able that in the majority of laboratory strains a high degree of receptivity depends upon the administration of estrogen and progesterone.

The method of counting the male's mounts, the females' lordosis responses, and the frequency of other estrus reactions is to be strongly recommended in all studies of heat behavior. Scores thus obtained provide the basis for a direct quantitative comparison of the effects of various types of hormone treatment. By employing the ratio of lordosis responses to mounts the degree of heat behavior induced by 100 R.U. of estrogen plus 0.5 mg of progesterone may be described as approximately 40% as intense as that which follows the administration of 500 R.U. of estrogen and 1 mg of progesterone. Receptivity induced by 500 R.U. of estrogen alone is only 19% as intense as that resulting from the administration of the same amount of estrogen plus 0.5 mg of progesterone. If these technics are applied to a sufficiently large number of animals results obtained will have high reliability. In the present instance the groups were small and comparisons must be regarded as approximate.

Summary. Three groups of spayed female rats were injected with various amounts of estrogen and of progesterone, and the sexual receptivity thus induced was measured. 500 R.U. of estrogen produced some receptivity in 2 of 7 cases. The same amount of estrogen followed by 0.5 mg of progesterone resulted in a much higher degree of receptivity in all 7 animals. 100 R.U. of estrogen and 0.5 mg of progesterone induced a low degree of receptivity in 6 of 7 cases. 500 R.U. of estrogen and 1 mg of progesterone caused the same animals to exhibit much more intense heat behavior. It is concluded that in the strain of animals studied the production of estrus behavior of normal intensity involves the synergistic action of estrogen and progesterone.

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Relation of Anesthetics to Experimental Shock Syndrome Produced by Venous Occlusion.

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A major difficulty encountered in studies of the factors affecting the onset and course of experimental traumatic or circulatory shock has been that of failure to bring about a fairly constant and readily reproducible syndrome. A partial solution, at least, seems to be offered by the technic reported by Perlow *et al.*[1] who modified the method of regional venous occlusion (Mann)[2] for this purpose. This approach to the problem has been utilized in our laboratory in a considerable number of animals with gratifying results, and certain experimental variables have been studied. The present report deals with the influence on the experimental picture of certain anesthetic agents.

Methods. The technic of venous occlusion as outlined by Perlow *et al.* consisted of ligation, under anesthesia, of the common and the internal iliac veins, followed by injection of a suspension of animal charcoal either into the external iliac or the femoral vein; the latter, in our hands, has seemed preferable in view of easier access. Anesthetics were given at dose levels biologically standardized for each animal (Wiggers);[3] these usually approximated the calculated doses, which were: (1) morphine sulfate, 2%, 1 cc subcutaneously followed by 1.5 g/kg urethane, 50% intravenously; (2) barbital sodium,

[1] Perlow, S., Killian, S. T., Katz, L. N., and Asher, R., *Am. J. Physiol.*, 1941, **134**, 755.

[2] Mann, F. C., *Am. J. Physiol.*, 1918, **47**, 231.

[3] Wiggers, C. J., *Physiol. Rev.*, 1942, **22**, 74.

TABLE I.
Effect of Anesthetic upon Survival Time in Shock Following Venous Occlusion.

Anesthetic	Duration of anesthetic	No. of exp.	Avg survival time and S.D.	Range in survival time	Wt. incr. of occluded leg, avg %	Range, %
Urethane	Throughout exp.	11	5'18" ± 2'7"	3'20"-10'45"	2.2*	1.0-3.2
Barbital sodium	" "	12	4'50" ± 1'32"	2'40"-7'15"	2.8	1.1-5.5
Pentobarbital sodium	" "	10	7'11" ± 1'40"	3'25"-9'20"	2.8	1.0-4.7
" "	During operation	12	8'16" ± 4'29"	3'45"-16'45"	4.6	2.1-7.9
Ether	" "	12	6'10" ± 3'8"	3'25"-16'	3.6	2.6-5.3

*Weight increase/body weight.

10%, 225 mg/kg intravenously; (3) pentobarbital sodium, 3%, 30 mg/kg intravenously; (4) ether, by mask.

Complete surgical anesthesia was induced in all cases. Moreover, it was maintained throughout the observational periods in the urethane, barbital sodium, and in one series of pentobarbital sodium experiments. Anesthesia during the surgical procedure only (aseptic technic) was employed in one pentobarbital series and in the animals in which ether was used.

In a representative group of experiments the following data were obtained prior to and at intervals following occlusion: Mean arterial blood pressure (by Hg manometer, from the common carotid); respiratory rate and heart rate; rectal temperature; hematocrit; hemoglobin (g %) and specific gravity (falling drop method). Blood samples were taken by femoral puncture.

Body weights ranged from 5 to 20 kg, most of the animals weighing from 7-10 kg.

An approximate value for the loss of fluid into the occluded leg was determined by separating the hind limbs (gluteal muscles included) along midline. Differences in weight are expressed in per cent of body weight.

Results. Number of experiments, average survival times and average weight increases of the occluded limbs are given in Table I.

A progressive acceleration in heart rate occurred from 1-2 hr after occlusion, and persisted until terminal stages. This was paralleled by a steady decline in arterial blood pressure which, however, frequently rose from 10-30 mm above control levels before the decrease began.

Blood studies invariably revealed a greater or less degree of hemoconcentration. Barbital sodium and pentobarbital sodium gave closely corresponding control values (Table II).

Changes in rectal temperature following occlusion were not consistent; in the barbital sodium experiments an increase occurred in 7, decrease in 2 and no change in 1; with pentobarbital sodium 5 showed an increase, 4 a decrease and 1 no change; with urethane, decreases in 4, increase in 2, and no change in 2.

Discussion. The average survival time was longest in the pentobarbital sodium series (Table I) and, as might be expected, the degree of hemoconcentration was most marked (Table II). Statistical analysis of the data failed to demonstrate any significant differences in the survival times. In view of this observation and because of its greater ease of administration, pentobarbital sodium is our choice for routine experiments. Maintenance of anesthesia throughout the experiment or for operation only appeared to have

TABLE II.
Effect of Anesthetics on Hemoconcentration in Venous Occlusion Shock.

Anesthetic	Hematocrit			G % hemoglobin			Specific gravity		
	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
Urethane	48.7	55.8	9.2	17.6	20.2	14.2	1.0560	1.0631	.62%
Barbital sodium	43.3	51.5	14.5	14.3	16.0	23.0	1.0542	1.0597	.52%
Pentobarbital sodium	43.3	56.3	29.7	15.6	21.9	40.3	1.0588	1.0767	1.2 %

*(a) control, (b) maximum, (c) % increase.

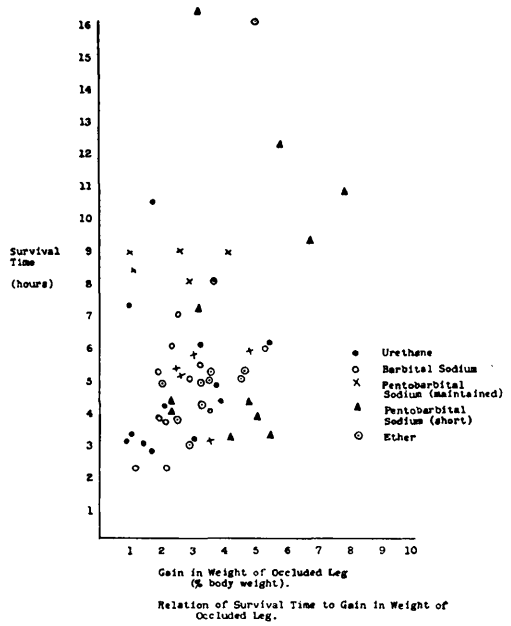

FIG. 1.

Relation of survival time to gain in weight of Occluded Leg.

little influence on the outcome as judged by the results of the 2 pentobarbital series. This circumstance may have been due to 2 factors: the long-lasting effect of the initial injection of pentobarbital sodium and the fact that additional anesthetic was not required when the mean arterial blood pressure had fallen to about 70 mm Hg.

The wide variation in the values for the loss of fluid into the occluded leg (1.0-7.9%) indicates that although reduced blood volume may be a contributing factor, it is probably not the only factor in this type of experimental shock. This is also supported by the lack of correlation between length of survival time and weight of the occluded leg (Fig. 1). The closest approach to a correlation is perhaps suggested in the ether series.

Summary. In this laboratory the method of venous occlusion with several types of anesthetics has consistently produced experimental shock characterized by hemoconcentration, circulatory failure and death. Although oligemia may be a contributing factor, it does not appear to be the only factor in this type of experimental shock. From the point of view of survival time there is little choice between short and maintained anesthesia. Using the same criterion, the anesthetic agents of preference are pentobarbital sodium, and ether.

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Crystalline Trypsin-Inhibitor and Blood-Clotting.

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Through the courtesy of Dr. T. E. Weichselbaum, Washington University, St. Louis, we were supplied with 10 mg of pure *crystalline* trypsin-inhibitor (1 mg $\equiv 4.0 \times 10^{-2}$ $[T.U.]_{Hb}$), a polypeptide which was originally isolated from pancreas by Northrop and Kunitz.[1] The sample, dissolved in 10 cc physiological saline, was brought from pH = 5.1 to pH = 7.2 with a trace of n/10 NaOH (= inhib.). The other clotting reagents and test methods have been described in previous publications,[2] the dialyzed crude albumin (= alb.) being prepared from *rabbit* plasma.

I. *Inhibition of clotting of recalcified citrated plasma (rabbit):* Whole plasma and several saline dilutions showed unequivocal retardation of clotting on recalcification in the presence of inhib., as compared to controls; *e.g.*, clotting-time of plasma (1:2 dilution) on adding $CaCl_2$: (a) control (with saline) = 3′ 20″; (b) with inhib. = 7′ 05″.

[1] Kunitz, M., and Northrop, J. H., *J. Gen. Physiol.*, 1936, **19**, 991.

[2] Ferguson, J. H., *J. Lab. and Clin. Med.*, 1938, **24**, 273.