sonically treated thromboplastin subsequently incubated with TPase lost its lethal potency for rabbits.

Discussion. The results of this study lend further support to the assumption(2) that TPase-labile moiety of thromboplastin and its *in vivo* toxicity are related. As in the case of heat inactivated thromboplastic suspensions, loss in clotting potency after ultrasonic treatment is not reflected by a concomitant loss in lethal potency for rabbits. In addition, the data indicate that there are at least 2 active thromboplastic moieties in tissue suspensions. By clotting measurements, the TPase-treated thromboplastin used in these studies was almost identical to that of the ultrasonically treated suspension yet the former was no longer toxic to rabbits while the latter had lost none of its toxicity. Quick (5,6) has presented evidence suggesting that brain thromboplastic extracts have a heat labile factor which acts directly with prothrombin, and a heat stable factor requiring a plasma factor to produce active thromboplastin. Whether the TPase labile factor is the same as, or distinct from, the heat stable factor is not known. However, the former appears to be important in producing the lethal effects seen upon injection of thromboplastin into experimental animals. In addition, it is apparent that clotting potency *per se,* as measured by *in vitro* clot acceleration, gives no indication of the physiological activity of tissue thromboplastins. The amount of phosphorus liberated by TPase under standard conditions appears to be a much better criterion.

Summary. The effects of ultrasonic treatment on human brain thromboplastic suspensions have been presented. Although causing a loss in clotting potency, ultrasonic treatment does not affect the toxicity of intravenous tissue thromboplastin nor has it destroyed the TPase-labile component. The results are discussed and possible implications presented.

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Method of Chorioallantoic Membrane Inoculation which Decreases Nonspecific Lesions. (23056)

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Since the original method of chorioallantoic membrane (CAM) inoculation was introduced by Goodpasture(1), numerous variations for preparing eggs for this route of inoculation have followed(2-5). The preparation of embryonated eggs for CAM inoculation with pock inducing viruses is of considerable importance if accurate counts of the pocks are to be made(5-7). Nonspecific lesions due to trauma invariably appear when eggs are prepared for CAM inoculation. They may be large enough to obscure the field to be counted or small enough to be mistaken for specific lesions. Their presence can be a source of variation in pock enumeration and differentiation and can influence the accuracy of pock titrations.

The purpose of this paper is to present a modification of the technic of CAM inoculation which greatly reduces nonspecific lesions. A statistical assessment of the accuracy of this method is reported.

Materials and methods. Virus and seed preparations. The virus used in this study

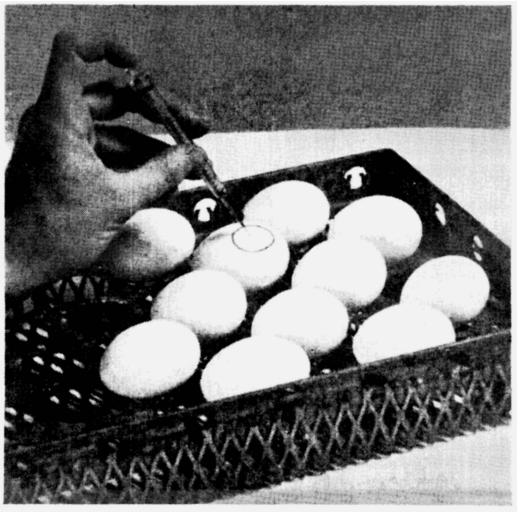

FIG. 1. Inoculation of CAM over rotated air space.

was the Yamada strain of variola virus obtained through the courtesy of Dr. Joseph E. Smadel, Walter Reed Army Institute for Research, Washington, D.C. It was isolated from a Japanese patient with a moderately severe case of smallpox and when received was in the second CAM passage. The virus was passed 3 times on the CAM of 11-12-day-old white leghorn eggs. On the sixth passage, eggs were inoculated with approximately 5000 infectious units per 0.05 ml and incubated at 35°C for 48 hours. A working pool of the virus was obtained by harvesting the infected membranes. A 20% suspension of harvested membranes in heart infusion broth, pH 7.0, was emulsified in a Waring blendor for 3 minutes and centrifuged for 10 minutes at 2000 rpm in an angle head centrifuge. The supernatant was stored in sealed ampoules in a dry ice chest at −70°C. Technic of preparing eggs for CAM inoculation. 1. Eggs were candled and an area of the CAM was selected which was well developed. Areas over large blood vessels were avoided. 2. Over the center of the air space a small hole was drilled through the shell with a Vibro-Tool* (V-3 hard tantalum carbide point). 3. A circle was outlined over the selected CAM area with the Vibro-Tool. The circle was drilled deep enough to loosen the flap of shell but not enough to penetrate the shell membrane. 4. The circular shell flap was detached from the shell membrane with a half spear point dissecting needle. 5. A drop of sterile saline was placed on the exposed shell membrane and a slit 1-2 mm long was made in the fibers of the membrane with a sterile dissecting needle. 6. After the saline had been in contact with the membrane for 1-2 minutes, the CAM was dropped by gentle suction with a rubber bulb thus creating an artificial air space. The suction was applied over the opening previously drilled in the air space. This was done in the beam of an egg candler so that the size of the air space could be visualized and controlled. The artificial air space was usually 20-25 mm in diameter. 7. The egg was rotated as soon as the artificial air space was formed. The newly formed air space was moved from the site of preparation to a well developed area of the CAM. This rotation was made to the left or right, depending on the location of the area of the CAM. The position of the artificial air space was then outlined. 8. A minute opening was drilled in the shell with the Vibro-Tool, over the area of the artificial air space. This opening was used for the introduction of inoculum. 9. The inoculum, 0.05 ml, was introduced into the egg (Fig. 1) from a 1 ml Luer-Lok tuberculin syringe fitted with a #26 or 27 gauge ¼ inch needle. The inoculated eggs were tilted slightly to distribute the inoculum evenly over the dropped area of the CAM. The eggs were then placed on their long axis in trays with the artificial air space uppermost and incubated undisturbed at 35°C for 72 hours. Leaving the openings in the shell unsealed in no way affected the viability of the egg or the formation of pocks. A few precautionary measures should be followed in this method. The slit in the shell membrane should be as small as possible, since the shell membrane is used as a natural seal when the eggs are rotated. In addition, in step 6, the artificial air space should be formed not later than 5 minutes after the application of the saline. It has been our experience that the exposed membranes dry rapidly. Undue delay makes the formation

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TABLE I. Statistical Evaluation of 2 Technics of CAM Inoculation Employed for Calculation of Infectious Units of Variola Derived from Pock Counts.

	Non-rotated air space	Rotated air space
	Infectious units/ml ($\times 10^7$)	
	6.0	5.4
	3.6	7.6
	8.6	10.0
	9.4	11.0
	5.0	4.0
	4.8	9.2
	3.6	11.0
	5.0	8.6
	5.8	6.0
	5.0	6.2
	2.4	8.0
	2.2	6.2
	3.4	5.2
	1.4	5.2
	4.0	9.8
	6.0	6.8
	4.2	8.4
	2.8	7.2
	6.0	5.4
	4.8	11.2
	5.2	7.4
Mean	4.7×10^7	7.6×10^7
Stand. dev.	$\pm$ 1.93	$\pm$ 2.14
Stand. error of mean	$\pm$ 0.42	$\pm$ 0.46

of the artificial air space difficult and often causes it to be irregular in shape. Eggs were also prepared for CAM inoculation by the conventional method. In this procedure, the artificial air space was not rotated after the CAM was dropped. The inoculum was introduced onto the CAM through the exposed shell membrane. Virus titrations were carried out as follows: serial 10-fold dilutions ranging from 10^{-1} to 10^{-5} were made in heart infusion broth which contained 500 units of penicillin and 100 μg of streptomycin per ml. Virus dilutions 10^{-4} and 10^{-5} were inoculated on the CAM of 11-12-day-old embryonated eggs in quantities of 0.05 ml per egg by the methods described. Seven to 8 eggs were inoculated per dilution and incubated at 35°C. After 72 hours incubation, the CAM was removed from the eggs, washed and floated in Petri dishes containing formol saline. The pocks were counted with the aid of an illuminator. This instrument facilitates the examination of membranes by passing a reflected light across a Petri dish and illuminates the pocks by the darkfield principle. The number of infectious units per ml of the suspension was determined by counting the number of pocks per membrane and calculating for the dilution. Usually 7-8 membranes per dilution were counted. The average number of pocks per membrane was multiplied by 20 (for conversion to a ml basis) and by the dilution factor. The membranes employed to determine the concentration of infectious units were those which contained between 0 and 100 pocks.

Results. Accuracy of method. A total of 42 titrations were made to test the difference between the treatments employing rotated and non-rotated eggs. Twenty-one titrations were made with each treatment. One virus pool was used throughout the test and the dilution technics, inoculation, incubation and pock counting were kept standard.

Results of the treatments and statistical calculations are shown in Table I. Different mean values were apparent for both treatments, with a higher mean being obtained for the rotated method. A test of significance was made to evaluate the difference between the means of both treatments. The t value obtained was 4.580 for 40 degrees of freedom. The probability was less than 1 in 1000 that this value could occur by chance alone. The difference between the means was highly significant. The incidence of non-specific lesions in the non-rotated treatment was 66% higher than in the rotated treatment.

Discussion. The mean value found when the method of rotating the artificial air space was used was significantly higher than the value for the non-rotated method of CAM inoculation. The higher mean value obtained was probably due to the low incidence (4%) of nonspecific lesions. The non-rotated treatment induced a 70% incidence of nonspecific lesions which probably retarded or obscured specific pock lesions. Traumatic lesions generally occur in the area where the CAM is initially dropped. By rotation of the artificial air space to a new area of the CAM, the nonspecific lesions which may appear are left in the initial CAM area. Consequently, they do not interfere with pock formation or counting. A great deal of experience is not re-

quired to prepare a CAM area free of traumatic lesions with this method.

The principal advantage of this new method is that it reduces nonspecific lesions due to trauma, thereby increasing the accuracy of titrations. The reduction of nonspecific lesions also facilitates detection of pock inducing viruses in diagnostic work which may be masked by nonspecific lesions.

Summary. A modified method of CAM inoculation is described which greatly reduces nonspecific lesions and increases the accuracy of titrations.

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Effect of Estradiol on Fibrinolytic Activity of Rat Uterus.* (23057)

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The rat uterus contains an activator of plasminogen(1). The present investigation demonstrates a correlation between concentration of the tissue activator in the rat uterus and the estrous cycle.

Methods. Concentration of the tissue activator of plasminogen in the uterus was estimated by the quantitative and selective method described previously(2). In principle the activator is extracted with potassium thiocyanate solution, precipitated at acid reaction, redissolved and the concentration estimated by fibrin plate method(3). The activator concentration is calculated in units of a standard preparation/gram fresh tissue. The estradiol dipropionate solution contained 20 μg/ml in olive oil. White rats weighing 200-300 g were used. The weight of animals, macroscopic appearance and weight of the uterus and microscopic appearance of the vaginal smear were recorded. Concentration and amount of tissue activator in 3 series of animals (total 32) are presented in Table I. Six animals were killed immediately with chloroform and tissue activator concentration in the uterus estimated as described. Twenty-six animals were ovariectomized under ether anesthesia. Eleven of these, after 4-7 weeks without further treatment, were killed with chloroform and the uterus immediately removed and investigated. Eight of the ovariectomized animals were given 1 ml estradiol dipropionate solution intramuscularly every third day (from 5-11 times). Animals were killed in early estrus some days after the last injection, after examination of the vaginal smear (Allen-Doisy). The uterus was then removed and investigated. Seven of the ovariectomized animals were given 1 ml estradiol dipropionate solution intramuscularly every third day (from 2 to 4 times). The animals were then kept until vaginal smear showed them to be in late estrus (after 17 to 56 days). They were then killed and the uterus analysed. Additional confirmative control experiments are not included in the Table.

Results. The present investigation concerns tissue activator concentration in the rat uterus during the estrous cycle in the hope of confirming experimentally previous results with samples of human endometrium (in different stages of menstrual cycle) and human

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