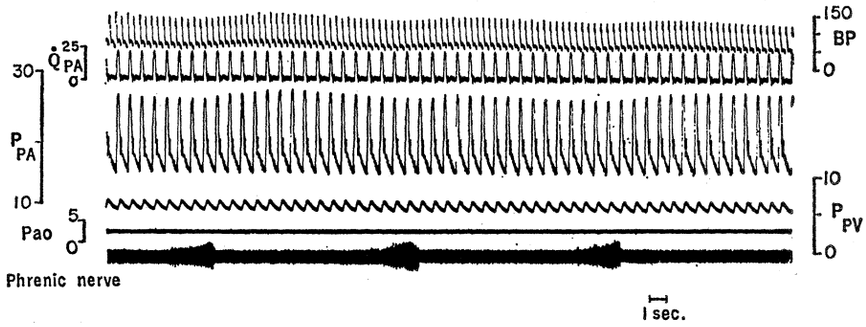

Fig. 2. Representative records of Traube-Hering vasomotor waves and phrenic nerve activity. The systemic arterial blood pressure (*BP*) and the pulmonary arterial blood pressure (P_{PA}) show regular oscillations of about 10 mm-Hg. The swings in pressure in both circuits are synchronous and are preceded by a volley of phrenic nerve activity. Blood flow through the isolated lobe ($\dot{Q}_{PA}$), pulmonary venous pressure (P_{PV}), and airway pressure (P_{ao}) are maintained constant. Pressures are shown in millimeters of mercury; $\dot{Q}_{PA}$, in milliliters per second.

respirators and connecting both sides of the tracheal divider to O_2 reservoirs). Since ventilation was arrested and airway pressure remained unchanged, the passive effects of lung inflation on pulmonary arterial pressure could not be involved in producing the waves in blood pressure. However, in order to determine whether the fluctuations in pulmonary arterial pressure were related to respiratory activity even though there were no respiratory movements, the neurogram of the cervical portion of the phrenic nerve, recorded with bipolar platinum electrodes, was used to indicate efferent respiratory activity.

Figure 2 shows an example of the cyclic variations in systemic and pulmonary artery pressure observed during apneic oxygenation in five dogs. Each wave in systemic and pulmonary artery pressure is preceded by a volley of phrenic nerve activity, indicating that the blood pressure oscillations are Traube-Hering waves (*10*). Figure 2 also shows that only the systolic pressure in the pulmonary artery is cycling while diastolic pressure remains constant. This is the characteristic pressor effect of sympathetic nervous stimulation on the pulmonary circulation, that is, an increase in pulmonary arterial systolic pressure despite constant pulmonary blood flow.

Except for potential anastomotic connections between the pulmonary and bronchial (systemic) vessels in the lungs, the pulmonary circulation in the isolated lobe was separate from the rest of the circulation. Such connections have never been shown to be of hemodynamic significance in the normal lung. Moreover, in the preparation used in the present study, ligation of the bronchial arteries consistently failed to produce any effect on the pulmonary arterial pressure. Conversely, during stimulation of the sympathetic nerves to the lungs, a pressor response could be demonstrated in the pulmonary circulation even though systemic blood pressure did not change (*9*). Because of the functional independence of the pulmonary and systemic circulations, and since pulmonary blood flow, pulmonary venous pressure, and airway pressure were constant, the pulmonary arterial pressor waves could not have been caused by the systemic arterial waves or by rhythmic changes in blood flow or by the passive mechanical effects of ventilation. Instead, they seemed to be caused by rhythmic changes in vasomotor tone, originating, as in the case of Traube-Hering waves in the systemic circulation, in the respiratory center and affecting the smooth muscle of the pulmonary arterial tree by way of the pulmonary sympathetic nerves.

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Mammary Tumor Virus Antigen: Sensitive Immunoassay

Abstract. *A rapid, sensitive immunoassay for mammary tumor virus antigen based on inhibition of passive hemagglutination has been developed. The method permits measurement of this antigen in mouse milk from which the fat has been removed.*

Quantitative biological assay of mammary tumor virus (MTV) involves the induction of nodules or tumors in virus-free mice after a latency period of several months. Other sensitive and specific in vitro immunological methods have been used to detect MTV (*1*), but none of these is adaptable to its titration. We developed a passive hemagglutination test in which tanned sheep erythrocytes coated with MTV antigen were agglutinated in the presence of antibody to the virus (*2*). The method provided a highly sensitive technique for measuring antibody in serums of animals immunized with purified MTV. We now describe the adaptation of this to a hemagglutination-inhibition test to measure MTV antigen.

The methods were based on those developed for the titration of a murine leukemia virus (*3*). The MTV antigen was prepared from samples of skimmed milk from C_3H/HeN mice by either of two methods. The first involves differential centrifugation and banding of MTV on Ficoll-sucrose density gradients (*2*); the second consists of rate-zonal centrifugation followed by isopycnic banding on linear gradients of Ficoll in heavy water (*4*). Examination of preparations with the electron microscope, especially with the latter technique, shows a preparation in which almost all of the observable forms are typical intact MTV B particles (Fig. 1).

For sensitization of the sheep cells, the purified virus suspension must be treated with ether at 4°C, three volumes of (anesthetic grade) ethyl ether being added to one volume of virus. The mixture was agitated for 30 seconds in a cyclomixer six times over a period of 30 minutes. Thirty minutes later, the ether was removed in a stream of nitrogen and the residual material was used for coating tanned sheep cells. Antiserums were prepared in rabbits or rhesus monkeys and absorbed with sheep erythrocytes and normal mouse tissues (*2*).

Cells coated with antigen at the optimum dilution of 1:2 were prepared and used on the same day. All titra-

tions were carried out by the microtiter technique with the use of disposable plastic plates (*5*). Standard rabbit antiserum (0.025 ml) to MTV was diluted serially in *p*H 6.4 phosphate-buffered saline containing 1 percent normal rabbit serum. To each dilution in the series was added 0.025 ml of 0.6 percent sensitized sheep cells. After incubation at room temperature for 2 hours, titers were recorded as the highest dilution of serum which produced complete hemagglutination. For the hemagglutination inhibition tests, the unit of antibody or highest dilution of serum necessary to produce complete hemagglutination was determined. Materials to be assayed for viral antigen were diluted serially in 0.025-ml portions in the phosphate-buffered saline and serum described above. Then 0.05 ml of serum, diluted to contain 2 units of antibody, was added to each dilution in the series. The mixture was incubated for 30 minutes at room temperature before the addition of sensitized sheep cells. The results of the tests were read after 3 hours at room temperature, and the highest dilution of the preparation showing definite inhibition was the endpoint. Control inhibition tests with standard purified virus were performed on each testing date; diluent and cell controls accompanied each test. All specimens were also tested by microimmunodiffusion for the presence of MTV antigen with rhesus monkey antiserum to MTV (*2*).

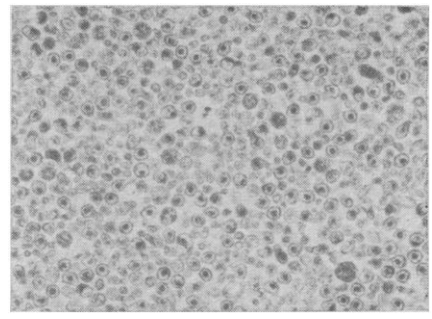

Fig. 1. Thin section of typical pellet from C_3H/HeN mouse milk obtained by high-speed centrifugation of material which banded at a density of 1.156 in Ficoll-D_2O gradient. The pellet is composed almost exclusively of mammary tumor virus (MTV) B particles (× 20,000).

Tests for viral specificity of the hemagglutination reaction showed that rabbit antiserum to MTV consistently gave titers of 1:256 or higher with sheep cells sensitized with several preparations of purified virus; normal rabbit serum failed to react. The reaction was inhibited to high dilution by purified virus and the skimmed milk from a virus-carrying mouse strain (C_3H/HeN), but not by the milk from BALB/c mice, which are virus free. Furthermore, rabbit antiserum to MTV did not react with sheep cells sensitized with a preparation of skimmed milk (purified by density-gradient centrifugation) from virus-free BALB/c mice.

With the viral specificity of the reaction confirmed, a total of 17 milk samples from MTV-positive and MTV-negative mice were obtained from three laboratories (*6*). These samples, tested as unknowns, were correctly identified as being derived from MTV-positive or MTV-negative strains. All results were confirmed by microimmunodiffusion tests with rhesus monkey antiserum.

After identification, titrations for viral antigen in milk samples were performed by hemagglutination inhibition, and the results are given in Table 1. Based on the known occurrence of MTV in the milk and the incidence of mammary tumors in the mouse strains, both the hemagglutination inhibition and precipitation reactions are consistent with those expected. The lack of close correlation between hemagglutination inhibition titers and mammary tumor incidence in certain highly susceptible mouse strains indicates that genetic susceptibility of the strain, as well as the amount of virus, have an important bearing on tumor incidence. The milk of the $C_3H \cdot A^{vy}fB$ strain, which has a 90 percent tumor incidence, apparently did not contain enough viral antigen to be detected by this assay. Nevertheless, the sensitivity of the inhibition reaction is attested to by the fact that diluted (1:256 to 1:2048) skimmed milk which contained the antigen inhibited the reaction. Parallel hemagglutination-inhibition tests with untreated and ether-treated milk containing the antigen showed that, in general, titers were somewhat increased after treatment; the immunodiffusion reaction was unaffected by ether treatment.

The capacity of tanned sheep cells to adsorb an antigen (or antigens) of MTV forms the basis of a system for the rapid assay of the antigen. Although we have not yet characterized the sensitizing substance, the fact that the quantity increases after ether treatment suggests that it is probably a subviral structural unit of the MTV virion.

Table 1. Hemagglutination-inhibition (HAI) assay of antigen in untreated mouse skim milks. The tests were performed with 2 units of rabbit anti-serum to MTV. The presence of B particles was determined by electron microscopic examination.

Mouse strain	Tumor incidence (%)	B particles	HAI titer (reciprocal)	Precipitation with monkey antiserum to MTV
	*Milk from IMR mice**			
BALB/c/Crgl	20	0	< 8	0
AfB	30	0	< 8	0
RIIIfB	3	0	< 8	0
A	94	+	2048	+
BALBcfC$_3$H/Crgl	84	+	1024	+
C$_{57}$BLfRIII	60	+	1024	+
DBA—RIII	97	+	1024	+
RIII/Haag	83	+	1024	+
C$_{57}$BL/Haag	0	0	< 8	0
C$_3$HfB/Crgl	44	+	1024	+
	Milk from NCI mice†			
$C_3H \cdot A^{vy}$	100	+	256	+
$C_3H \cdot A^{vy}fB$	90	0	< 8	0
BALB/c	20	0	< 8	0
BALB/cfDD	97	+	2048	+
	Milk from NIH mice‡			
BALB/c	2	0	< 8	0
DBF-1	?	0	< 8	0
C_3H/HeN	95	+	1024	+

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Lysergic Acid Diethylamide: Dissociation of Its Behavioral and Hyperthermic Actions by DL-α-Methyl-p-Tyrosine

Abstract. *Rabbits treated with LSD 25 exhibit characteristic signs of hyperexcitability, increased peripheral sympathetic activity, and hyperthermia. When the rabbits received prior treatment with* DL-α-*methyl*-p-*tyrosine, the excitation and sympathetic actions of LSD 25 were abolished or attenuated, but the hyperthermia was unchanged from that of the controls. Concentrations of norepinephrine in brain stems of treated rabbits were greatly decreased. The excitation of central nervous system and sympathomimetic actions of LSD 25 in the rabbit are apparently mediated by norepinephrine, whereas the hyperthermic action functions through a nonadrenergic mechanism.*

In experimental animals lysergic acid diethylamide (LSD 25) produces a dose-related hyperthermia (*1*). In rabbits this response is accompanied by physiological and behavioral changes, such as increased motor activity, excitation, pupillary dilatation, and hypersalivation. This excitatory syndrome (*2*), including the hyperthermia, is in some ways similar to the psychic effect in man (*3*). Although the mechanism of behavioral action of LSD 25 is unknown, it has been suggested by indirect evidence only that the catecholamines might take part in an intermediate step (see *4*).

The inhibitor of tyrosine hydroxylase, DL-α-methyl-*p*-tyrosine (*5*) (α-MT), when given in repeated doses, causes the depletion of norepinephrine in a variety of tissues by inhibiting the conversion of tyrosine to dihydroxyphenylalanine. If norepinephrine is involved in the excitatory syndrome, animals so treated should respond to LSD 25 in a modified manner.

Male albino rabbits (1.5 to 2.5 kg) were divided into six groups as follows: (i) those given only saline, (ii) those given polyethylene glycol solvent and saline, (iii) those given LSD 25, (iv) those given solvent and LSD 25, (v) those previously treated with α-MT and then given saline, and (vi) those previously treated with α-MT and then given LSD 25. Rabbits restrained in open stanchions (*6*) were intraperitoneally injected with α-MT (80 mg/kg), suspended in a 10 percent solution of polyethylene glycol 300, every 4 hours for 24 hours; LSD 25 was then given to control and previously treated animals, as well as to animals previously treated with solvent, and their behavior was recorded. Changes in rectal temperature were also monitored by a YSI model 47 telethermometer. Concentrations of norepinephrine in brain stems of animals treated with α-MT were compared with those of controls and of rabbits treated with solvent. Norepinephrine was determined by a modification of the method of Moore and Rech (*7*).

The intravenous injection of LSD 25 (100 μg/kg) to control rabbits results in pupillary dilatation, hyperventilation, increased motor activity, and an increase in rectal temperature (Fig. 1). Although the hyperexcitability subsides about 2 hours after administration of LSD 25, the hyperthermia gradually returns to normal in approximately 6 hours. Animals first treated with α-MT are sedated, but they exhibit functional reflexes and movements. The administration of LSD 25 to such animals results in very little behavioral change. A slight degree of increased respiration occurs in some cases, but generally the animals remain sedated. The effect of LSD 25 on rectal temperature, however, is present to the same extent as in the control animals; the peak response of 1.5° to 2.0°C occurs within 2 hours, the effect gradually subsiding within the subsequent 4 to 6 hours.

Between the rabbits that received only LSD and those treated with α-MT and LSD, there were no significant differences in temperature changes at any of the designated times, when analyzed by the *t*-test. Although the behavioral and sympathomimetic actions of LSD 25 were not affected in the controls that received polyethylene glycol, the hyperthermic response was slightly but consistently lower in those rabbits. Statistically, however, rabbits treated with the LSD and those treated with the glycol and LSD differed significantly ($P < .05$) only at the 15-minute and 4-hour readings. The nature of the apparent antipyretic action of polyethylene glycol is not known, but by itself it does not seem to produce a hypothermic action (Fig. 1). Rather, animals first treated with solvent tend to have slightly higher initial temperatures.

The amounts of norepinephrine in brain stems of controls and animals first treated with α-MT were assayed to

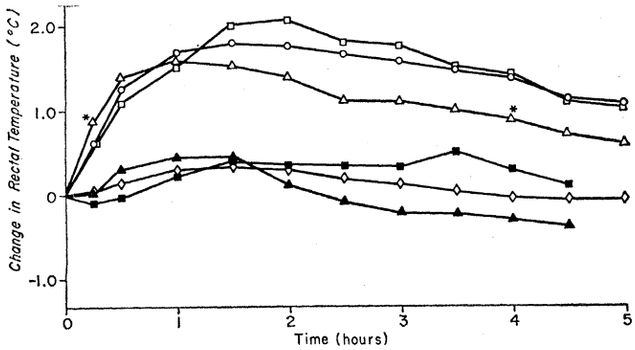

Fig. 1. Changes in rectal temperature in response to treatment with LSD in controls and rabbits first treated with α-MT. Solvent (10 percent polyethylene glycol 300) or α-MT suspended in solvent was injected intraperitoneally every 4 hours for 24 hours before administration of LSD or saline at zero time. Each curve represents the mean changes observed in 6 to 14 rabbits: (open circles) LSD ($n = 14$), (open triangles) solvent first then + LSD ($n = 10$), (open squares) α-MT first then LSD ($n = 9$), (open diamonds) saline ($n = 8$), (solid triangles) solvent first then saline ($n = 9$), and (solid squares) α-MT first then saline ($n = 6$). Asterisk denotes values which are significantly different from controls ($P < .05$).