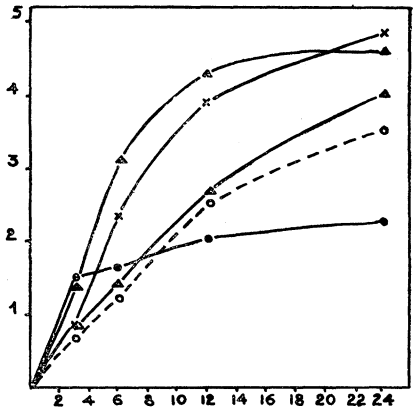

Fig. 1. The effect of coumarin in various concentrations on the elongation of segments of sunflower hypocotyl. Ordinate, increase in length, in millimeters; abscissa, time, in hours. (△) Coumarin, 10 ppm; (×) coumarin, 100 ppm; (▲) coumarin, 500 ppm; (●) coumarin, 1000 ppm; (○) water.

straight-growth test or the split pea test (*4*), though in low concentrations ($10^{-5}M$ to $10^{-3}M$) it acts synergistically with indoleacetic acid. In the course of an investigation of the latter effect in sunflower hypocotyls I have observed that coumarin is itself a strong promoter of elongation.

The experiments described in this report were carried out on *Helianthus*, variety Pole Star. Seedlings were grown in vermiculite at 26°C in the dark. One-centimeter sections of the hypocotyl, taken about 1 cm below the cotyledonary node of 6-day-old plants, were placed in the solutions to be tested in red light. The subsequent increase in length of the sections was determined under a binocular dissecting microscope with a micrometer scale.

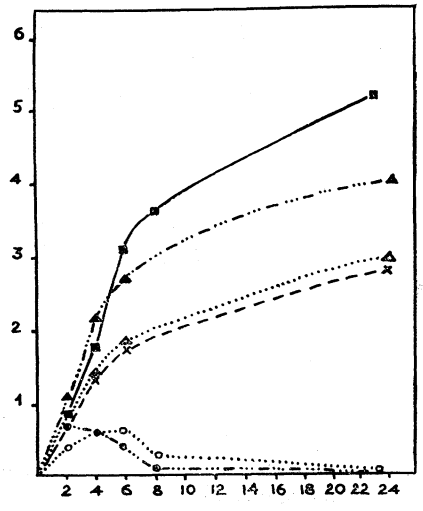

Fig. 2. The effect of coumarin and of 4-hydroxycoumarin on the elongation of segments of sunflower hypocotyl. Ordinate, increase in length, in millimeters; abscissa, time, in hours. (■) Coumarin, 250 ppm; (○) 4-hydroxycoumarin, 250 ppm; (△) 4-hydroxycoumarin, 50 ppm; (●) coumarin, 250 ppm, plus 4-hydroxycoumarin, 250 ppm– (▲) coumarin, 250 ppm, plus 4-hydroxycoumarin, 50 ppm; (×) water.

It was found, when a comparison was made with controls placed in water, that coumarin very markedly stimulated elongation of the sections. Measurements made after 24 hours indicated that the magnitude of the stimulation was related to the concentration of coumarin, the optimum curve resembling that for stimulation induced by auxins. Maximum stimulation was produced by a concentration of coumarin of about 250 parts per million. Measurements made after shorter time intervals, however, revealed that even the supraoptimal concentrations of coumarin strongly stimulated growth during the first few hours; there was then a decline in rate of elongation (see Fig. 1). The sharpness of this decline increased with increase in coumarin concentration.

Previous workers (*2, 3*) have investigated the relation between the structure and the activity of coumarin and its derivatives. Usually, alteration of the coumarin molecule caused a decrease in the effect on germination and root growth. In the investigation reported here, the effect of substitution was found to be greatest in positions 3 and 4, adjacent to the unsaturated bond of the lactone ring. As measured after 24 hours, the increase in length of segments in 250-ppm solutions of coumarin, 4-hydroxycoumarin, 3-chlorocoumarin and 3-methylcoumarin was 295, 0, 120, and 130 percent, respectively (relative to 100 percent in water). Application of 4-hydroxycoumarin in concentrations which did not inhibit elongation partly abolished the stimulating effect of coumarin when the two compounds were applied together (Fig. 2). The effect of substitution demonstrated here for hypocotyl elongation is considerably more marked than that reported for seed germination (*2*) and root growth (*3*).

The growth-promoting effect of coumarin is not confined to *Helianthus* hypocotyls. It has also been observed in preliminary experiments with *Avena* coleoptiles (see *5*), *Pisum* epicotyls, and *Phaseolus* hypocotyls. This activity, when considered in conjunction with the fact that coumarin in the concentrations usually applied inhibits root growth but at very low concentrations may stimulate it (*6*), suggests that this substance should be regarded as a naturally occurring growth regulator (*7*).

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7. The work described in this report was done in connection with a thesis for the M.S. degree at the Hebrew University, Jerusalem. I should like to thank Dr. A. M. Mayer and Dr. A. Poljakoff-Mayber for their interest and guidance.

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Antidromic Cortical Response to Stimulation of Isolated Pyramidal Tract

Abstract. Direct electrical excitation of the intact medullary pyramids evokes a complex cortical response. When the pyramidal tract was dissected away from the bulb and stimulated in isolation, the antidromic cortical response consisted of a simple, positive potential, regardless of the stimulus parameters. This finding necessitates a reinterpretation of previous results obtained by stimulation of the intact pyramids.

Stimulation of the medullary pyramids has been widely used to study the behavior of various parts of Betz cells (*1*)—for example, apical dendrites, recurrent collaterals and others—and to map the origin of the corticospinal tract. Studies purporting to demonstrate ascending fibers in the medullary pyramids have proved to be in error because of the spread of current to adjacent ascending fibers (*2*). It appears that several other results obtained by antidromic pyramidal stimulation need to be reviewed in the light of the following experiments (*3*).

The bulbar pyramids and the anterior cerebral hemispheres were exposed in cats anesthetized with α-chloralose (35 mg/kg, intraperitoneal) and paralyzed with decamethonium bromide (1 mg/hr, intravenous). The preparation was placed supine, and bipolar silver-wire electrodes were placed on one exposed pyramid. Monopolar surface recordings were made from the ipsilateral pericruciate cortex. Stimulation of the intact pyramid yielded the cortical potential shown in Fig. 1*A*. The positive (downward) wave *a* (latency, 0.4 to 0.6 msec; duration, 1 msec) had the lowest threshold, faithfully followed repetitive shocks

in excess of 200 per second, and survived longest following death by asphyxiation. This wave is due to antidromic conduction in Betz cell axons. Wave *b* (latency, 1.5 msec; duration, 1–2 msec) was usually monophasically positive and had a higher threshold than wave *a*. Wave *b* was unaltered at stimulus rates up to 20 to 30 per second but decreased at higher rates and failed completely at 80 to 100 shocks per second. This component was also attenuated by prior shocks to the contralateral forepaw. The interpretation of *b* is still uncertain. Components *c* and *d* were highly variable, wave *c* (latency, 3 to 5 msec; duration, 4 to 5 msec) being sometimes positive and sometimes positive-negative in configuration, and *d*, which immediately followed *c*, being similar to the primary cortical response following stimulation of the contralateral forepaw. Waves *c* and *d* both interacted strongly with the primary cortical response, the time-course of the interaction being approximately that of a primary cortical response similarly conditioned. Increasing the intensity or duration, or both, of the stimulus recruited the components sequentially from *a* to *d*; however, *b*, *c*, and *d* differed only slightly in threshold. Gradually increasing the frequency of repetitive stimulation sequentially eliminated the components from *d* to *b*, leaving *a* unaltered.

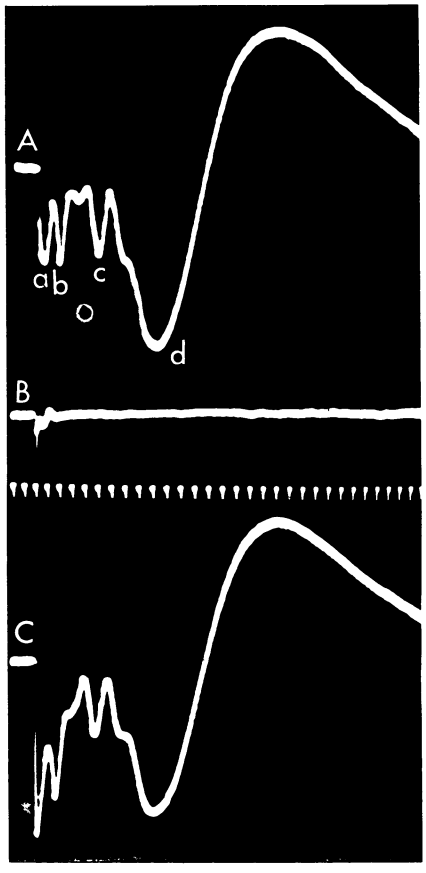

Fig. 1. (*A*) Response from lateral tip of cruciate fissure following stimulation of intact medullary pyramid. (*B*) Response from same point following stimulation of isolated pyramidal strand; *a* wave is 25 μv peak-to-peak. This record shows the greatest negativity in the *a* wave that was ever observed and is among the smallest isolated responses observed. (*C*) Response after contact of strand with brain stem. The slow component of the shock artifact is larger in *C* than in *A*. Time, 1 msec. Shocks were 50 v square pulses of 0.05-msec duration, led through an isolation transformer.

After these observations, the pia mater was dissected away from the ventral medullary surface. A section of pyramidal tract approximately 3 mm long and less than 1 mm in diameter was cut transversely at its caudal end. This strand was then lifted onto bipolar electrodes, free from the underlying bulbar tissue. Such isolation reduced to negligible proportions any spread of stimulating current to structures outside the strand. Stimulation of this isolated strand produced the response shown in Fig. 1*B*. This response consisted of a positive potential which was identical in properties to component *a*, except that the threshold was slightly elevated. This positive wave was sometimes followed by a brief (0.5 msec) negative wave and a prolonged positive wave, both of exceedingly small amplitude. The amplitude of the *a* wave (20 to 120 μv) varied from one preparation to another according to the amount of pyramid included in the strand. That loss of viability of the strand was not responsible for the absence of *b*, *c* and *d* was indicated by the fact that the strand conducted the direct and indirect pyramidal discharges from cortical stimulation (*4*) and the reflex corticofugal discharge (*5*) from stimulation of the contralateral forepaw. Increased strength (up to 50 v) or pulse duration (up to 1 msec), or repetition (up to 500 per second for 5 sec) of the stimulus did not alter the response seen in Fig. 1*B* except in amplitude. When the electrodes carrying the strand were lowered into contact with the underlying bulbar tissue, the fully developed complex reappeared (Fig. 1*C*).

At the end of each experiment, the animal was perfused with formalin, and the bulb was removed, serially sectioned, and Luxol-fast or Weil stained to determine the amount of pyramidal tract dissected (Fig. 2). Evidently stimulation of the intact medullary pyramids excites a host of elements in addition to pyramidal axons. Component *d* has generally been recognized as resulting from spread of the stimulus to adjacent corticopetal fibers of the medial lemniscus,

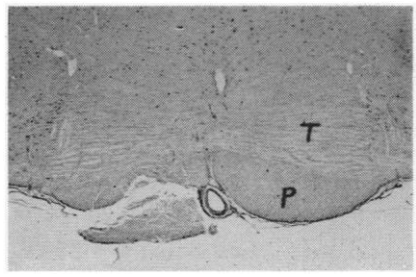

Fig. 2. Luxol-fast section through bulbar pyramid showing extent of dissection; this dissected strand is larger than usual: *P*, intact pyramid; *T*, trapezoid body. Medial lemniscal fibers are interspersed through the trapezoidal fibers and between the pyramid and trapezoid body.

but the first three components have been regarded as the cortical consequence of activity in pyramidal fibers exclusively. The experiment reported here leads to the conclusion that the sole cortical consequence of antidromic pyramidal activation is the *a* wave (and, perhaps, the inconstant, prolonged positive potential of extraordinarily minute amplitude following the *a* wave). The prominent *b* wave and the variable *c* wave must result from activation of hitherto unsuspected elements in the vicinity of the medullary pyramidal tracts.

Such a finding calls for a reevaluation of those studies in which antidromic stimulation has been used in an attempt to map the cortical origin of the pyramidal tract (*6*) and of many other experiments depending upon the original interpretation of the antidromic potential (*7*). Stimulation of the isolated pyramidal strand fails to yield a large, prolonged surface-negative potential; such a potential, which is observed when the intact pyramidal surface is stimulated, has been ascribed to activity in apical dendrites (*8, 9*). Moreover, the concept of recurrent collateral activation of apical dendrites directly or via interneurons does not find any support (*9, 10*); no delayed activity could be detected following stimulation of the strand.

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1. We are using the term "Betz cells" to indicate those cells, and only those cells, whose axons are contained in the pyramidal tract.
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15 April 1959

Effect of Diisopropylfluorophosphate on Sulfhydryl Proteases

Abstract. Diisopropylfluorophosphate inhibited all the sulfhydryl proteases studied in our tests. This inhibition was most pronounced at *p*H 6.0. By first blocking the sulfhydryl group with *p*-chloromercuribenzoate, inhibition could be prevented. Neither cysteine nor choline gave appreciable reactivation of diisopropylfluorophosphate-inhibited bromelain.

Although the organic phosphate nerve gases are well established as specific inhibitors of certain esterases, trypsin and chymotrypsin, activated plasmin and phosphoglucomutase (*1*), no reports of specific inhibition of the sulfhydryl enzymes have been made (*2*).

In a comparative study of plant proteases we found that moderate concentrations of diisopropylfluorophosphate inhibited many preparations of bromelain (*3*), papain, and ficin. With commercial bromelain, $4 \times 10^{-4}M$ diisopropylfluorophosphate inhibited 50 percent of the protease activity within 2 hours at room temperature. Some of our fractionated bromelain preparations, on the other hand, showed only slight inhibition at high concentrations of reagent.

The effectiveness of diisopropylfluorophosphate as an inhibitor depended not only on the type of enzyme and the degree of purity of the enzyme but also on the *p*H of the solution (Fig. 1). Bromelain and papain, which presumably have similar active sites, showed an entirely different behavior at all *p*H values below *p*H 5.0. On the other hand, Rhozyme P-11, a fungal protease which does not require a free sulfhydryl group for enzymatic activity, showed a *p*H-inhibition curve which was remarkably similar to that of bromelain. These similarities and differences may provide clues to the nature of the active sites on these enzymes, or to the effect of unknown materials in these preparations which mediates the action of diisopropylfluorophosphate on sulfhydryl groups.

That the sulfhydryl group is the site actually being affected is shown by an experiment summarized in Table 1. Blocking the sulfhydryl group with *p*-chloromercuribenzoate before exposing the enzyme to diisopropylfluorophosphate gave complete protection against inhibition. Another sulfhydryl blocking technique, and one which may frequently occur with enzyme preparations, is mild oxidation. This also protected against diisopropylfluorophosphate.

Attempts to reactivate diisopropylfluorophosphate-inhibited bromelain with cysteine, choline, or a combination of the two reagents at either *p*H 5.0 or *p*H 7.0 have been only slightly successful. Cysteine regenerated no more than 10 percent of the original activity.

Our finding that diisopropylfluorophosphate, under the proper conditions, will react with sulfhydryl enzymes now makes this chemical a fairly general protein reagent. It will react directly with the nitrogen of imidazole or the hydroxyl of tyrosine (*4*); it will react directly or indirectly with amino (*5*) and sulfhydryl groups; it will react indirectly with the hydroxyl group of serine (*1, 6*).

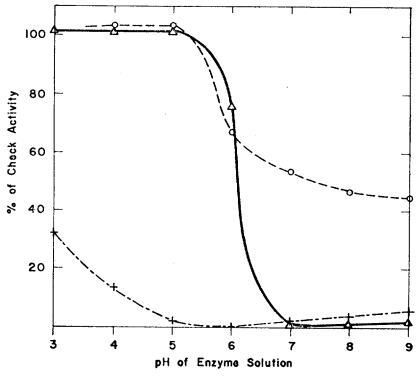

Fig. 1. Effect of *p*H on the inhibition of bromelain (solid line), papain (dashed line with crosses), and Rhozyme P-11 (dashed line with circles) by diisopropylfluorophosphate. The enzymes were incubated for 1 hour at 25°C in $1 \times 10^{-3}M$, $1 \times 10^{-3}M$, and $1 \times 10^{-4}M$ diisopropylfluorophosphate, respectively.

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6. We wish to thank Mrs. A. Chun, Mr. S. Nakata, and Mr. R. Sugai for technical help in running certain of these assays.

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Table 1. Effect of protecting the sulfhydryl group of a purified bromelain sample on inactivation by diisopropylfluorophosphate (DFP).

Treatment	Milk-clotting unit*/g	Percentage of 4660
Enzyme in *p*H 7.0 buffer (no PCMB)†	4660	100
Enzyme in 25 percent isopropylalcohol (IPA)	4520	97
Assayed without cysteine after dialysis	2620	56
Assayed with 0.005*M* cysteine after dialysis	4500	97
Enzyme in 25 percent IPA with $10^{-3}M$ DFP	706	15
Assayed without cysteine after dialysis	52	1
Assayed with 0.005*M* cysteine after dialysis	450	10
Enzyme in *p*H 7.0 buffer with PCMB†	80	2
Enzyme in 25 percent isopropylalcohol	0	0
Assayed without cysteine after dialysis	552	12
Assayed with 0.005*M* cysteine after dialysis	4900	105
Enzyme in 25 percent IPA with $10^{-3}M$ DFP	0	0
Assayed without cysteine after dialysis	486	10
Assayed with 0.005*M* cysteine after dialysis	4900	105

* Milk-clotting unit: 1 min to clot 5 ml of a 5-percent skim milk solution adjusted to *p*H 5.3 and incubated at 37.5°C. † *p*-Chloromercuribenzoate.

Influence of Adrenalectomy and Hypophysectomy on Cerebral Serotonin

Abstract. Changes in serous and encephalic serotonin in hypophysectomized or adrenalectomized rats have been observed. Adrenalectomy produces a decrease of serous serotonin and an increase of the serotonin of hemispheres, base, and medulla oblongata; with hypophysectomy, serotonin is also reduced in serum and increased only in the base and medulla oblongata.

It is known that the cerebral serotonin content can be varied by artificial means, either by stimulating the formation of