butyldithiocarbamate, applied either to cloth covers or directly to plants, eventually may be useful to protect crops other than tobacco from ozone damage (*6*).

SAUL RICH
GORDON S. TAYLOR
Connecticut Agricultural Experiment Station, New Haven

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28 March 1960

Serial Lactic Dehydrogenase Activity in Plasma of Mice with Growing or Regressing Tumors

Abstract. Close correlation has been observed between the serially tested plasma level of a glycolytic enzyme, lactic dehydrogenase, and the growth of several transplanted mouse tumors. The character of the lactic dehydrogenase curve determined by serial blood sampling of the animals during the development of such tumors is found to consist of five separate phases. The first three appear before any visible evidence of tumor growth. Inhibition or regression of tested tumors, induced by therapeutic compounds, was accurately reflected in a corresponding reduction of lactic dehydrogenase activity in the peripheral plasma of the host.

Lactic dehydrogenase activity has been found to be elevated in the serum or plasma of animals with various experimental tumors, and in some patients with malignant neoplasia (*1–3*). It is also increased in the serous effusions that bathe cancer tissue in human beings, and in the media surrounding various malignant cell lines in tissue culture (*2, 3*).

The serial assay of this enzyme in the blood plasma of mice during the growth of such diverse transplanted tumors as Ehrlich solid carcinoma, sarcoma T-241, mammary adenocarcinoma E-0771, or the Cloudman S91 melanoma shows a close correlation between increasing tumor mass and the progressive elevation of lactic dehydrogenase activity in the peripheral blood. This confirms and elaborates earlier observations of elevation of this enzyme in the serum or plasma of tumor-bearing animals (*1–3*). In addition to an overall correlation of enzyme activity and tumor volume, five phases of the lactic dehydrogenase time curve have been observed, as illustrated in Fig. 1: (i) a latent period between tumor implantation and initial appearance of elevated levels of enzyme in the plasma lasting 24 to 96 hours, depending upon the tumor type and its growth rate; (ii) a rapid increase of the enzyme activity from normal values (approximately 500) to 2000 to 6000, giving a 5- to 10-fold enzyme increase prior to detectable growth of tumor implant (this increase takes place usually during the 2nd or 3rd day after implantation); (iii) a plateau following the initial increase and remaining essentially level for several days; (iv) a second rapid increase in plasma enzyme closely correlated with the logarithmic growth phase of the tumor, but usually initiated prior to measurable growth of the new tumor mass. Such an enzyme increase continues with growth of the tumor up to values of 25,000 to 50,000 units—an increase over normal enzyme values of 50- to 100-fold. The final phase (v) frequently observed is an abrupt fall in enzyme level in the plasma just prior to the death of the tumor-bearing animal. Such a fall has also been seen in terminal cancer patients (*2*). Although Fig. 1 is diagrammatic, it accurately represents the composite data for the control mice in 20 individual experiments. The lactic dehydrogenase activity curve was derived from 707 individual enzyme determinations on 163 untreated Swiss ICR mice implanted subcutaneously with the Ehrlich carcinoma. The bleeding was done serially on the same animals by a modification of the orbital bleeding technique (*4*). The plasma enzyme was assayed by a microtechnique modification of a spectrophotometric procedure described previously (*5*). The tumor growth curve in Fig. 1 is an expression of the average tumor volume, with time, determined by a total of 1172 three-dimensional caliper measurements on the solid Ehrlich carcinomas of these mice, taken from the palpable appearance of the lesion to the death of the animal.

Appropriate correlative alterations of the lactic dehydrogenase activity curve occur when the growth of the tumor is modified by various antitumor compounds. Figure 2 illustrates the development and regression of an Ehrlich carcinoma implanted in an animal undergoing treatment with orthophenylenediamine (*6*) and indicates the corresponding increase and decrease of the enzyme activity in the blood plasma as a function of tumor behavior.

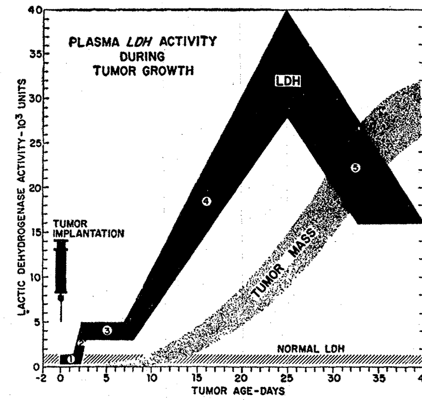

Fig. 1. Correlation of increasing lactic dehydrogenase activity (LDH) in plasma with progressive growth of the solid Ehrlich carcinoma, and illustration of five distinct phases of the enzyme response curve.

The first three phases do not show in this plot since the lactic dehydrogenase determinations were not started until the 4th day following tumor implant, at which time the phase (iii) plateau had already been reached. The data of Fig. 2 were obtained from a single mouse and illustrate the possibility of serial accumulation of enzyme information with these techniques on individual experimental animals, analogous to the close clinical following of a patient.

Although the tumor regressed completely in this experiment and the animal remained tumor free during several months of subsequent observation, the enzyme activity never returned to a completely normal level. This has been confirmed in several experiments in which the enzyme activities in the plasma of mice with regressed tumors slowly approach normal levels after the original precipitous decrease, but never-

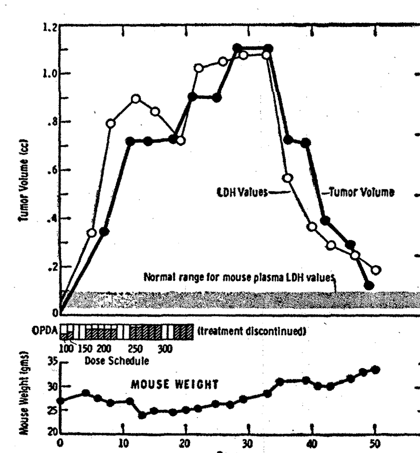

Fig. 2. Growth and regression of a treated Ehrlich carcinoma and the corresponding plasma levels of lactic dehydrogenase activity (LDH).

theless remain significantly elevated for periods of at least 1 year. This postregression plateau is in most cases within the 2000 to 6000 unit level, which corresponds quantitatively to the first host-response plateau seen shortly after tumor implantation but prior to the appearance of the tumor.

Since the values for lactic dehydrogenase activity in plasma have responded to the successful treatment of the established tumors which we have tested, and since they have also reflected tumor inhibition prior to visible or measurable changes in the tumor mass, these enzyme methods are being explored to determine their potential usefulness as additional indices in the screening of antitumor compounds and of extending the understanding of tumor-host-enzyme relationships (*7*).

VERNON RILEY
FELIX WROBLEWSKI

Divisions of Experimental Chemotherapy and *Clinical Investigation, Sloan-Kettering Institute for Cancer Research, New York, New York*

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Communicative Mandible-Snapping in Acrididae (Orthoptera)

Abstract. *Paratylotropidia brunneri* Scudder is the first insect known to possess a long-range mandibular sound signal. This signal probably evolved through a stage in which feeding noises were significant; it is believed to be a functional analog of other insect calling sounds.

Many insects with chewing mouthparts make audible noises while feeding, but only among the short-horned grasshoppers are cases known in which sounds made by movements of the empty mandibles operate as intraspecific communicative signals. This ability has appeared in scattered genera in three subfamilies of Acrididae: Acridinae, Oedipodinae, and Cyrtacanthacridinae (*1, 2*). In most cases the sounds appear to be no more than relatively nonspecific reactions to disturbances, produced by nymphs and adults of both sexes when they contact one another or when they are disturbed by the activities of other animals. However, in *Calliptamus italicus* (L.) (Cyrtacanthacridinae), several mandibular noises occur as significant signals in situations similar to those which are regulated by tegmino-femoral stridulation in the Acridinae and Oedipodinae. Mandibular sounds are produced by adults of both sexes when they are disturbed, and by males during aggressive encounters with other males, during courtship, when courtship is interrupted, and during copulation (*2,3*). The single finding that keeps this series of situations from paralleling those in which tegmino-femoral stidulation is significant in Acridinae and Oedipodinae is that *C. italicus* has no calling sound—no signal produced by lone males in the absence of other individuals which results in the coming together of the sexes or has any of the other side effects of this signal in various Orthoptera and Homoptera (see *4*).

On 20 June 1959 I tape-recorded a mandible-snapping noise made by *Paratylotropidia brunneri* Scudder (Cyrtacanthacridinae), which is not only the first sound recorded for this species but also appears to represent a close parallel of the calling sound in other Orthoptera and in Homoptera. This large grasshopper was abundant in a hill prairie along the crest of the Mississippi River bluff south of Valmeyer, Ill., in Monroe County. A similar prairie just south of this one has been described and illustrated (*5*). The dominant plant is *Andropogon scoparius* Michx.; there are occasional clumps of *A. gerardi* Vitman present, along with several other native prairie plants and animals. During the day in late spring and early summer, the principal sounds in the prairie are the calling songs of three Acridinae: *Chloealtis conspersa* Harris, *Pseudopomala brachyptera* (Scudder), *and Eritettix simplex* (Scudder) (*6*). The tiny grassland cicada, *Beameria venosa* (Uhler), and three largely nocturnal crickets, *Acheta fultoni* Alexander, *Miogryllus verticalis* (Serville), and *Oecanthus argentinus* Saussure, were the only other singing insects heard in the prairie on three separate visits at this time of year. This is perhaps the only habitat in eastern North America in which slant-faced grasshoppers are at any time the dominant noisemakers.

Several *Paratylotropidia brunneri* were collected before it was discovered that series of soft ticks heard almost continually here and there across the prairie were being produced by this species. A male was approached and watched as he made the noise; his mandibles could be seen moving in time with the sound. Individuals spaced a few feet apart seemed to be responding to one another by repeating series of ticks in rapid succession, each one beginning about a second after his nearest neighbor had finished. My attempts to get a response by tapping various metal objects together were unsuccessful until finally a nearby male delivered a series of ticks immediately following an imitation made by striking a metal thermometer case against a brass belt buckle. In each of many subsequent trials, the insect responded to the imitation after an interval of about 1 second (0.9 to 1.0 second in five tape-recorded trials). This was the same interval as was occurring between successive series of ticks by neighboring grasshoppers, and an irregular juggling of the time of delivery of the imitation left no doubt that the grasshopper was responding to it.

The mandible-snapping of *Paratylotropidia brunneri* is a simple sound, resembling a low-intensity abbreviated version of the ticking song of the katydid, *Microcentrum rhombifolium* (Saussure) (*4*). It is audible from a distance of several yards. Audiospectrographic analysis shows that the ticks have a nearly continuous frequency spectrum up to at least 15 kcy/sec, with intensity peaks at about 3, 5, and 8 kcy/sec. The ticks are delivered at rates of 6 to 7 per second (*7*); of 16 tape-recorded series, 12 series were comprised of 4 ticks each, and the other four, of 2, 3, 5, and 6 ticks each (*8*).

Every aspect of this observation suggests that the ticking of *P. brunneri*—produced by lone males and elicited consistently by auditory stimuli—is functionally analogous to the calling songs already known for Acridoidea, Tettigonioidea, and Auchenorhynchous Homoptera. This is a significant addition to our knowledge of insect acoustics, representing another instance of parallel evolution in the development of long-range sound signals. Further observation on this species is likely to reveal that mandibular sounds function in several situations, as they do in *Calliptamus italicus*.

Communicative mandible-snapping has probably evolved in every case through a stage in which the noise made by feeding grasshoppers was the initial auditory stimulus. Visually significant motion of the mandibles seems a less likely precursor, though it may have appeared as an intermediate stage in some cases; Acrididae are generally most active in bright sunlight, and vision is important in their close-range behavioral interactions. Lépiney (*9*) has shown that the odor of crushed leaves acts as an attractant to migratory lo-