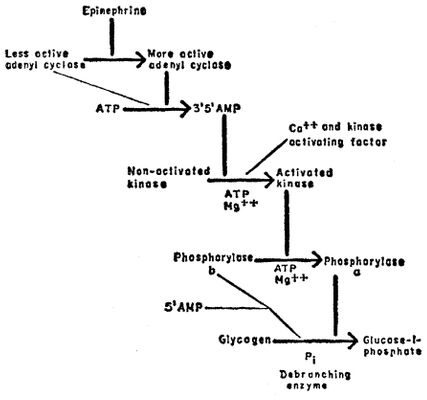

Fig. 1. Arrows indicate known chemical changes; lines connect chemical agents and the changes they are known to influence or catalyze. Abbreviations: ATP, adenosine triphosphate; 3'5'AMP, adenosine cyclic 3'5'-monophosphate; 5'AMP, adenosine 5'-monophosphate; P_i, inorganic phosphate.

the total is converted to 3'5'AMP. Other data (*6*), showing that $1.5 \times 10^{-5}M$ epinephrine and $1.5 \times 10^{-5}M$ 3'5'AMP produce almost exactly the same quantitative response in rabbit-liver slices in terms of glucose output and glycogen breakdown, also suggest that there is no rapid amplification of the hormonal signal at this stage in this tissue. However, by use of the pair of rat gastrocnemius muscles in place in the body, intracardiac injection of 0.55 μmole epinephrine per kilogram was found to increase the cyclic AMP content on one muscle within 1 minute by 0.54 to 1.13 μmole/kg beyond that of the other muscle, which was removed just before the injection (*8*). This represents 10- to 20-fold amplification, if one assumes an even distribution of epinephrine ($5.5 \times 10^{-8}M$) throughout the body during the whole time.

Using phosphorylase-*b* kinase extracted from rabbit muscle, Krebs, Graves, and Fischer (*9*) found increase in activity from 200 unit/ml in the absence of 3'5'AMP to 3100 unit/ml in the presence of $1 \times 10^{-4}M$ 3'5' AMP. If one assumes that the glycogen-synthesizing units of Illingworth and Cori (*10*) also indicate the catalytic activity of phosphorylase in glycogen breakdown, this may be calculated to represent formation of about 1600 μmole glucose-1-phosphate per minute in response to 1 μmole of 3'5'AMP.

One may estimate overall amplification in the phosphorylase system from the figures of Timms *et al.* (*11*): $5.4 \times 10^{-7}M$ epinephrine reduced the mean level of glycogen in guinea pig intestinal smooth muscle from 1.46 to 1.01 mg/g (wet wt) within 2 minutes. This reduction represents formation of glucose-1-phosphate at 2320 μmole/min per micromole of epinephrine. Using frog sartorius muscle at 20°C, Helmreich *et al.* (*12*) calculated the glycogen decrease in $5 \times 10^{-7}M$ epinephrine from the glucose-6-phosphate and lactate formed. The maximum rate of loss may be estimated, from their graph of loss versus time, at about 0.1 μmole of glucose ml^{-1} min^{-1}, which figure gives an amplification factor of 200. These figures are within the range to be expected from combination of the amplifications (given above) of the signals of the "first messenger" epinephrine and the "second messenger" 3'5'AMP. In view of the variety of "second messenger" functions that have been ascribed to 3'5'AMP, it is interesting that, at least in the phosphorylase system, this signal is amplified more than that of the "first messenger."

By analogy with visual excitation Wald (*3*) has suggested application of Poisson curves to the relation between log of concentration of activating agent and frequency of response in a cascade system. While the phosphorylase response, unlike visual excitation or blood clotting, is not usually considered in terms of an all-or-none phenomenon, one may note that the curves, obtained for the relation between log epinephrine concentration and extra 3'5'AMP formed by liver adenyl cyclase (*7*), are of a sigmoid shape that corresponds with the dose-response curves for many drugs (*13*).

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Mast Cells and Necrosis

Abstract. *Subcutaneous injections of normally well tolerated amounts of hypertonic NaCl or urea solutions produce extensive topical necroses in rats systemically treated with various mast-cell dischargers and mast-cell products. This response is considered to be closely related to mast-cell function, for it cannot be duplicated by systemic treatment with a variety of other agents.*

Two hundred and forty female Sprague-Dawley rats with an average body weight of 100 g (range, 90 to 110 g) were subdivided into 24 equal groups for two experiments. Half the animals received 2 ml of a hypertonic aqueous solution of NaCl (10 percent), the other half a solution of urea (20 percent), always subcutaneously on the back in the thoracolumbar region. One group of each series was not otherwise treated and served as controls; the other groups were treated with various mast-cell dischargers, mast-cell products, and severe stressor agents as indicated in Table 1.

In all instances the systemic treatment was applied just before the topical treatment. The animals of groups 9

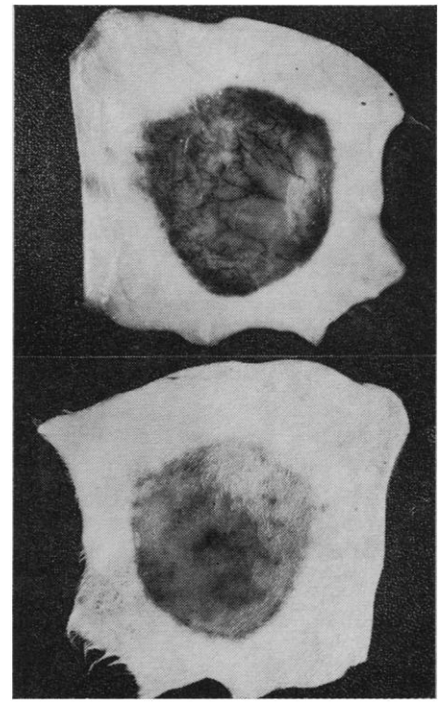

Fig. 1. Sharply delimited area of cutaneous necrosis (with thromboses in small veins and hemorrhages) at the site of topical treatment with hypertonic NaCl in a rat given compound 48/80 at a distance from this region. External (top) and internal (bottom) aspect of same skin flap.

and 10 were restrained for 24 hours before subcutaneous injection of the hypertonic solutions.

The injection sites of the hypertonic NaCl or urea solutions showed no evident sign of damage beyond transient edema in the control rats. The same was true of those treated with such severe stressors as just-sublethal doses of epinephrine or norepinephrine. Even spinal cord transection conducive to complete paralysis of the hind quarters or prolonged immobilization on a board caused only occasional small patches of cutaneous necrosis at the sites where hypertonic NaCl was applied. On the other hand, serotonin and histamine (two mast-cell products) as well as polymyxin, compound 48/80, dextran, and dextrin (all potent mast-cell dischargers) produced extensive topical necrosis, with thrombosis and hemorrhage in and around the necrotic area, within a few hours after injection (Fig. 1). On the following day, the surface layer of the affected skin region showed signs of total disintegration with exulceration. This response involved virtually the whole area treated with NaCl or urea, respectively; only in the two groups given histamine was this reaction less pronounced. Heparin, another mast-cell product, was totally ineffective in this respect although in some animals it caused minor hemorrhages at the sites where the hypertonic solutions were applied.

Table 1. Effect of various systemic treatments upon the ability of hypertonic NaCl and urea solutions to elicit topical necrosis at subcutaneous injection sites.

Groups	Systemic treatment*	Necrosis at site of topical treatment with	
		NaCl	Urea
1,2	None	0	0
3,4	Epinephrine bitartrate (1 mg s.c.)	0	0
5,6	Norepinephrine bitartrate (1 mg s.c.)	0	0
7,8	Spinal cord transection (by thermocautery above 1st lumbar vertebra)	+	0
9,10	Restraint (24 hr on a board just prior to topical treatment)	+	0
11,12	Heparin (1 mg i.v.)	0	0
13,14	Serotonin creatinine sulfate (2 mg s.c.)	+++	+++
15,16	Histamine phosphate (30 mg i.v.)	++	++
17,18	Polymyxin-B sulfate (2 mg s.c.)	+++	+++
19,20	Compound 48/80 (1 mg s.c.)	+++	+++
21,22	Dextran (60 mg i.v.)	+++	+++
23,24	Dextrin (250 mg i.v.)	+++	+++

* All subcutaneous (s.c.) injections given in 0.2 ml water on belly, all intravenous (i.v.) injections in 1 ml water into jugular vein with the animal under light ether anesthesia.

It may be significant that the above mentioned mast-cell dischargers and mast-cell products (again with the notable exception of heparin and unlike nonspecific stressors) are also potent elicitors of the tissue calcification in the phenomenon of "mastocalcergy" (*1*). A recent review of the literature on mast cells lists more than 30 theories concerning their function (*2*). It would be premature to speculate upon the intimate mechanism of the phenomenon just described, but it appears justified to conclude that close relationships exist between mast cells and tissue resistance to necrotizing agents.

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Actinomycete: Isolation and Identification of Agent Responsible for Musty Odors

Abstract. *A compound produced by certain actinomycete cultures is responsible for a persistent musty odor. It has been isolated in high purity and identified by chemical and spectroscopic properties. Possible structures are discussed.*

The musty odor of old potato bins, damp cellars, aged straw piles, stagnant ponds, and so forth, may be attributed in large part to the growth of actinomycetes. Although their normal habitat is soil, these organisms grow abundantly on most types of biological materials. They have in some instances adapted themselves to aquatic environments and have long been known to render potable water supplies nonpalatable by imparting musty and earthy tastes and odors (*1*). Simple amines, short chained aldehydes, and saturated fatty acids have been found in these organisms (*2*). Romano and Safferman (*3*) grew *Streptomyces griseoluteus* and obtained an unidentified odoriferous material which gave a characteristic odor even at a dilution of one to a million. Gaines and Collins (*4*) found that *S. odorifer* produced several simple compounds such as acetic acid, acetaldehyde, ethyl alcohol, isobutyl alcohol, isobutyl acetate, and ammonia. We have cultured certain types of actinomycetes and isolated a unique chemical substance which we believe to be responsible for a very persistent musty odor.

In the spring and summer of 1961 the water of the Cedar River in Iowa had a severe musty taste and odor. At that time Morris (*5*) isolated two genera of actinomycetes (*Micromonospora* and *Streptomyces*) from the river. The predominating *Streptomyces* were later cultured in mass quantities, and Morris *et al.* (*6*) isolated a chemically neutral fraction which contained a musty-odor compound. Briefly, their method consisted of an initial steam distillation that was followed by solvent extraction of the distillates. The neutral fraction from gas chromatography consisted of seven detectable components (Fig. 1). By peak-area analysis, one peak represented a concentration of more than 95 percent of the sample. Because of the low yield of the neutral fraction (approximately 2 g per 500 liters of culture) and the capacity of this material to produce musty odors in extremely high dilutions, the component represented by this peak was considered to be the musty compound. Further attempts at purification of this fraction by microdistillation (1 cm path) at 0.3-mm pressure did not significantly remove all of the impurities represented by the minor peaks in Fig. 1. However, this distillation method did produce approximately 500 mg of a clear, colorless liquid having a specific gravity of 0.987 and an index of refraction (N_D^{25}) of 1.5022, both at 25°C. The N_D^{25} of the neutral fraction before vacuum distillation was 1.5001. In view of this slight change in refractive index after vacuum distillation and the observance of no additional components by modified gas-chromatography procedures, we concluded that this colorless distillate