mouse succumbed 4 days following the second injection. Note the severe degree of injury to the liver in the form of marked vacuolation within the hepatic cells. ×560.

were C H and dba mice.§ Repeated subcutaneous injections of necrosin were[3] likewise followed by some degree of tumor necrosis with concomitant necrotizing effect on some of the lung metastasis. In these animals, however, many more injections of this toxic material were necessary, and the effect was generally not as pronounced as on the Swiss strain of mice. The extensive initial vascularity of the tumor has probably something to do with the ultimate effect of necrosin administration. Finally, in view of Shear's[9] recent work on bacterial polysaccharides necrosin was tested for the Molisch reaction, and it was found on a sample utilized to be negative. Culturing necrosin for bacteria in broth yielded negative results within eleven hours, and negative growth on agar slants within 24 hours. The necrosin samples employed ranged from fresh material (only several hours old) to material prepared over 2 years previously and preserved in a refrigerator. The effects obtained were identical. Finally, the subcutaneous injections of 0.2 cc of a slightly acid exudate (pH 6.9 to 7.0), in which presumably necrosin was present, induced hemorrhagic necrosis in the tumor and in some of the metastatic foci of Swiss mice.‖

Conclusions. Necrosin is a substance associated with the euglobulin fraction of usually acid inflammatory exudates. Its liberation by injured cells offers a reasonable explanation for the basic pattern of injury in inflammation.

The subcutaneous injections of canine necrosin, at a distance from the site of spontaneous tumors, in a strain of Swiss mice produce, after varying intervals, hemorrhagic or other types of necrosis in the tumor substance.

A necrotizing effect is also seen to occur in some of the metastatic lesions encountered in the lung.

Necrosin injections in these mice induce severe injury to the liver.

None of the effects on the tumor or on its metastasis are usually encountered with the use of the euglobulin fraction of canine blood serum, the leukocytosis-promoting fraction of canine exudates, or with one of the proteolytic enzymes adequately studied, namely papain.

9 Shear, M. J., *J. National Cancer Inst.*, 1944, **4**, 461.

‖ My thanks are due to Mrs. Ruth Lewin for technical aid in the course of this study.

16757

Effect of Adrenocorticotrophic Hormone on Anaphylaxis in the Guinea Pig.

JACQUES LEGER,* W. LEITH AND BRAM ROSE. (Introduced by J. S. L. Browne.)
From the McGill University Clinic, Royal Victoria Hospital, Montreal, Quebec.

Adrenal cortical hormones are known to cause dissolution of lymphoid tissue,[1,2] and it has recently been shown that in sensitized animals, the rate of lymphocyte disintegration is paralleled by an increase of circulating antibodies.[3-6] This phenomenon may be elici-

* Fellow National Research Council, Canada.

1 Dougherty, T. F., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 132.

2 Selye, H., *J. Clin. Endocrinol.*, 1946, **6**, 117.