abraded but not in normal spores (*3*), an effect consistent with the usual influence of temperature upon solubility product. Spore sections previously floated on 10 percent acetone in water were relatively electron transparent in the cortical region; when floated on a dilute aqueous solution of lanthanum, the cortical region was much more dense (*4*), showing a strong adsorption of the trivalent cation.

The notion of a contractile cortex unifies the problems of development and release of a low-water condition. On the other hand, the stability of the vital cores of dormant spores to heat and the development of germinated spores now do not appear more mysterious than the behavior of lyophilized cells.

The hypothesis is not grossly inconsistent with free energy requirements for dehydration and with known strengths of materials. Wool keratin may again be taken as representing the spore core. The free energy required in the change of wool with 36 percent water (equilibrated by vapor transfer with pure water) to 15 percent water (equilibrated against a salt solution with water activity 0.5) is about 1 cal/gm of dry keratin (*8*), and for complete dehydration about 10 cal. A relatively small amount of substrate should suffice for the observed dehydration of the spore even with considerable inefficiency in the contraction.

To approximate the strength required for compressive dehydration of wool to 15 percent water, we equate the pressure energy (resilience) to the free energy of dehydration: 1 cal/cm^3 of keratin = 43 kg/cm^2. (This ignores density and osmotic pressure. The pressure obtained is well above measured osmotic pressures of vegetative cells; that of the specialized spore core may be very low.) A 1-μ sphere of keratin held by a tensile coat 0.1-μ thick requires a strength of about 90 kg/cm^2; a 0.5-μ sphere with a 0.25-μ coat requires about 35 kg/cm^2. Muscle and Kuhn's copolymers (both wet) have tensile strengths of 5 to 20 kg/cm^2; rubber and plastics have strengths to 700 kg/cm^2.

We present this speculation in hope that a simple definitive test will be suggested, and also for the insights to be expected from a novel view. If sporulation involves the walling off of a minute vegetative cell *within* the cytoplasm of the parent cell (the newly germinated spore is osmotically stable), some factors involved in cell wall formation should be essentially similar in sporulation and in vegetative growth. Thus in Collier's (*9*) elegant system with *Clostridium roseum*, penicillin (an inhibitor of cell wall synthesis) inhibits an early stage of sporulation but not the conversion of dipicolinate-rich immature spores to heat-stable spores, a stage that might involve primarily the active-transport of divalent cations across the previously-formed external membrane (*10*).

J. C. LEWIS
N. S. SNELL
H. K. BURR

Western Utilization Research and Development Division, U.S. Agricultural Research Service, Albany, California

References and Notes

1. M. R. J. Salton and B. Marshall, *J. Gen. Microbiol.* **21**, 415 (1959).
2. W. G. Murrell and W. J. Scott, *Nature* **179**, 481 (1957); ———, *Abstr. Intern. Congr. for Microbiology, 7th Congr., Stockholm, Sweden* (1958), p. 26.
3. S. J. Rode and J. W. Foster, *Proc. Natl. Acad. Sci. U.S.* **46**, 118 (1960).
4. B. H. Mayall and C. F. Robinow, *J. Appl. Bacteriol.* **20**, 333 (1957).
5. W. Kuhn, A. Ramel, D. H. Walters, *Proc. 4th Intern. Congr. Biochemistry*, O. Hoffman-Ostenhoff, Ed. (Pergamon, New York, 1959), vol. 9.
6. J. F. Powell and R. E. Strange, *Biochem, J.* **54**, 205 (1953).
7. T. Hashimoto and H. B. Naylor, *J. Bacteriol.* **75**, 647 (1958).
8. J. L. Morrison and J. F. Hanlan, *Proc. 2nd Intern. Congr. Surface Activity*, J. H. Shulman, Ed. (Academic Press, New York, 1958).
9. R. E. Collier, thesis, University of Illinois (1958).
10. We thank the many persons who have discussed this concept with us.

15 July 1960

Transmissible Agent Associated with 26 Types of Experimental Mouse Neoplasms

Abstract. A transmissible agent or factor has been found to be associated with all transplanted and spontaneous experimental tumors examined. This observation was made possible by utilization of a biochemical response of normal animals when inoculated with plasma or organ extracts from tumor-bearing hosts. This transmissible enzymic "lesion" is expressed by a five- to tenfold increase in the plasma lactic dehydrogenase activity of the injected normal test animals. The factor is heat labile, passes through bacteria-retaining filters, but is nondialyzable. It is partially sedimented by centrifugation at 100,000*g* for 1 hour.

With the aid of a new technique, a transmissible factor has been detected in the blood of all animals bearing transplanted and spontaneous tumors so far examined. This includes a spectrum of 25 standard transplanted mouse tumors, and one variety of a "spontaneous" neoplasm of uncertain etiology.

The agent is detected by its induction of an abnormal enzyme response in the peripheral blood of normal test animals after injection of plasma from a tumor-bearing donor. This enzyme indication of the presence of an otherwise "silent" agent is in the form of a five- to tenfold increase of lactic dehydrogenase (LDH) activity in the recipient blood plasma. This usually appears within 48 hours and can be transmitted serially to other normal recipients apparently indefinitely.

The factor is transmitted through injection of plasma, whole blood, tumor extracts, or organ extracts from a tumor-bearing or leukemic animal. No detectable enzyme alteration or transmission occurs with similar materials from analogous normal animals or with heated materials from tumor-bearing animals. The factor may be transmitted by intraperitoneal, subcutaneous, intramuscular, or intravenous injections, or by dermal application.

Plasma, or organ extracts, from the tumor-bearing host may be employed in crude form or they may be clarified by centrifugation at relatively high speed, or passed through bacteria-impervious Selas filters of 03 porosity. Some agent was still present in the centrifuged supernatant after 1 hour at 100,000*g*, but the highest activity was associated with the resuspended pellet. The factor did not pass through standard Visking cellophane dialysis tubing which retains particulates exceeding a molecular weight of approximately 20,000.

With this simple enzymic technique, the presence and the transmissibility of the factor can be followed even though no immediate overt pathological or other physical manifestations are readily detectable when the factor is injected into adult animals.

In general, Swiss ICR male mice were used as recipient test animals for testing mouse tumors, although appropriate strains matching the requirements for tumor transplantation were also employed. However, the transmissibility of the factor crosses all mouse strain lines so far tested, so that Swiss

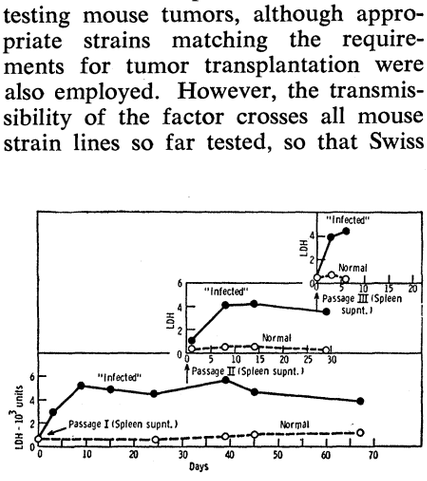

Fig. 1. Serial transmission of enzyme-detectable agent in normal mice. The factor originated in an Ehrlich carcinoma–bearing mouse and was transmitted by way of spleen extract supernatants.

mice may be used for detecting the agent from tumors that require other host strains for successful cellular transplantation.

Lactic dehydrogenase was determined by previously reported spectrophotometric methods (*1*), and serial blood samples were obtained by a modification of the orbital bleeding technique (*2*).

Figure 1 illustrates the serial transmissibility of the factor through several passages of Swiss ICR mice. In this instance, the agent was transmitted through the supernatant of centrifuged spleen extracts. The original spleen was from a mouse implanted subcutaneously with an Ehrlich carcinoma. Analogous spleen extracts from normal animals had no influence on the lactic dehydrogenase, and spleen extracts from tumor-bearing mice behaved the same as normal extracts when they had been heated at 70°C or higher for 15 minutes. The same is, of course, true for other organ extracts and for infected blood plasma.

Table 1. Serial transmission of plasma factor from a mouse implanted with the Lewis lung carcinoma through sufficient passages to dilute the original inoculation to 10^{-18}.

Passage	Mouse strain	Day*	Average plasma LDH†		Dilution‡ of original plasma
			Normal	Tumor	
1	C577BL (F)	2	370	3700	10^{-3}
2	ICR (M)	5	610	3300	10^{-6}
3	ICR (M)	4	410	3300	10^{-9}
4	ICR (M)	2	770	2800	10^{-12}
5	ICR (M)	2	360	3100	10^{-15}
6	ICR (M)	3	370	3700	10^{-18}

* Number of days elapsing between plasma injection and LDH determinations. † Five mice per group; intraperitoneal injection of 0.1 ml of infected plasma. ‡ The dilution is somewhat greater than this value (0.1 ml of 1/10 diluted plasma injected intraperitoneally into a 20-gm mouse is approximately 5×10^{-4} dilution per passage, assuming full carcass distribution).

Table 2. Typical plasma LDH values with time in normal recipient mice following injection of blood plasma from tumor-bearing mice compared with controls receiving normal plasma. There were five mice in each group. The plasma LDH ranges were similar to those in Table 3.

Tumor	Mouse strain	Average plasma LDH			
		0*	2*	7*	16*
Lewis lung carcinoma	C57BL (F)	460	3,700	8,100	6,200
None	C57BL (F)	380	370	610	740
Carcinoma 1025	AKR (F)	450	2,200	5,000	4,400
None	AKR (F)	320	260	310	350

* Number of days elapsing between plasma injection and LDH determinations.

Table 3. Spectrum of 26 mouse tumors exhibiting presence of a transmissible factor in host blood by intraperitoneal passage of "tumor" plasma to normal mice.

Tumor	Host*	Day†	Plasma LDH	
			Range	Average
None	ICR (M)	0	260– 510	380
None	C57BL (F)	0	260– 470	310
None	AKR (F)	0	400– 560	430
Sarcoma 180 (solid)	ICR (M)	4	3400–5900	4400
Sarcoma 180 (ascitic)	ICR (M)	4	2200–4400	3600
Mammary Adca EO-771	C57BL (F)	2	5800–6700	6400
Mecca lymphosarcoma	ICR (M)	3	3000–3400	3200
Miyono adenocarcinoma	ICR (M)	3	1600–2200	1700
Bashford carcinoma 63	ICR (M)	3	1700–3000	2200
Lewis lung carcinoma	C57BL (F)	2	3200–4200	3700
Carcinoma 1025	AKR (F)	2	1500–3000	2200
‡Friend virus leukemia	ICR (M)	5	2600–3800	3200
‡Friend virus leukemia (solid)	ICR (M)	2	2000–2900	2400
Cloudman S91 melanoma	ICR (M)	3	2900–4400	3700
Ehrlich carcinoma (solid)	ICR (M)	2	2000–2400	2300
Ehrlich carcinoma (ascitic)	ICR (M)	2	2400–3300	2800
Sarcoma MA 387	ICR (M)	4	2500–4000	3300
Leukemia L-1210	ICR (M)	4	3300–7800	4600
Leukemia L-4946	ICR (M)	4	2700–4600	3400
Nelson ascites	ICR (M)	4	3900–4900	4200
Harding-Passey melanoma	ICR (M)	2	1600–3300	2400
Glioma 26	ICR ((M)	2	1900–3200	3100
Lewis bladder carcinoma	ICR (M)	5	4200–5400	4500
Sarcoma T-241	ICR (M)	2	2000–3300	2700
Ridgway osteogenic sarcoma	ICR (M)	13	2500–4100	3000
Wagner osteogenic sarcoma	ICR (M)	13	2500–3100	2800
‡C3HBA mammary adenocarcinoma	ICR (M)	6	3200–4600	4000
‡Moloney leukemia	ICR (M)	4	2400–4000	3000
‡Spontaneous mammary tumors§	ICR (M)	3	2300–2900	2600

* Five mice per group. Mouse strain indicated is normal recipient but not necessarily tumor-bearing donor strain. † Number of days elapsing between plasma injection and LDH determination. ‡ Known or presumed virus-induced neoplasms. § Example of eight spontaneous tumors tested. Primary mammary adenocarcinomas arising in Swiss O'Grady females, old breeders.

Table 1 illustrates the serial transmission of the agent from another tumor type (Lewis lung carcinoma), transmitted by blood plasma only. The total dilution of the plasma at each passage is more than 1/1000, which means a theoretical dilution of the original inoculum of more than 10^{-18} after six consecutive transfers of the agent into normal animals. It would therefore appear that the agent must be replicated.

Table 2 illustrates the typical course of lactic dehydrogenase in the blood of normal mice that were injected intraperitoneally with 0.1 ml of a 1-to-10 dilution of the plasma from a mouse carrying either the Lewis lung carcinoma or carcinoma 1025. In this experiment the host strain required for the tumor was employed as detector, but similar qualitative results were obtained with the Swiss ICR strain. These data also show that the control animals receiving normal plasma were unaltered in their LDH blood titer.

Table 3 lists experimental tumors which have undergone tests for this transmissible enzyme-detectable factor. Control values are listed in the table for the various normal mouse strains employed. The data show that a five- to tenfold enzyme increase occurs in the plasma of the test mice receiving a dilute plasma injection from any of the various tumor-bearing animals. The illustrative values shown are typical of several hundred similar determinations. It may be of interest to note that no increase of plasma lactic dehydrogenase, or other abnormal alteration, was observed at any stage during mouse pregnancy. This suggests that rapidly growing embryonic or fetal tissue is distinct from malignant growth in respect to this parameter.

The possible implications of these findings to cancer etiology, diagnosis, and immunological and other rational approaches to therapy and prevention are self-evident to those inclined toward speculation. It would be premature, and possibly presumptive, however, to engage in such discussions in this brief report.

It might be appropriate, however, to discuss the aspect of these studies which has made the experimental observations possible. Heretofore, initial detection of virus, or virus-like agents, has been largely dependent upon the

production of disease, or of pathological lesions, either in the host or in cells in vitro. Likewise, searches for cancer agents have relied on manifestations of overt malignancy, probably under biologically unfavorable conditions. The finding that transmissible, replicating agents can be detected by the induction of a biochemical "lesion" in a normal host, in the form of an abnormal alteration in concentration of a blood enzyme, has apparently permitted the uncovering of agents masked or "silent" in reference to standard criteria. The tumor-producing potential of this factor is unknown at present, although it has been studied only in adult animals and for limited periods in respect to latency. The quantitative correlation of plasma lactic dehydrogenase with experimental mouse tumor growth and regression, however, is now firmly established (*3, 4*) and it seems quite clear that the transmissible enzyme elevation described here is identical to the phase-three plateau previously described as an initial part of the multiphase curve associated with tumor implantation and growth (*4*). The transmissible, enzyme-elevating factor probably also explains the failure of the host plasma lactic dehydrogenase to return to completely normal levels following tumor regression (*4*). Such a five- to tenfold abnormal elevation has persisted for periods exceeding a year following "complete" tumor regression and has been transmissible when such host plasma was injected into normal recipients (*5*).

VERNON RILEY, FRANK LILLY,
EDITHA HUERTO, DAVID BARDELL
Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, New York

References and Notes

1. O. Warburg and W. Christian, *Biochem. Z.* **287**, 291 (1936); F. Wroblewski and J. S. LaDue, *Proc. Soc. Exptl. Biol. Med.* **90**, 210 (1955).
2. V. Riley, *Proc. Soc. Exptl. Biol. Med.*, in press.
3. B. R. Hill and C. Levi, *Cancer Research* **14**, 513 (1954); K. M. Hsieh, V. Suntzeff, E. V. Cowdry, *ibid.* **16**, 237 (1956); C. Manso, K. Sugiura, F. Wroblewski, *ibid.* **18**, 682 (1958); F. Wroblewski, *Cancer* **12**, 27 (1959); V. Riley and F. Wroblewski, *Federation Proc.* **18**, 310 (1959).
4. V. Riley and F. Wroblewski, *Science* **132**, 151 (1960).
5. We are indebted to Drs. K. Sugiura, P. C. Merker, L. J. Old, D. W. Woolley, D. A. Clarke, H. Balner, G. S. Tarnowski, M. N. Teller, E. Hirschberg, D. J. Hutchison, and J. A. Jacquez for providing us with various tumor-bearing animals. The observations reported here are a by-product of collaborative studies with Felix Wroblewski on tumor-enzyme relationships, such as that recently reported (*4*). This is to acknowledge the importance of the catalytic role of Drs. Wroblewski and C. Chester Stock in making these findings possible. Valuable assistance was also provided through the SKI Volunteer Office by Eleanor Hevesi, Charles Forman, Henry Abrahams, and Winifred Powers.

18 July 1960

Effect of Pyramidal Tract Activity on Dorsal Column Nuclei

Abstract. The response of single units in cuneate and gracile nuclei to cutaneous stimulation can be modified by prior stimulation of the motor cortex of the cat. Both excitation and inhibition of these neurons can be effected via the pyramidal tract.

Direct cortical projections to various sensory nuclei of higher mammals have recently been described (*1*). Although the anatomical descriptions differ slightly, it is evident that the gracile, cuneate, and spinal trigeminal nuclei are abundantly supplied with corticofugal fibers coursing within the pyramidal tract. Concurrent physiological studies (*2*) have shown a depressive effect of central structures on peripherally evoked potentials in the dorsal root (DR reflex), dorsal column relays, dorsal column nuclei, spinal trigeminal nucleus, cochlear nucleus, and the olfactory bulb. Single unit analysis (*3*) of the spinal afferent paths and of the retina, however, have shown not only an inhibition but also an excitation from central structures. We have previously shown (*4*) that the motor cortex of the cat has both an excitatory and an inhibitory influence on cuneate neurons. Responses in some cuneate neurons could be evoked by single shocks (0.02 to 2.0 msec in duration) to the pericruciate cortex, the latencies ranging from 5 to 30 msec. Other cuneate neurons were rendered less excitable for periods of 100 to 200 msec, beginning 10 to 30 msec after a single shock or a train of shocks (300 per second) to the motor cortex. The inhibition was manifested by changes in the response properties of the affected neuron when the footpad was stimulated (decreased probability of response, increased initial spike latency

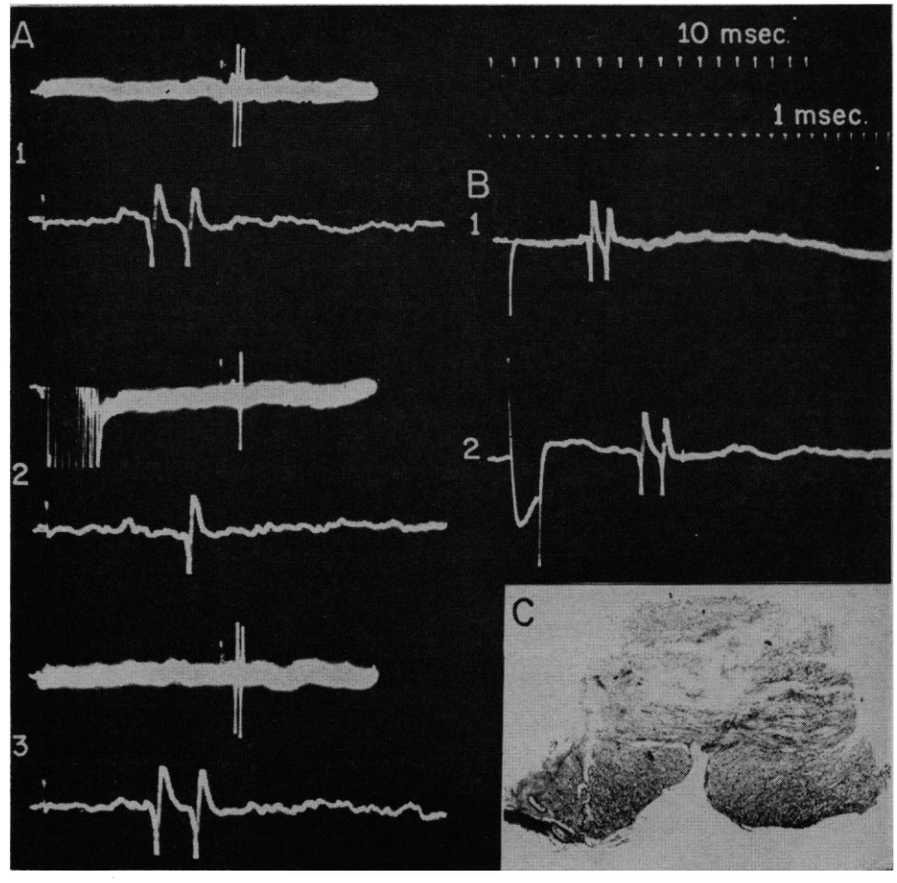

Fig. 1. Responses of two cuneate units recorded after brain-stem transection. (*A*) Inhibitory interaction. Each pair of sweeps shows same event; the upper, slow sweep was triggered 85 msec before the lower, fast sweep. Traces (1) and (3) response of unit in left cuneate nucleus to left forepaw stimulation. (2) Increased latency and decreased number of spikes per discharge to the same forepaw stimulus after a conditioning train (eight shocks at a rate of 312 shock/sec) to the right motor cortex. (*B*) Response of another unit in left cuneate nucleus to peripheral and cortical stimulation. (1) Fired by ipsilateral forepaw shock. (2) Fired by shock to right motor cortex. The 1 msec time trace applies to the fast sweeps in (*A*) and all sweeps in (*B*). (*C*) Luxol-fast section of what remained of the pyramidal tract after transection. Note that lateral borders of the pyramidal tract are damaged (× 17).