Reports

Tumor-Inducing Factor in Drosophila

Abstract. Attempts to repeat experiments reported to demonstrate the existence of a tumor-inducing factor in the *tu-e* mutant of *Drosophila* were not successful.

In view of the continued references (*1*) to earlier work in this laboratory (*2*) concerning the purification and properties of a reputed tumor-inducing factor (TIF) from the *tu-e* mutant of *Drosophila*, it seems desirable to comment on the investigations described and on additional experiences.

Initially I served as adviser to F. Friedman and L. Burton on the purification of TIF with the assumption that the biological assay was satisfactory. However, shortly before the departure of these investigators from this laboratory they were presented with coded samples containing only buffer solution or buffer plus various concentrations of "purified TIF." The results of their assay demonstrated clearly that they could not distinguish buffer solution from TIF solutions. Because of the possible validity of some of the explanations offered for this, and because of some subsequent experiments of a similar nature, I undertook a repetition of tumor transmission experiments with both crude and purified extracts. Control series totaling 1047 animals, surviving to adults after injections, yielded 216 (20.4 percent) with melanotic inclusions, whereas 207 (18.5 percent) were observed among 1120 animals surviving injections of preparations from the *tu-e* mutant. Percentage survival varied widely, as did percentage "tumors" (2 to 80 percent), in different experiments, but controls did not differ significantly from experimentals when injections were made at the same time.

Since various arguments can be invoked, neither these results, nor other details not given here, prove that a tumor-inducing factor does not exist in the *tu-e* mutant of *Drosophila*. Nevertheless, I would be pleased to be forgotten as a collaborator in the work described earlier (*2*).

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2 December 1960

Rapid Effect of Sodium Cyanide and Dinitrophenol on Mammalian Nerve

Abstract. Strips of rat nerve from dorsal roots were immersed in solutions containing sodium cyanide and α-dinitrophenol. The inhibitors extinguish the action potential of such strips within minutes. The rapid action on mammalian nerve is contrasted with the slow effect of inhibitors on amphibian and invertebrate nerve.

The application of cyanide to the squid giant axone (*1*) or to frog peripheral nerve (*2*) does not block conduction or depress the resting potential for at least 2 hours. Presumably the effect of inhibitors on sheathed nerve that has been reported (*3*) may be explained as resulting from the increased leakage of ions into the extracellular space around nerve fibers; the ions having been restrained from diffusion by the envelope of sheaths surrounding the nerve fibers would become more concentrated and cause depolarization. The slow action of the inhibitors on invertebrate and frog nerve has been offered as evidence that the action potential is not directly dependent upon metabolic energy. We have attempted to assess the generality of these results by applying sodium cyanide and α-dinitrophenol to mammalian nerve.

Rats were anesthetized with ether, the lumbar roots were exposed, and thin strips of nerve were dissected from these roots. The rootlets were placed on silver stimulating and pickup electrodes; they were then stimulated with a 0.1-msec pulse, and the action potential was photographed with a Polaroid Land camera from a Dumont 304H oscilloscope after preamplification with a Grass P-4 amplifier. The strips were kept in Ringer's solution (20° to 25°C) for a 1-hour control period and immersed in Ringer's solution to which an inhibitor was added. The strips were tested periodically until the potentials disappeared; they were then placed quickly in Ringer's solution to check the reversibility of inhibition.

Strips were immersed in 0.005*M* solutions of sodium cyanide and 0.001*M* solutions of α-dinitrophenol within a *p*H range of 6.8 to 7.6. Figure 1 is a graph of the amplitude of the action potentials obtained for 17 strips immersed in cyanide (open circles) and ten strips placed in α-dinitrophenol (solid circles). The effect on individual strips may occur more rapidly than the average values indicate. The shortest disappearance of the action potential occurred in about 1 minute with a strip in cyanide solution. The longest survival time was 15 minutes. Such a range may be attributed to the variability in strip diameters.

After disappearance of the potentials, the strips were again immersed in Ringer's solution. The cyanide-poisoned nerves recovered almost the full amplitude of the action potential within 2 to 3 minutes; the strips treated with α-dinitrophenol did not recover at all, even after prolonged washing in Ringer's solution. Nerves may be treated with cyanide and reversed repeatedly, provided washing is performed immediately after disappearance of the potential.

The inhibitors cyanide and α-dinitrophenol, in contrast to their action on frog and squid nerve, rapidly extinguish the action potential of mammalian nerve. The experiments of Dettbarn and Stämpfli (*4*) have shown that α-dinitrophenol does not affect the resting potential of rat nerve. If cyanide is similarly ineffective, the inhibitors do not act by depolarizing nerve. Schoepfle and Bloom (*5*) have suggested that the effect of cyanide on frog nerve is to alter the *h* factor before altering the sodium or potassium equilibrium poten-

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

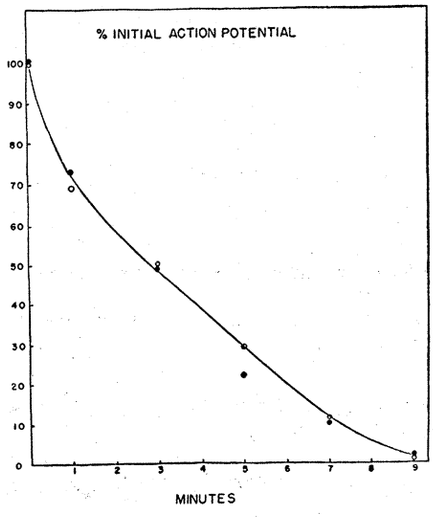

Fig. 1. Amplitude of action potentials obtained for strips immersed in cyanide and α-dinitrophenol.

tials. This mechanism of action implies that cyanide acts physically on the membrane in addition to having an effect on the cytochrome enzymes.

The inhibitors may act on mammalian nerve by blocking energy sources immediately necessary for impulse transmission. This hypothesis assumes that mammalian nerve differs from frog and squid nerve in containing a system in which the action potential is linked to the energy sources. Inhibition of the sodium extrusion mechanism, definitely dependent upon metabolism, appears to be excluded. The action of cyanide and α-dinitrophenol within minutes precludes the possibility that enough internal sodium or potassium ion accumulation occurs to depress the action potential. It is not yet possible, however, to decide whether the inhibitors act by binding physically with a membrane component or whether they act upon a metabolic system within mammalian nerve which generates the action potenial (*6*).

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Self-Absorption Correction for Isotopes Emitting Weak Beta Rays

Abstract. The shape of the self-absorption correction curve of β-emitters is not universal but depends on the geometrical arrangement of the sample and counting device. This may explain why the correction factor defined by Hendler (*1*) is not always linearly related to the thickness of the sample.

In determining the radioactivity of samples of radioactive isotopes emitting weak β-rays, such as C^{14}, S^{35}, and H^3, one has to take into account that part of the radiation is absorbed in the sample itself. R. W. Hendler has described a method for calculating the self-absorption correction factor for C^{14} assay (*1*). A series of planchets of constant area containing different known quantities of the same radioactive material is prepared, and the weight m (in milligrams) and the specific count rate (in counts per minute per milligram) are recorded for each planchet. A standard weight m_{std} is chosen arbitrarily and the correction factor F (as a function of m) is defined as the ratio of the specific count rate R at weight m_{std} to the specific count rate at weight m. Hendler found experimentally that F was related to m by a linear function from infinite thinness to a weight of several times saturation thickness. This relation was contested by J. Katz (*2*), who calculated F for several sets of experimental data and found that F was not linearly related to m.

The treatment of experimental data on self-absorption is mostly based on the fact that the absorption curves of many β-emitting isotopes are approximately exponential up to a certain thickness of the absorber (*3*). If the exponential equation is applied to the set of data for $BaS^{35}O_4$, published by Katz and Golden (*4*) and quoted by Hendler in his reply (*5*), F is not found to be a linear function of m, although within a limited range of values of m the deviation from a straight line is not great. The experimental data are fitted much better by a function of m which is derived from the exponential absorption equation, provided the absorption coefficient is chosen appropriately. The parameters used for these data were $R/I_\infty = 0.0569$ and $\alpha = 0.0956$ (see below). The reference weight was 12 mg over an area of 3.14 cm^2. Within the range given by Katz and Golden ($m = 4$ to 25 mg), the deviation of the function F was smaller than 1 percent, whereas the straight line, which was suggested by Hendler (*5*), deviates by as much as 2 percent from the curve given by Katz and Golden.

From a mathematical treatment of the exponential absorption equation it follows that the slope of the curve of F plotted against m increases by a factor of two when going from $m = 0$ to $m = \infty$.

The absorption of radiation can be measured by interposing a filter of weight m over a fixed area between a sample containing a certain isotope and the detecting device. It can be seen for many isotopes that

$$I = I_0 \cdot e^{-\alpha m} \qquad (1)$$

where I is the observed count rate in counts per minute, I_0 is the count rate without filter, and α is the absorption coefficient in $milligrams^{-1}$ (*6*). This means that each layer dm of the filter decreases the radiation by the same fraction (Beer's Law). For the self-absorption it follows (*4*) that

$$I = I_\infty (1 - e^{-\alpha m}) \qquad (2)$$

where I_∞ is the observed count rate at infinite thickness. The specific count rate is I/m, and the correction factor F is defined as

$$F = (R/I)m \qquad (3)$$

where R is the specific count rate at weight m_{std}. Substituting Eq. 2 in Eq. 3 we obtain

$$F = \frac{R}{I_\infty} \cdot \frac{1}{1 - e^{-\alpha m}} \cdot m \qquad (4)$$

By definition, F is 1 for $m = m_{std}$, and

$$R = \frac{I_\infty}{m_{std}} (1 - e^{-\alpha m_{std}}) \qquad (5)$$

F can be analyzed for small values of m by expanding the exponential term into a series and by again expanding the resulting fraction into a binomial series, cutting off after the terms of the second degree:

$$F = \frac{R}{I_\infty} \cdot \frac{1}{\alpha m}\left[1 + \frac{\alpha m}{2} + \frac{\alpha^2 m^2}{12}\right] \cdot m$$

$$= \frac{R}{\alpha I_\infty} + \frac{R}{2 I_\infty} \cdot m + \frac{R \cdot \alpha}{12 I_\infty} \cdot m^2 \qquad (6)$$

It follows that the intercept of the curve of F against m on the ordinate is $R/(\alpha \cdot I_\infty)$, that the initial slope is $R/(2 \cdot I_\infty)$, and that the curve is bending upwards.

For high values of m (region of infinite thickness) the exponential term in Eq. 4 can be neglected against 1, and we get

$$F = \frac{R}{I_\infty} \cdot m \qquad (7)$$

which is a straight line through the origin with a slope of R/I_∞, that is, two times as high as at $m = 0$.

Whereas the exponential function fits very well the data of Katz and Golden (and many other sets of data published earlier), it is evident that the data cited