gether in the suitable habitats and are harvested indiscriminately by crowfoot brails dragged across the river bottom. The harvested shells are considered essential to the cultured pearl industry (*11*). Annual yields of high quality shells have ranged from 5,000 to 10,000 tons, but recently yields decreased because of overfishing, pollution, or siltation of the habitats.

Large quantities of shells are harvested and discarded because they have undesirable color or structural characteristics for the pearl industry. The discarded shells have desirable chemical qualities, *E. crassidens* being an example. The suggestion of using a biogenic calcareous substance as a raw material in manufacturing is not unusual (*12*), although production of a high quality chemical reagent from these shells would be unique. Current research (*11*) on the life histories and management of this valuable, renewable natural resource is indeed opportune.

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Epinephrine: Cascade Reactions and Glycogenolytic Effect

Abstract. *The concept of a cascade reaction may serve to indicate the underlying chemical similarity between several biological amplification processes, and as a basis for the formulation of units that emphasize amplification phenomena in reaction kinetics. This approach is discussed in relation to the glycogenolytic effect of epinephrine.*

Blood coagulation has been represented by Macfarlane (*1*) as a cascade reaction that can provide a series of amplifications of a weak initial stimulus; by Davie and Ratnoff (*2*), as a waterfall sequence. Wald (*3*) has drawn an analogy between blood coagulation and visual excitation and has suggested that amplification systems of this type may occur elsewhere in living systems.

Elevation of blood glucose by epinephrine is now known to be associated with a rather complex chain of events that connects the hormone and the glycogenolytic enzyme phosphorylase (*4*). The events divide into at least five stages, in four of which a chemical agent acts by influencing the rate of a reaction whose product is an agent that influences the stage immediately following. There is some justification for classification of these events as a cascade amplification system and for their arrangement in the form of Fig. 1. First, the same physiological necessity for amplifying a weak signal exists here as in blood coagulation or vision; and second, three of the individual stages are similar to the conversions of proenzyme to enzyme that occur in blood coagulation.

However, in the phosphorylase system there are additional complications: First, various agents, such as 5′-adenosine monophosphate (5′AMP), glucose-6-phosphate, and adenosine triphosphate (ATP), that are not produced specifically in response to the original hormonal stimulus also act by influencing one or more of the enzymic stages; they appear to act for control systems that are distinct from or complementary to the hormones. At the phosphorylase stage, for instance, 5′AMP acts by stimulating the activity of phosphorylase *b* (*5*), thus producing the same effect as epinephrine but at the same time decreasing the amplification of a hormonal signal to the extent that it may have no effect on the production of glucose from glycogen; calcium and a kinase-activating factor act similarly at the kinase stage (*4*). Second, 3′5′AMP appears to act as a 'second messenger' for various hormones other than epinephrine, as an agent to accomplish either the same end (as with glucagon and the elevation of blood sugar) or different ends [as with vasopressin (*6*)]. However, if we consider the glycogenolytic response to epinephrine as an isolated (and well documented) system, it appears that the cascade manner of arrangement may be acceptable as an indication of the chemical similarity between this and several other biological amplification systems.

The question then arises of an appropriate unit to be used in describing amplification at each stage. The number of molecules of product obtained in unit time, in response to one molecule of the agent influencing the enzymic reaction, appears to be one way of expressing the information required. Although enzyme kinetics has been concerned with the rate of change of substrate or product under various conditions, this particular quantitative relation has received little attention. At present, amplifications can be expressed in this way only in terms of epinephrine, 3′5′AMP, and glucose-1-phosphate, each of which has a well-determined molecular weight. The range of numerical values for this expression, obtained with the individual stages in the postulated chain of events and compared with the values obtained for the overall response, in terms of micromoles of final product per micromole of hormone, may provide evidence on the validity of the theoretical scheme.

Although reported experiments were not designed for this type of approach, they yield some relevant data. Numerical values for this expression would be expected to vary somewhat with the ratio of quantity of agent to quantity of enzyme; this variation has been demonstrated with particulate preparations of adenyl cyclase from dog myocardium by Murad *et al.* (*7*). From their figures one may calculate that with $4 \times 10^{-3}M$ ATP and $1 \times 10^{-5}M$ epinephrine about 1/10 molecule of extra 3′5′AMP was formed in 12 minutes at 30°C in response to 1 molecule of epinephrine. At the lowest concentration of epinephrine shown on their graph (about $6 \times 10^{-7}M$) about ⅓ molecule of 3′5′AMP was formed in 12 minutes in response to 1 molecule of epinephrine. In view of other, more general requirements of the tissues for ATP, it may be physiologically significant that only a small proportion of

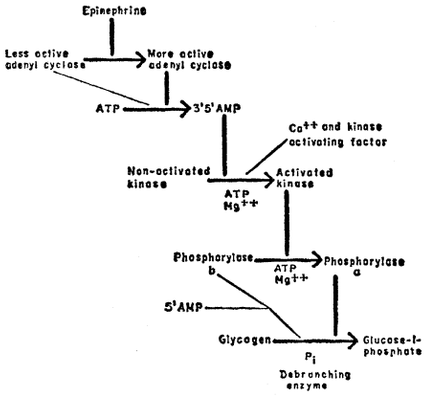

Fig. 1. Arrows indicate known chemical changes; lines connect chemical agents and the changes they are known to influence or catalyze. Abbreviations: ATP, adenosine triphosphate; 3'5'AMP, adenosine cyclic 3'5'-monophosphate; 5'AMP, adenosine 5'-monophosphate; P_i, inorganic phosphate.

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

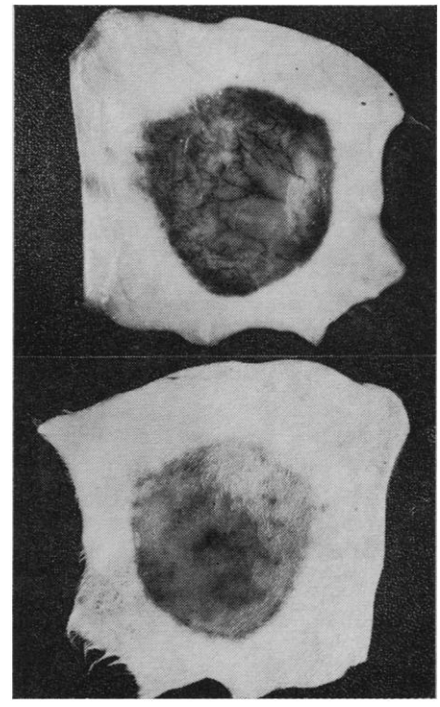

Fig. 1. Sharply delimited area of cutaneous necrosis (with thromboses in small veins and hemorrhages) at the site of topical treatment with hypertonic NaCl in a rat given compound 48/80 at a distance from this region. External (top) and internal (bottom) aspect of same skin flap.