connecting strand is easily broken yielding fairly dispersed virus.

Summary. Growth curve studies indicate that the m.o.i. plays a major role in the replication and resultant quantity of VSV. Consistent high yields of the virus can be obtained when the m.o.i. is kept at approximately 1. Natural aggregation studies using the count technique indicate that the virus is extruded from the cells in loosely connected chains. The agar sedimentation procedure for counting virus particles can be readily adapted to vesicular stomatitis virus with high efficiency.

The author is most grateful for the excellent technical assistance of Mary-Martin Bratcher.

1. Berlin, B. S., Proc. Soc. Exp. Biol. and Med., 1964, v117, 864.
2. Galasso, G. J., Sharp, D. G., J. Bact., 1964, v88, 433.
3. Hackett, Adeline, Virology, 1964, v24, 51.
4. Pittman, D., Shechmeister, I. L., St. John, R. C., Radiation Res., 1965, v24, 337.
5. Galasso, G. J., Sharp, D. G., J. Immunol., 1962, v88, 339.
6. Sharp, D. G., Lab. Invest., 1965, v14, 831.
7. Sharp, D. G., Smith, K. O., Proc. Soc. Exp. Biol. and Med., 1960, v104, 167.
8. Pittman, D., St. John, R. C., Shechmeister, I. L., Nature, 1965, v206, 1228.
9. Franklin, R. M., Virology, 1958, v5, 408.
10. Wagner, R. R., Levy, A. H., Snyder, R. M., Ratcliff, G. A., Jr., Hyatt, D. F., J. Immunol., 1963, v91, 112.
11. Howatson, A. F., Whitmore, G. F., Virology, 1962, v16, 466.
12. Mussgay, M., Weibel, J., J. Cell Biol., 1963, v16, 119.
13. Stone, R. S., Sellers, M. I., Hiramoto, T., J. Appl. Phys., 1961, v32, 1639.
14. Reczko, E., Arch. Ges. Virusforsch., 1960, v10, 588.
15. Hackett, Adeline, Madin, S. H., Schaffer, F. L., Bact. Proc., 1966, v66, 123.
16. Huang, Alice, Wagner, R. R., Fed. Proc., 1966, v25, 491.

Received July 25, 1966. P.S.E.B.M., 1967, v124

A Comparison of Calcium Dependent Contraction in Glycerinated Uterine Smooth Muscle and Cardiac Muscle. (31663)

SYDNEY R. JONES AND ARTHUR H. BRIGGS
Department of Pharmacology, University of Mississippi Medical Center, Jackson

Initially work in this laboratory showed that adenosine triphosphate (ATP) and magnesium (Mg) were necessary for contraction in glycerinated guinea pig uterine fibers(1). It was noted that in approximately 25% of freshly extracted fibers (4 days to 1 month) but not in older fibers calcium (Ca) produced a slight increase in tension following maximum contraction with ATP and Mg. It was suggested that this may be the result of the presence of a small amount of a labile relaxing factor which survived the extraction procedure. However, subsequent studies revealed that not only ATP and Mg were necessary for contraction but a small amount of Ca had to be present and that Ca contaminating the fibers was enough in most cases to produce maximum contraction in the presence of ATP and Mg. This suggested the possibility that the Ca dependent contraction in uterine muscle may be the result of a factor(s) similar to that described in skeletal and cardiac muscle (3,4). This factor(s) which is probably protein in nature can be extracted from skeletal and cardiac muscle and is lost during extraction in glycerol in cardiac muscle(5,6). For these reasons a study was done to compare the effects of prolonged extraction in glycerol on the calcium dependent contraction in uterine smooth muscle and cardiac muscle.

In addition, it was noted that glycerinated uterine fibers from estrone pretreated guinea pigs developed tensions of approximately 100 g/cm^2. It has been reported that estrogen treatment not only increases the concentration of extractable actomysin but also increases the synthesis of proteolytic enzymes in the lysosomes. Szent-Gyorgyi reported that

progesterone in high concentrations prevents the release of these enzymes from the lysosomes and thereby prevents the breakdown of contractible protein and can increase the amount of extractable actomysin from the uterus(7). Thus he suggested the possibility that the low tension generated by the glycerinated uterine fibers could be the result of lysosome breakdown and that progesterone in high concentrations may prevent this breakdown and thus increase the tension generated. For this reason in this study we also investigated the tension generated by glycerinated uterine fibers extracted with and without high concentrations of progesterone.

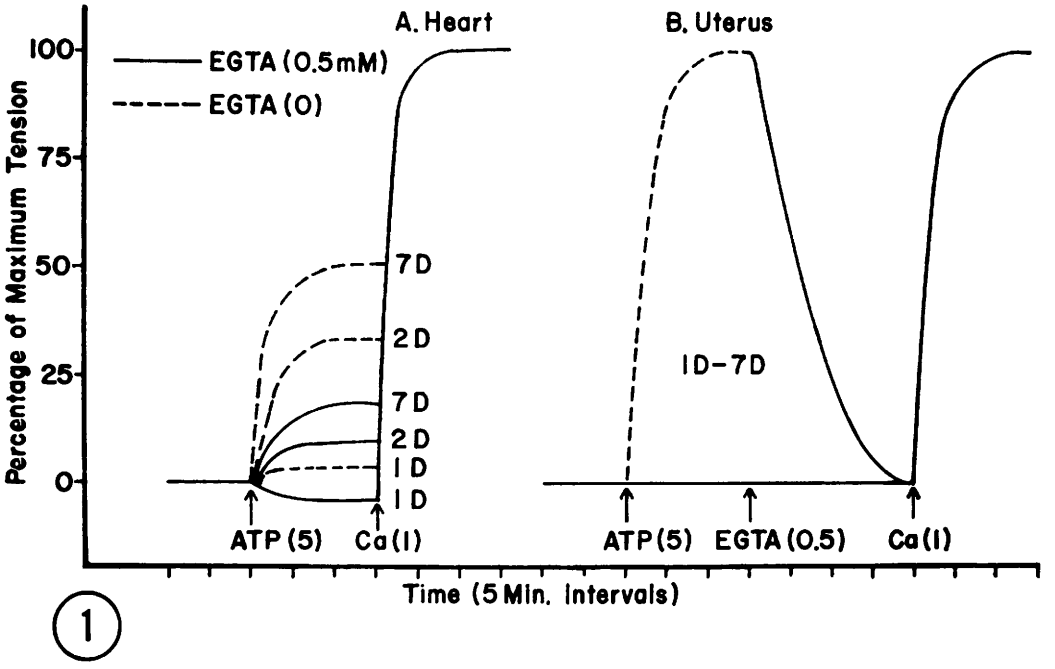
Methods. Mature non-pregnant guinea pigs were used. The animals were injected with 12 mg esterone in oil intramuscularly daily for 3 days. On the evening of the last day of treatment the guinea pigs were stunned by a blow on the head and both horns of the uterus were rapidly removed. The uterine horns were then tied to hardwood applicator sticks at the approximate *in vivo* length and placed in extraction solution containing 50% glycerol (v/v) with 100 mM histidine buffer pH 7.0 with and without progesterone (1 mg/ml). All procedures were carried out in the cold room at 4°C. The preparations were kept in the cold room from 2-27 hours prior to storage at —20°C or use. Preparations extracted in progesterone were used after extraction in the cold room for 10 minutes to 24 hours (no differences were noted). Strips were prepared for contraction as described previously(1,2). The contraction solution in which the fibers developed tension contained 20 mM histidine, 50 mM potassium chloride and various concentrations of other ions and substances as indicated. All experiments were carried out at room temperature 24°C. Stock solutions (100 mM) of Tris-ATP, [Ethylenebis (oxyethylenenitrilo)] tetraacetic acid (EGTA) at the desired pH's were prepared and added to the contraction solutions to attain the desired concentrations. Under the conditions of our experiments EGTA has a high affinity for Ca, but the binding of Mg is negligible (pK of Mg for EGTA at pH 6.65 is 0.882)(8). Double distilled water was used throughout for all solutions.

In the experiments with fibers which had been extracted with progesterone, progesterone (1 mg/ml) was also present in the contraction solution.

Dog glycerinated cardiac muscle fibers were prepared as previously described(6), and the extraction and contraction solutions were the same as above.

Results. Fig. 1 shows the typical effects of ATP, EGTA and Ca on glycerinated cardiac and uterine fibers extracted at —20°C from one to 7 days. The contraction solution contained 5 mM Mg. In this figure and the following one, we have expressed the tension as the percentage of maximum tension that can be obtained in the presence of ATP, Mg, and Ca. Each line represents a typical result obtained in the series of 5 to 12 experiments. Note that in cardiac fibers extracted for one day, in the absence of EGTA ATP produces a small contraction, and 1 mM Ca produces maximum contraction (Fig. 1A). On further extractions (2 and 7 days) the percentage of maximum tension increases on addition of ATP alone. Note that the addition of the calcium chelating agent EGTA decreases the percentage of maximum tension developed with the addition of ATP alone in each case. In contrast, in glycerinated uterine fibers, Mg and ATP produce essentially 100% of the maximum tension. EGTA produces complete relaxation and Ca maximum contraction. No differences occurred among fibers extracted from one to 7 days.

Fig. 2 compares the effects of extraction in glycerol from one day to 12 weeks in cardiac and uterine muscle fibers. In these experiments the contraction solutions contained 5 mM Mg and 0.5 mM EGTA. Note that in cardiac fibers ATP produces initial relaxation in fibers extracted for one day and 1 mM Ca produces maximum contraction. However, as fibers are extracted for longer periods of one week to 12 weeks the percentage of maximum tension increases on addition of ATP alone and Ca has less effect. At approximately 12 weeks, ATP alone produces 100% of the maximum tension and Ca has no effect. In contrast, in glycerinated



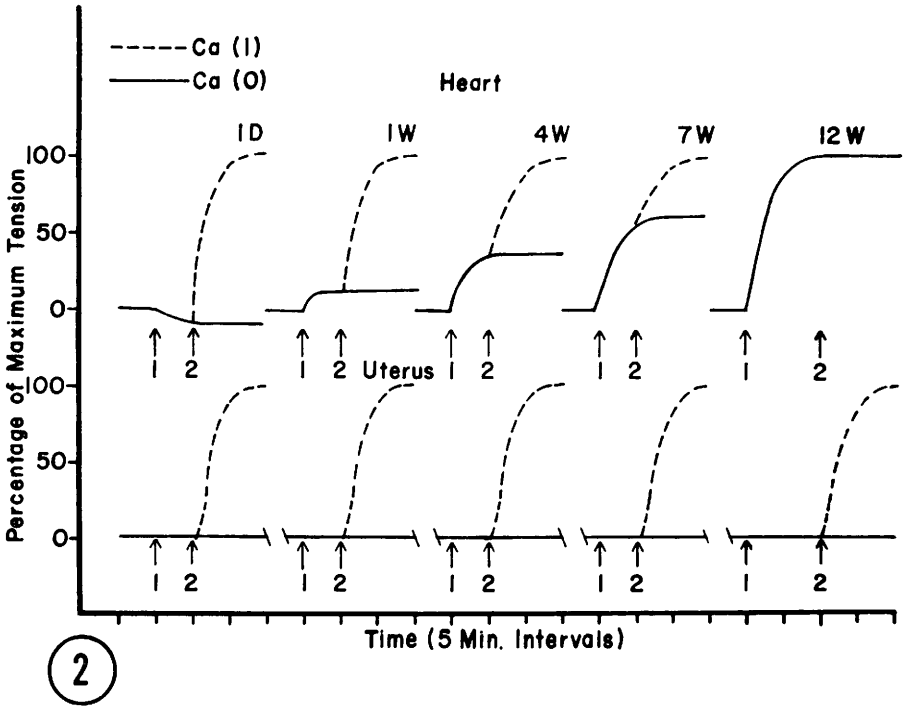


FIG. 1. Effects of ATP, EGTA and Ca on tension in glycerinated cardiac and uterine fibers extracted from 1 to 7 days (D). Final mM concentration in parenthesis. Each line is a typical results obtained in a series of 5-12 experiments.

FIG. 2. Effects of extraction in glycerol from 1 day (D) to 12 weeks (W) on tension produced by ATP and Ca in cardiac and uterine muscle fibers. Contraction solutions contained 5 mM Mg and 0.5 mM EGTA. 1 = addition of ATP (5 mM). 2 = addition of Ca (1 mM). Each line is a typical result obtained in a series of 5-12 experiments.

TABLE I. Effects of Extraction in Progesterone on Maximum Tension Developed in Glycerinated Uterine Fibers.

Conditions	No. of exp	Maximum tension developed g/cm² (mean ± SEM)
Extracted without progesterone	6	100 ± 6.10
		P >.90
Extracted with progesterone	6	90 ± 7.7

uterine fibers no contraction or relaxation occurs when ATP is added, Ca gives maximum contraction, and further extraction has no effect on these parameters. Glycerinated fibers extracted up to 6 months still display this calcium sensitivity.

Table I shows that the maximum tension developed in guinea pig uterine fibers from estrone treated animals which have been extracted in glycerol without progesterone developed a tension of 100 g/cm². Note that the maximum tension in glycerinated fibers extracted in high doses of progesterone shows no significant difference in the amount of tension developed.

Discussion. The results of these experiments and previous studies(6) indicate that in freshly extracted cardiac glycerinated fibers Ca is necessary for contraction, but that with long term extractions the fibers lose this Ca sensitivity. Thus, Fig. 1 indicates that in the presence of EGTA, ATP produces a relaxation in fibers which have been extracted for one day but in fibers extracted for longer periods, the initial contraction produced by ATP alone is increased. Fig. 2 indicates that with EGTA present this initial contraction produced by ATP continues to increase so that by 12 weeks 100% of maximum tension is developed by ATP alone and Ca has no effect. The exact nature of the system which renders the fibers Ca sensitive is not known. However, in skeletal muscle a protein has been isolated which confers EGTA sensitivity on actomysin made with highly purified action (3,4). The loss of a similar protein during glycerol extraction in cardiac muscle fibers could account for the observed results.

In contrast, although uterine fibers which have been freshly extracted show calcium dependent contractions, this is not lost during prolonged extractions (Fig. 1-2). This suggests either that the Ca dependent contraction in uterine smooth muscle is produced by a fundamentally different factor(s) than in cardiac muscle or that the uterine smooth muscle contains a factor(s) which produces calcium sensitivity similar to other muscles, but it is so tightly bound to the other proteins that it is not extractable. Further work is obviously necessary to explain this striking difference between glycerinated cardiac fibers and uterine fibers.

Another interesting difference should be pointed out. Note that the glycerinated uterine fibers produce maximum contraction after ATP alone in one- to 7-day extracted fibers without EGTA present (Fig. 1B). This contrasts with the cardiac fibers in that the initial percentage of maximum tension induced by ATP in fresh cardiac fibers increases rapidly with extraction during the first days (Fig. 1A). Since a large part of this initial tension induced by ATP can be inhibited by addition of EGTA (Fig. 1A) it suggests that either more fiber Ca is becoming available or the ability of some subcellular system (such as the sarcoplasmic retricular or mitochondria) to reduce the fiber calcium concentration is becoming impaired as extraction proceeds. It is possible that the explanation of this difference is due to the fact that smooth muscle appears to contain much less mitochondria and very little sarcoplasmic reticulum(9).

Finally, the suggestion that the glycerinated uterine fibers develop low tension because of release of proteolytic enzymes from lysosomes would seem to be unlikely since progesterone which has been reported to inhibit the release of these enzymes(7) has no effect on the maximum tension developed in these fibers.

Summary. A comparison of Ca dependent contractions in glycerinated cardiac and uterine muscle fibers and the effects of extraction in progesterone on the maximum tension developed in glycerinated uterine fibers were made. It was concluded that cardiac muscle contains an extractable factor(s) which results in Ca dependent contractions. Uterine muscle contraction, however, is either made

Ca dependent by a fundamentally different factor(s) or similar factor(s) which are so tightly bound to the contractile proteins that they cannot be extracted. In addition it was concluded that the low tension generated by glycerinated uterine fibers was probably not due to breakdown and release of proteolytic enzymes from lysosomes since progesterone which supposedly inhibits lysosome breakdown had no effect on the maximum tension generated.

1. Briggs, A. H., Am. J. Physiol., 1963, v204, 739.
2. Briggs, A. H., Hannah, H. B., Muscle, Proceedings of symposium held at Faculty of Medicine, Univ. of Alberta, Pergamon Press, New York, 1965, 287.
3. Ebashi, S., Nature, 1963, v200, 1010.
4. Katz, A. M., Fed. Proc., 1965, v24, 208.
5. Lee, K. S., J. Pharmacol. & Exp. Ther., 1960, v132, 149.
6. Embry, R., Briggs, A. H., Am. J. Physiol., 1966, v210, 826.
7. Szent-Györgyi, A. G., Muscle, Proceedings of symposium held at Faculty of Medicine, Univ. of Alberta, Pergmaon Press, New York, 1966, 345.
8. Weber, A., Winicur, S., J. Biol. Chem., 1961, v236, 3193.
9. Rhodin, J. A. G., Physiol. Rev., 1962, v42, Suppl. 5, 48.

Received July 25, 1966. P.S.E.B.M., 1967, v124.

Differential Mortality of Male and Female Germfree C3H Mice Introduced into a Conventional Colony.* (31664)

HENRY C. OUTZEN AND H. IRA PILGRIM (Introduced by Thomas F. Dougherty)
Department of Anatomy, and The Germfree Laboratory, Department of Surgery, University of Utah Collge of Medicine, Salt Lake City

A difference in mortality rates between germfree male and female C3Hf/Pi mice exposed to a conventional colony was noticed in this laboratory. This was at variance with published observations(1). A similar discrepancy existed with respect to the effect of age on survival.

Strains and species of germfree animals appear to differ in ability to respond to environmental contamination(2). Germfree mice are, however, capable of responding to antigenic stimulation(3,4). The purpose of this report is to document the effect of age and sex on the ability of one strain of germfree mice to survive exposure to a conventional colony.

Materials and methods. Male and female germfree C3Hf/Pi mice were maintained in aluminum, autoclavable isolators(5). They were then removed from the isolators at ages ranging from 12 to 498 days and transferred into our conventional mouse colony (an ordinary, barrier maintained colony; not derived from a germfree source). While in a germfree state the mice were fed a formula consisting of ground liver, bean meal, corn oil, powdered milk, wheat germ, salt, water, and supplemental vitamins. When removed they were placed on Old Guilford high-fat mouse breeder diet.

For analysis, males and females were divided into 3 groups by age; under 2 months, 2 to 6 months, and over 6 months of age. These age categories were established prior to their being removed from the germfree environment, the purpose being to test the hypothesis, derived from earlier subjective observations, that males died more readily than females, and that old mice died more readily than young ones.

The data were evaluated by the method of Pilgrim and Dowd(6). This method makes a continuous correction for death due to extraneous causes (*e.g.*, sacrifice for histological study). Animals dying of extraneous causes are utilized up to the time of death, and are then mathematically eliminated from subsequent consideration. The corrected data

* Supported by USPHS Grant CA 05771 from Nat. Cancer Inst., and Contract PH43-64-925 from Cancer Chemotherapy National Service Center, Nat. Inst. Health.