after 11 days incubation was 235, 130, and 63, respectively. Only a few eggs were found within the host plants grown at 2 percent oxygen.

The large reduction in number of galls at the lower oxygen levels appears to be due to the reduced hatching rate and development of the eggs of the original females and the reduction in infectivity of the larvae in the soil. The possibility that soil aeration affects nematodes has long been recognized; however, it has been difficult to establish direct evidence that inadequate oxygen in the soil limits the activity of nematodes except under rather extreme conditions. These results indicate that the activity of some nematodes in the soil phase is more dependent upon the availability of oxygen than previously thought.

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References

1. H. R. Wallace, *Ann. Appl. Biol.* **44**, 57 (1956).
2. L. H. Stolzy, S. D. Van Gundy, J. Letey, Jr., *Phytopathology* **50**, 656 (1960).
3. C. Overgaard Nielsen, *Natura Jutlandica* **2**, 1 (1949); R. A. Rohde, *Proc. Helminthol. Soc. Wash., D.C.* **27**, 160 (1960); P. H. Santmyer, *ibid.* **23**, 30 (1956).
4. L. H. Stolzy, O. C. Taylor, J. Letey, Jr., T. E. Szuszkiewicz, *Soil Sci.* **91**, 3 (1961).
5. A. F. Bird, *Nematologica* **4**, 31 (1959).
6. J. Letey, Jr., O. R. Lunt, L. H. Stolzy, T. E. Szuszkiewicz, *Soil Sci. Soc. Am. Proc.* **25**, 183 (1961).

3 April 1961

Simultaneous Appearance of Free Arginine and Deoxyribosidic Compounds during Mitosis

Abstract. Sequential measurements were made of the free amino-acid pool in the premitotic region of microspores of *Lilium longiflorum*. The basic amino acid arginine appears in the pool and then disappears. Arginine appears at the same developmental stage at which it has been reported that the free deoxynucleosides appear.

The mitotic division in the microspores of *Lilium longiflorum* presents a particularly favorable subject for the study of the chemistry of mitosis. It is especially favorable for two reasons. First, a morphological index of development (*1*) orders the sequence of events during the interphase preceding mitosis; second, the microspores undergo this mitosis in synchrony (*1*). Using this material, Foster and Stern (*2*) and Taylor (*3*) have been able to show the precise time, during the interphase preceding mitosis, at which deoxyribonucleic acid (DNA) is synthesized. Nasatir, Bryan, and Rodenberg (*4*) showed changes in the composition of the soluble proteins during interphase and mitosis. The large quantitative change in the free deoxyribosidic compounds (*2*) and the correlation of this peak value with the doubling of one of the soluble proteins (*4*) led us to an examination of the associated changes in the free amino acids.

Table 1. Change in concentration of the free amino acids in the anther, with respect to the length of the flower bud in *Lilium longiflorum*. Mitosis of the microspores occurs at 60 mm.

Bud length (mm)	Change in concentration (μmole/anther)		
	Lysine	Histidine	Arginine
50	4.67	0.32	
52.5	1.25	5.10	2.25
54	2.40	0.31	
55	1.62	2.10	
60	3.40	7.30	

Flower buds of *Lilium longiflorum* Thunb. cv. "Croft" of varying lengths were removed, and the anthers were excised. The anthers were homogenized in 70-percent ethanol and centrifuged. The supernatant fraction was evaporated and the residue was dissolved in 2.5 ml of *p*H 2.2 buffer. The amino acids were separated by column chromatography by the methods of Moore and Stein (*5*). The quantity of each amino acid was determined by the ninhydrin reaction described by Moore and Stein (*6*).

Changes in many amino acids were found, but the most striking were the changes of the basic amino acids, lysine, histidine, and arginine. A measurable amount of arginine was present only at a bud length of 52.2 mm. This is the same bud length at which Foster and Stern (*2*) found free deoxyribosidic compounds and at which Nasatir, Bryan and Rodenberg (*4*) found the doubling of one of the soluble proteins. Histidine and lysine, in contrast to arginine, were present at all the bud lengths measured: 50, 52.5, 54, 55, and 60 mm. At the time of DNA synthesis, arginine appeared and histidine increased over tenfold, but lysine decreased by a factor of four. Later, at the time of mitosis, arginine was absent, but the concentrations of both lysine and histidine tripled. These results are summarized in Table 1.

Because of the small number of buds analyzed, it is difficult to say definitely how the pattern of the free amino-acid pool changes with development. For example, it is not possible to decide whether the high level of arginine is present during all the premitotic fluctuations in free deoxyribosides or whether the concentration follows these fluctuations in detail. If the concentration fluctuates, there may be more than one peak. To demonstrate such a second peak would require the use of a much larger number of buds.

On one point, however, the data are quite unambiguous: Two amino acids, distinctively present in chromosomal protein, undergo marked concentration changes close to the time of DNA doubling. This result leads us to speculate that the pertinent protein synthetic mechanisms undergo a corresponding change related to the requirements of chromosome duplication.

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References and Notes

1. R. O. Erickson, *A. J. Botany* **35**, 729 (1948).
2. T. S. Foster and H. Stern, *Science* **128**, 653 (1958); *J. Biophys. Biochem. Cytol.* **5**, 187 (1959).
3. J. H. Taylor, *Exptl. Cell Research* **4**, 164 (1953).
4. M. Nasatir, A. M. Bryan, S. D. Rodenberg, *Science* **132**, 897 (1960).
5. S. Moore and W. Stein, *Anal. Chem.* **30**, 1185 (1958).
6. ———, *J. Biol. Chem.* **211**, 907 (1954).
7. This investigation was supported in part by a grant (G-4084) from the National Science Foundation and in part by a fellowship to one of us (M.N.) from the National Cancer Institute.
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5 June 1961

Virus–Tumor Synergism

Abstract. More than 30 mouse tumors are associated with virus-like agents that may readily be detected by enzymic techniques. Radiation and chemically induced tumors do not ordinarily give evidence of such activity. The present report is of experiments in which a synergistic effect has been observed to occur when animals were inoculated with both the filtrable agent and a virus-free tumor. Synergism was shown by accelerated growth of the tumor and by elevation of a glycolytic enzyme (lactic dehydrogenase) in the host plasma.

Most transplantable mouse tumors and their hosts have been shown recently to have a virus-like agent, or agents, associated with them (*1, 2*). While these tumor-host-virus associations now include over 30 varieties of tumor types as revealed by enzymic techniques, the relationship of these agents to the neoplastic process is still uncertain (*2*).

Radiation-elicited and chemically induced primary tumors, in contrast, do not ordinarily exhibit evidence of such agents by these methods, and the significance of the exceptions which we have observed are obscure and require further critical studies. However, synergistic effects have been observed when a lactic dehydrogenase elevating agent obtained from a transplantable tumor, such as the Ehrlich carcinoma, was added to an apparently "virus-free" tumor-bearing host. Such newly derived primary tumors or their subsequent "virus-free" passage transplants, or other "virus-free" neoplastic equivalents, appear to be required for detection of such viral effects on tumor growth and metabolic response.

As shown in Fig. 1, the growth rate of the virus-infected 20-methylcholanthrene induced tumor was substantially increased in subsequent transplant passages compared with its nonviral counterpart. Appropriate histological differences consistent with more rapid cell division were also observed, and a glycolytic enzyme, lactic dehydrogenase, was substantially elevated in the host blood plasma when the Ehrlich-associated virus was inoculated into "virus-free" tumor-bearing mice (Fig. 2). This synergistically induced blood enzyme elevation was in considerable excess over that produced additively by the tumor or the virus alone.

Although the metabolic and other alterations appear to be permanent in the sense of being transmitted by repeated tumor transplant, it is unknown whether transduction or transformation phenomena are involved, since the intact agent continues its association with the tumor during such passages. In the present experiments, the enhanced tumor growth rate and the exorbitant increase in lactic dehydrogenase was a consequence of the controlled addition of a virus, while in the previous studies (*1*) covering a spectrum of 30 long-established transplanted mouse tumors the viral agents were already present through an unknown process.

The present findings provide a synthetic reproduction of the previously reported five separate phases of the serial plasma lactic dehydrogenase curve found associated with the malignant process in a wide assortment of standard transplanted mouse tumors (*3*). The numbers within circles in Figs. 1 and 2 indicate the various induced five-phase counterparts in these studies: Phase 1 constitutes the normal lactic dehydrogenase value during the post-

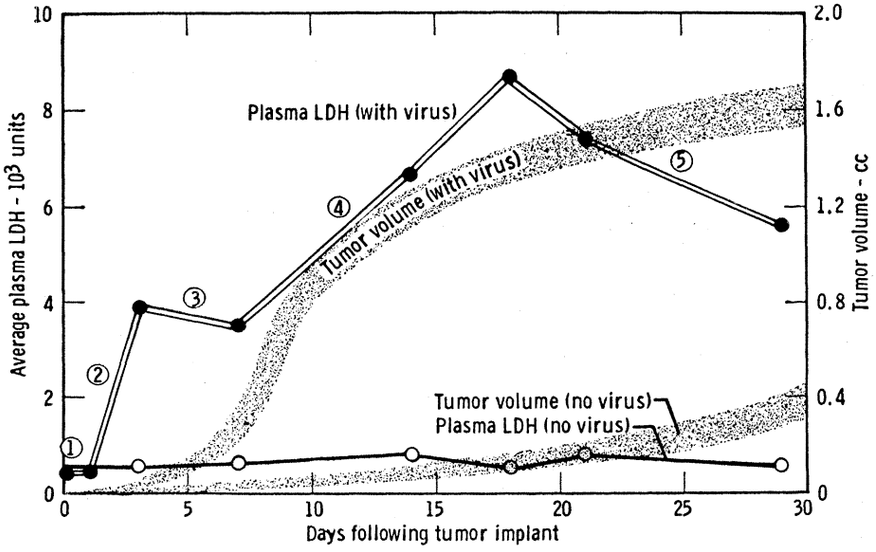

Fig. 1. Comparison of host plasma lactic dehydrogenase activity (LDH) and tumor growth rate in a 20-methylcholanthrene induced transplanted tumor with and without added virus obtained from a mouse bearing the Ehrlich carcinoma. Tumor in third transplant generation, agent added during primary induction.

injection latent period, phase 2 is the abrupt increase in this enzyme occurring between 24 and 72 hours after injection of virus in a normal host, phase 3 is a five- to tenfold elevated lactic dehydrogenase plateau and represents host-virus equilibrium when only the virus is present, phase 4 indicates the synergistic metabolic reaction between virus and established or growing tumor, and phase 5 reflects a quantitative reversal of this synergistic effect during the terminal stage of the host and the deteriorating stage of the tumor. This experimentally controlled virus-tumor recombination may therefore constitute a general explanation of the previously reported correlations observed between mouse tumor growth and alterations in the host plasma lactic dehydrogenase. These data also demonstrate the ability of certain virus-like agents to affect the biological behavior and the biological and the metabolic characteristics of tumors irrespective of the question of their viral etiology.

Such virus-tumor synergism suggests new possibilities and approaches for examining the mechanism of lactic dehydrogenase and other enzyme eleva-

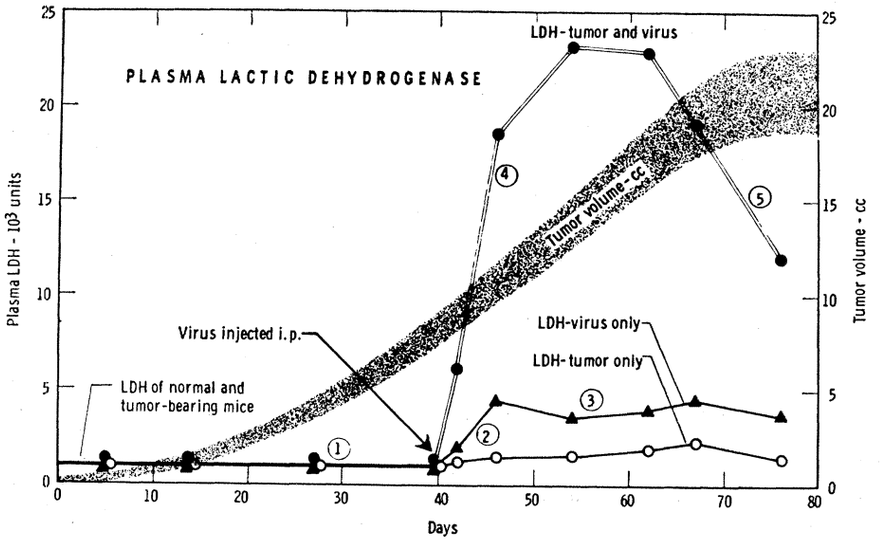

Fig. 2. Synergistic production of high plasma lactic dehydrogenase activity (LDH) in a "virus-free" tumor-bearing mouse after intraperitoneal injection of a virus-like agent associated with the Ehrlich carcinoma. The "virus-free" tumor is a radiation-induced transplantable pituitary neoplasm.

tions found in the serum of some cancer patients, and possibly other clinical states such as viral hepatitis. However, it should be mentioned that in mice we regularly observe moderate plasma lactic dehydrogenase elevation in certain stages of the development of radiation-induced leukemia and chemically induced primary tumors where, in most instances, we have been unable to detect the presence of transmissible agents (*4*).

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References and Notes

1. V. Riley, F. Lilly, E. Huerto, D. Bardell, *Science* **132**, 545 (1960).
2. V. Riley, E. Huerto, F. Lilly, D. Bardell, J. D. Loveless, M. A. Fitzmaurice, *Proc. Am. Assoc. Cancer Research* **3**, 262 (1961).
3. V. Riley and F. Wroblewski, *Science* **132**, 151 (1960).
4. We are indebted to Dr. William Money for providing the pituitary tumor employed in some of these studies, and to Editha Huerto, David Bardell, James D. Loveless, and Mary Anne Fitzmaurice for valuable assistance. Appreciation is expressed for the counsel of Dr. C. Chester Stock and Dr. Gilbert Dalldorf. This study was partially supported by a grant from the U.S. Public Health Service (National Cancer Institute).

22 May 1961

Transreplication and Crossing Over in Sordaria fimicola

Abstract. A study of the segregation of markers closely linked to the *gray* ascospore color locus in *Sordaria fimicola* reveals that there is a high incidence of crossing over very near the locus when it transreplicates, which is much more pronounced in 5:3 than in 6:2 asci. Also, a single 7:1 and several aberrant 4:4 asci are described. At a different spore color locus, transreplication yields only 6:2 ratios, while other spore color loci fail to transreplicate altogether.

Transreplication or "gene conversion" is easily detected in asci of *Sordaria fimicola* heterozygous for loci affecting ascopore color (*1*). Recently (*2*), a linkage group has been worked out for the chromosome bearing the *gray* (*g*) color marker, and several of the mutant loci are near the *g* locus (Fig. 1). This has made possible a study of the relationship between crossing over in the *g* region and transreplication at that locus. The present report is based on a study of 146 aberrant asci.

An abnormal ratio for spore color appears among asci heterozygous for the *g* locus with a frequency of 1 in 800 to 1000 asci, regardless of the combination of other markers involved. Most of the aberrant asci have 6:2 and 5:3 spore color ratios. These are generally believed to have resulted from DNA miscopying during pairing prior to meiotic separation of chromosomes in the ascus (Fig. 2). The frequency of the two types is very nearly the same, the 5:3 asci being only slightly more abundant. However, transreplication does not proceed with equal frequency to the mutant and wild type alleles, the $g \rightarrow g+$ event occurring about five times more frequently than the $g+ \rightarrow g$ event.

An attempt to explain the 5:3 octads requires utilization of an eight-strand model of paired homologs at meiotic prophase, such as that recently proposed by Taylor (*3*). A single half-chromatid temporarily switching from its own strand to copy for a short distance off the homologous strand can readily account for the 5:3 ratio (Fig. 2*A*) without the complication of chromosome breakage, rejoining, and obligately increased crossing over required by Taylor's explanation of the 6:2 event.

An examination of the data on crossing over in the region of transreplication shows that none of the linked loci is altered in character by the transreplication event at *g*. Furthermore, the frequency of crossing over in the *mi-cor* interval of 4.4 units, which includes the *g* locus, is much higher in the aberrant asci—especially those with a 5:3 ratio—than in normal ones, and these crossovers in most cases involve the transreplicating strand. This relationship has been previously explained on the basis of intimate chromosomal pairing within a restricted region, under which condition both crossing over and transreplication are favored. The present studies support this concept as well as the hypothesis that the two are separate events and not obligately interdependent, since a number of aberrant asci fail to show crossing over in the area of transreplication.

By variously deploying the linked markers among the strains crossed, it was found that the transreplication-related crossing over was occurring very close to the *g* locus and within the *mi-cor* interval. For example, among 61 asci with a 5:3 ratio, 41 (67 percent) showed crossing over in the *mi-cor* interval (4.4 units), 26 showed a crossover between *g* and *cor* (3.4 units), and 29 between *mi* and *g* (1 unit), with 14 (23 percent) of

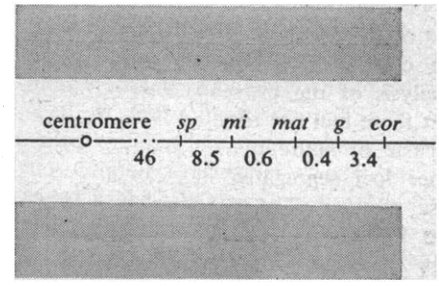

Fig. 1. Part of *g* linkage group showing morphological markers. Abbreviations: *sp*, spotty; *mi*, milky; *mat*, matted—all referring to mycelial characters; *g*, gray ascospore character; *cor*, corona, referring to the ring that appears near the center of the colony.

these having crossovers in both intervals simultaneously where only 0.14 percent of the asci would be expected to show double crossovers. When the marker *mat* just 0.4 crossover unit to the left of *g* was used, it was discovered that transreplication-related crossing over on that side was limited to the shorter interval.

In 6:2 asci the frequency of crossing over in the region of transreplication, though somewhat higher than normal, is less than half that found in 5:3 asci. This significant difference is not predicted by previous explanations of aberrant tetrads.

Six 4:4 asci, each with a pair of

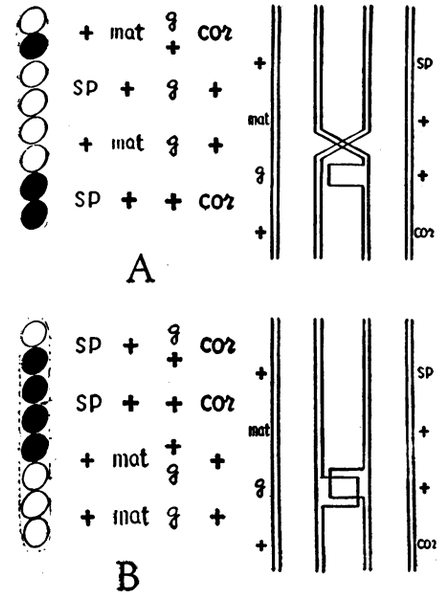

Fig. 2. Two types of aberrant asci with explanatory diagrams. (*A*), 5*g*:3*g*+ ascus, whose progeny can be explained by a half-chromatid miscopying at the *g* locus and a crossover between *mat* and *g*. (*B*), Aberrant 4:4 ascus that is most simply explained by reciprocal miscopying by two homologous half-chromatids.