servations recorded in the Tables I and II, that inoculations of embryo skin into rats of the same genetic constitution, fail to elicit a resistant state to grafts of a tumor autogenous to the strain. This observation is in accord with findings of previous investigators who likewise failed to elicit immunity in a pure line of animals with embryo skin of the same genetic line.[14]

Discussion. There is no intention here to discuss generally the genetic principles involved in the problem of induced resistance to malignant growth. As indicated in the previous communication[15] the results obtained are specifically concerned with the reticulum cell type lymphosarcoma, originating in a pure line of rats of Dr. Bagg. It was suggested that the type of tumor may be responsible for the specific immune action in an inbred strain of rats. The results obtained from the experiment described in the present paper brought further evidence, in this case, that the induced resistance is specific to the type of tumor and independent of the genetic inter-relation between the host and the immunizing agent, inasmuch as embryo skin of the same strain proved to be ineffective in eliciting the same phenomenon.

On the other hand the results of the present paper are in full agreement with those obtained by other investigators, showing that no resistance to tumor grafts can be elicited in animals of a pure line with embryo skin of the same strain.

Summary. Rats of a pure inbred line treated with embryo skin of the same strain failed to produce a resistant state to grafts of a tumor autogenous to the strain.

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Shock Produced by the Application of Tourniquets to the Hind Limbs of Rats.*

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The tourniquet method has been accepted as a valid means of producing experimental shock and numerous investigators have used some variety of tourniquets on laboratory animals, including albino rats. Some investigators have used rubber bands,[1,2] cord tied tightly or wrapped in numerous turns about the leg,[3-8] and compression from clamp devices to cut off the circulation to a limb.[9,10] We were interested in standardizing the tourniquet method so that graded and reproducible degrees of trauma could be effected. Animals shocked by this method were to be used in making biochemical studies for comparison with similar studies[11,12] on rats shocked by the

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Wisconsin.

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Noble-Collip[13] technic.

The purpose of this paper is to report: (1) the method developed for producing graded tourniquet shock, (2) the effect of re-application of tourniquets on the percentage survival in rats, and (3) the hemoconcentration which occurred in tourniquet-shocked rats.

Materials and Methods. The animals used for this study were adult male rats obtained from Sprague-Dawley, Inc. The animals were kept in our laboratory for at least 5 days before being used, at which time they weighed 250-325 g. They were maintained on the regular stock diet and tap water *ad libitum.*

The tourniquets used were designed so that a set screw could be adjusted against a metal bar in such a way as to tighten a loop of cord slipped over the leg of the rat. The metal portions of the tourniquets were of brass and the cord was ordinary fish line. The tourniquets were applied to both hind legs as high on the thighs as possible and tightened until the screws could no longer be turned by the normal amount of force applied by the fingers. When this was accomplished the muscles of the legs were rigid and the circulation was occluded as shown by cyanosis. The tourniquets were left in position for varying periods of time, depending upon the degree of shock desired.

The rats were anesthetized by Nembutal (Abbott Laboratories) given intraperitoneally just before the tourniquets were adjusted. The usual dose required was 0.2 cc. During the time the tourniquets were in place the animals were given additional amounts (0.05-0.10 cc) of Nembutal as needed to keep them in a semi-conscious state, but the anesthetic was not given after the tourniquets were removed.

As an indication of the course of hemoconcentration occurring after the removal of the tourniquets, hemoglobin determinations were made on tourniquet-shocked rats by the method of Evelyn[14] for oxyhemoglobin. Blood for these studies was obtained by cutting off the tip of the tail.

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Results and Discussion. Survival after tourniquet shock: The percentages of animals surviving application of tourniquets for various periods of time are summarized in Table I.

TABLE I.
Survival of Rats Subjected to Tourniquets for Varying Periods of Time.

No. of rats	Tourniquet applied		% survival
	hr	min	
7	3	30	100
8	3	35	75
15	3	40	47
12	3	45	33.3
33	4	0	3.0
4	4	15	0
6	4	30	0
4	8	0	0*

* None of these rats survived more than 3 hours after removal of the tourniquets.

It can be seen that the number of animals surviving decreased as the period of tourniquet application increased. The exact length of the survival period was not determined for every animal that died since some animals died during the night. The usual period of survival was 6 to 18 hours after removal of the tourniquets, but there seemed to be no correlation between the length of the period that the tourniquets were in place and the length of the survival period in those animals which died, except that when the tourniquets were applied for 8 hours the rats all died within 3 hours after removal of the tourniquets (Table I). The usual course of events after tourniquet removal was a period of approximately 4 or 5 hours in which the animal seemed to be in good condition, in spite of the fact that the legs showed increasing edema. The circulation was restored soon after the release of the tourniquets except in an occasional rat in which the vessels to the limb were permanently occluded as indicated by the continued cyanosis of the limb and the absence of edema. In some cases cyanosis remained yet edema developed. This could be due to venous occlusion or loss of whole blood into the traumatized area. During the first 1 or 2 hours after release the legs showed the greatest increase in size, but they continued to become gradually larger during the course of the first 5 or 6 hours. During that period fluid was lost into the tissue adjacent to the tourni-

TABLE III.
Hemoglobin Concentration in Rats in Tourniquet Shock.*

No. of rats	1† Hb– before tq. removal	2 Hb– after tq. removal	3 Hb– increase	4 Hb– last sample	5 Hb– increase	Survival +/—	Hour of death
	%	%	%	%	%		
1	84.0	111.5	27.5	111.5	27.5	—	7
2	85.0	137.2	52.2	137.2	52.2	—	7
3	92.0	126.4	34.4	126.4	34.4	—	8
4	79.0	117.3	38.3	117.3	38.3	—	4
5	98.1	130.1	32.0	121.8	23.7	Sacrificed	5
6	81.6	126.4	44.8	118.6	37.0	"	5
Avg	86.6	124.8	38.2	122.1	35.5		

* 100% hemoglobin equals 15.6 grams per 100 cc.
† The values in column 3 were obtained by subtracting the values in column 1 from those in 2; the values in column 5 by subtracting those in 1, from the ones in 4.

quet-traumatized tissue, and into the genital region, particularly the scrotum. After these first hours changes in gross size could not be detected by observation. Haist and Hamilton[10] found that the swelling of the limb reached its maximum during the second hour after removal of the clamps, but in their rats the clamps had been left on overnight (12-15 hrs) and the average survival time was only 3 hours 12 minutes. It might be expected that maximum edema would be reached more rapidly after longer periods of tourniquets.

Hemoconcentration in tourniquet shock: Hemoconcentration is usually an accompaniment of shock and it was of interest to follow the course and extent of hemoconcentrations in normal rats during the period following tourniquet removal when shock was developing. Hemoconcentration may be determined by measurement of hematocrit, by increase of specific gravity, by hemoglobin determinations and by red cell counts. In our laboratory hemoglobin determination was the most feasible method to use as an index of hemoconcentration.

It is known that splenic contraction occurs in shock, and that it contributes to hemoconcentration. Taylor and Page,[15] using dogs, found that "the splenic contribution to changes in the hematocrit index occurs during the first one or two hours of the experiment." The authors state that splenectomy abolishes the early sharp rise of the hematocrit index seen in shock. On the basis of these studies it is probable that the early increase in hemoglobin observed after the removal of tourniquets can be ascribed only in part to fluid loss. This fact in no sense invalidates the use of hemoglobin determinations as indications of hemoconcentration since we were interested in relative rises in hemoconcentration extending over a number of hours.

Hemoglobin determinations were made on rats which had tourniquets in place for 4 hours. The first determinations were made at the time of the removal of the tourniquets, and each hour thereafter for periods as long as 7 hours. Two of the animals for which data are given were sacrificed at the end of 5 hours to obtain blood for analyses to be reported in connection with other blood studies.[12] Table III is a summary of the hemoglobin values found during the period after the tourniquets were removed, and shows the highest concentration found for each of the animals during the experimental periods, that found in the last sample, and the increases of each of these over the original concentration. In the shocked rats hemoconcentration developed progressively after the removal of the tourniquets until the death of the animal. Increases in hemoglobin in rats which were followed to the hour of death ranged between 27 and 52%. From these data the conclusion can be drawn that marked hemoconcentration is an accompaniment of shock produced by tourniquets and that the wide variation in degree of hemo-

15 Taylor, R. D., and Page, I. H., *Arch. Surg.*, 1943, **47**, 59.

TABLE II.
Survival of Rats After Reapplication of Tourniquets Following Varying Periods of Release.

No. of rats	Tourniquet applied	Tourniquet released	Tourniquet reapplied	% survival
	hr	hr	hrs	
6	4	1	Through 24	100
6	4	2	" 24	100
6	4	3	" 24	100
6	4	4	" 24	100
8	4	5	" 24	100
8	4	6	" 24	100
8	4	7	" 24	87.5

concentration in animals at the time of death would preclude the use of it as an indication that the irreversible stage had been reached in tourniquet shock.

Reapplication of tourniquets: Experiments were designed to find the stage at which the irreversible condition is reached in tourniquet shock, and to determine if it is the period of ischemia alone or some subsequent change that is the decisive factor in this type of shock. Adult male rats were subjected to tourniquets for 4 hours, then the tourniquets were released for periods varying from 1 to 7 hours, then replaced and left in position through 24 hours from the time of first release. The rats were lightly anesthetized with ether for the replacement of the tourniquets, but they were not further anesthetized during the period of reapplication. The tourniquets were then removed and the rats were observed for the next 24 hours to determine survival rates. The data in Table II summarize the results of this experiment and by comparison with those in Table I it can be seen that only 3% of the rats survived 4 hours of tourniquets when there was no replacement, but in the case of temporary release for 1 to 6 hours 100% of the animals survived during the following 24 hours of reapplication. The tourniquets were then released and the animals were observed for an additional 24 hours. Two animals died after the second release of the tourniquet. Death in one of these rats followed severe hemorrhage from the traumatized tissue. The rats were regularly sacrificed after 48 hours because the limbs were necrotic. After replacement of tourniquets the legs had become cyanotic and remained so after the tourniquets were released. The rats were otherwise alert and energetic. When the release period was increased to 7 hours 87.5% of the animals survived when tourniquets were reapplied. The irreversible stage of shock was probably prevented from developing by the elimination of circulation to the injured limbs and the prevention of any further loss of fluid into the injured area and the escape of metabolites from the injured tissue. Thus the animal could successfully cope with the first loss of fluid and the first of the metabolites from the injured area. Green *et al.*[9] found that after clamping both legs of rats for 7 hours removal of limbs up to 2½ hours saved all animals, but "from 2½ hours onward the survival rates steadily fell, to reach zero, on the average, at 4 hours." They further observed that the rat could withstand repeated sublethal doses of clamping totalling at least 12 out of 32 hours.

It would appear that local fluid loss to the limb after release of the tourniquets cannot be the only factor responsible for the development of irreversible shock as reapplication of the tourniquets after fluid loss has occurred prevents death. The importance of fluid loss in decreasing the ability of the animal to recuperate cannot be denied, but it is to be emphasized, as blood[12] and muscle analyses show,[11] that after tourniquet removal substances from the limb escape into the circulation. The accumulation of these metabolic breakdown products probably interferes with cellular metabolism and damage to vital organs results. The beneficial effect following reapplication of tourniquets suggests that the total quantity of the injury substances, as well as the length of time over which they act, is of importance in determining the fate of the animal.

Summary. 1. A method for producing

tourniquet shock in rats is described. The method yields graded and reproducible results.

2. The percentage survival of rats to tourniquet shock was progressively decreased as the time of tourniquet application was increased. Following 3½ hours of tourniquets 100% of the rats survived, while after 4 hours of tourniquets only 3% survived.

3. Rats which had tourniquets applied for 4 hours with periods of release for 1 to 6 hours, followed by a second application of tourniquets, showed 100% in contrast to 3% survival for those without replacement, while 87.5% survived when the tourniquets were replaced after 7 hours.

4. The degree of hemoconcentration developing in rats in tourniquet shock was approximated by hemoglobin determinations. Increases in hemoglobin ranged between 27 and 52%.

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Inactivation of Estrone by Liver. Assay Method *In vivo* for Dietary Hepatic Injury in Rats.*

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Pellets of estrone implanted in the spleen will not induce estrus in ovariectomized rats fed a normal diet.[1] Estrone draining from the pellet through the splenic vein into the liver is inactivated by the normal functioning liver parenchyma, either through direct chemical destruction[1] or through a closed "hepato-intestinal cycle."[2] Failure of the liver to inactivate estrogen has been demonstrated by Biskind and Biskind[1] in rats maintained on a diet deficient in the vitamin B complex. Estrus in such animals appeared within 1 to 3 weeks and was interpreted as an indication of impaired liver function. Such functional change resulting from avitaminosis is in its etiology and pathogenesis different from the specific hepatic injury (necrosis and cirrhosis) produced in rats by the administration of a synthetic diet low in lipotropic factors (methionine and choline),[3] but containing all known vitamins including the members of the vitamin B complex. On the other hand, however, it has been shown[4] that with regard to inactivation of estrogen by the liver, rations deficient in lipotropic factors will have the same effect as rations free of vitamin B complex. In spite of this analogy only the disturbance elicited by rations deficient in lipotropic factors with its characteristic pathological manifestations is considered the true dietary hepatic injury.[3]

In previous experiments[4] the onset of functional disability of the liver, measured by the failure of inactivation of estrone, became evident in rats fed a diet low in lipotropic factors after an interval when severe histological changes are known to make their appearance. This interval was much longer than that observed in rats fed a vitamin B-free diet.[1,4] Whereas the impaired inactivation of estrone in rats kept on a vitamin B-free diet could be reversed and normalized by supplements of vitamin B complex,[1,4] large doses of yeast were needed to achieve the same result in rats

* Aided by a grant from Wyeth, Incorporated, Philadelphia.

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