sion or no change.[5] Similarly respiration is reduced in tissues and isolated enzymes irradiated *in vitro* (Barron *et al.*,[5] Dale[6] and others). Apparently the response of an intact animal where the tissues are bathed by the complex body fluids differs from the response of tissues in simpler media. The body fluids of irradiated animals contain products of tissue breakdown, and there is much evidence of toxemia during the acute radiation reaction. It is not likely that all the toxin is liberated at the time of irradiation. It is more probable that some cell injury remains latent for days and results, over an extensive period, in production of materials which have a specific dynamic action, reflected in elevated metabolism. It is of interest that thyroid metabolism, as indicated by the iodine turnover, is increased 2 to 3 days after irradiation.[7]

Summary. 1. Rats which died 3 to 5 days after total-body irradiation of 809 - 972 r showed an elevation of oxygen consumption of about 35% occurring within 24 hours.

2. Rats which died 9 to 10 days after doses of 648 to 972 r showed an increase in metabolism of about 58% and a decline prior to death.

3. Rats which survived doses of 648 r showed an average increase in oxygen consumption of 32% during the first week, and less pronounced increase extending over a period of at least 16 days after irradiation.

4. At doses from 54 to 432 r there was an increase in metabolism by about 8½%. The maximum oxygen consumption was reached later at sublethal rather than at lethal doses.

5. Increased metabolism did not coincide with weight loss either in time course or in dose dependence.

[5] Barron, E. S. G., to be published in *Nat. Nucl. En. Ser.*, 1949, IV, 22B.

[6] Dale, W. M., *Brit. J. Rad.*, 1943, **19**, 171.

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17226. Differential Blood Counts on Rats During Shock Induced by Tourniquets.

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The hematological changes which occur during secondary shock have been reported by Cannon, Frazer, and Hooper,[1] and other workers as reviewed by Moon.[2] Among the changes reported are variations in the red and white cell counts, but the relative values of the different white cells in particular have been neglected. White and Dougherty[3] have shown that in instances of stimulus or stress there is a dissolution of lymphocytes. It is the purpose of this study to show the changes which occur in the percentages of lymphocytes and polymorphonuclear neutrophils during induced experimental shock. Through the course of the experiment, it was found that the changes in the percent of monocytes, basophils, and eosinophils were too small to be significant.

Materials and methods. Albino rats, Sherman strain, including both sexes, and ranging in weight from 98 to 292 g were used. Rubber bands, tightly wrapped, were placed at the highest possible point on both thighs. The rats were kept in a semi-conscious state with intraperitoneal injections of nembutal (Abbott). Blood was obtained by puncturing a tail vein and then subjected to the usual methods for determining gross and differential counts.

Preliminary experiments on 12 rats were

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[2] Moon, V. H., *Shock*, 1942, Lea and Febiger, Philadelphia, Pa.

[3] White, A., and Dougherty, J. F., *Ann. N. Y. Acad. Sci.*, 1946, **46**, 859.

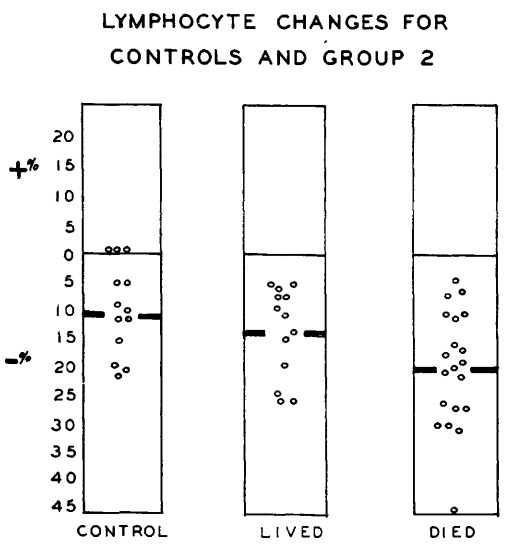

FIG. 1.
Each circle represents the change in lymphocytes which occurred between 2 and 3 hours after the release of 5-hour tourniquets in experimental animals and 2 hours after a 5-hour period of anesthesia for the control animals. Broken horizontal bars indicate the average change for each group.

conducted with both gross and differential counts being made at the same time to obtain the actual values of the cells present. The gross counts were discontinued in subsequent experiments after the trend had been established. Another group of 33 rats was used for differential counts alone. To establish the normal count, one smear was made just after the tourniquets were applied, and other smears were made every hour after the removal of the tourniquets for 3 hours if the animal lived. A third group of 11 rats was injected with dibenamine hydrochloride,* an adrenolytic drug, just before the tourniquets were applied. The procedure, otherwise, was the same as described above. In a fourth group of 9 animals, smears were made from blood taken from the toe at the end of the 5 hour period, just before the removal of the tourniquet. The procedure, otherwise, was the same as described for the second group.

In the present series of shocked rats, an average of 4 counts were made on 144 animals. For each differential count at least 100 cells were counted while in the second group of 33 animals used for differential counts alone and in the control group at least 200 cells were counted. This procedure would appear to establish the validity of the date presented.

Results. The significant feature of the studies reported is a comparison between the blood counts of fatal and non-fatal cases of shock. Undoubtedly, the procedures employed, anesthesia, tourniquet trauma, and handling, of themselves will produce some changes in the white blood cell count. However, the decrease in lymphocyte count found in animals that died is significantly greater than variations in the blood count of normal rats. This can be seen in Fig. 1. There are two obvious factors which *per se* may bring about the degree of scatter seen in Fig. 1. First, it should be emphasized that the onset of profound shock varies in the experimental animals and that death occurs anywhere from 1 to 10 hours after release of the tourniquets. Similarly, the greatest lymphocyte decrease is not always 2 to 3 hours after tourniquet release. However, in order to permit a direct comparison between the different animals, the blood samples recorded here were all taken between 2 to 3 hours after tourniquet removal. This factor may be the cause of some of the variability encountered, as illustrated in Fig. 1. Secondly, the animals begin to revive soon after the anesthesia wears off and the stress resulting from continued attempts to escape from the animal-boards may cause a greater temporary decrease in lymphocytes than would be seen if the animals were free. In five rats subjected to pentobarbital anesthesia for a period of 4 hours only a slight decrease in lymphocyte count from 5 to 11%, an average of 9%, was encountered. The average decrease in lymphocytes in controls carried for 2 hours past a 5-hour period of anesthesia (comparable to a 5-hour tourniquet period) was 12.9%. In contrast, the average decrease in fatal cases of shock was 18.5%.

Group 1. Gross and Differential Counts in Tourniquet Shock. In preliminary experiments on 12 rats, all of the animals showed a decrease in lymphocytes after removal of the

* Supplied by Dr. W. Gump, Givaudan-Delawanna, Inc., Delawanna, N. J.

TABLE I.
Percentage of Lymphocytes and Time of Death in Dibenamine Treated Rats 2 Hours after the Release of Hind-Limb Tourniquets.

No.	Normal % of lymphocytes	Lymphocytes 2 hr after tourniquet release, %	Time of death after tourniquet release, hr
1	75	37	3
2	78	61	2
3	66	26	3
4	74	47	3
5	75	57	2
6	64	44	2
7	76	58	2
8	78	49	2
9	66	46	3
10	73	52	2
11	67	46	2

TABLE II.
Percentage of Lymphocytes in the Leg Prior to Tourniquet Release and 2 Hours after Release.

No.	Normal % of lymphocytes	Lymphocytes in leg before tourniquet release, %	Lymphocytes in blood 2 hr after tourniquet release, %
1	71	75	20
2	65	84	29
3	60	88	54
4	60	56	45
5	94	86	66
6	73	84	53
7	82	92	82
8	79	90	54
9	90	87	50

tourniquets, irrespective of whether the gross count increased or decreased. In all of these cases the *actual* number of lymphocytes was decreased. Six of the rats showed a decrease in gross count following the tourniquet removal. In these the actual number of lymphocytes decreased more than the actual number of polymorphonuclear neutrophils were increased. For example, the normal count of one animal showed a total of 7700 cells, of which 79% (6083) were lymphocytes and 21% (1617) were polymorphonuclear neutrophils. Two hours after tourniquet removal, the gross count was 5800 with 54% (3132 cells) lymphocytes and 46% (2668 cells) neutrophils. Therefore, the decrease in lymphocytes was 2951 cells and the increase in neutrophils was 1051 cells. A similar relationship was found in all instances where there were decreases in gross counts. On the other hand, there was an increase in the gross count in 6 of the rats. In these, the actual increase in the number of neutrophils was on the average greater than was the decrease in the number of lymphocytes. For example, one animal showed a normal gross count of 6300 cells, of which 90% or 5670 were lymphocytes and 10% or 630 were neutrophils. Two hours after tourniquet removal, the gross count was 6500 cells with 50% or 3250 lymphocytes and 50% or 3250 neutrophils. Therefore, the actual decrease in lymphocytes was 2420 cells while the actual increase in neutrophils was 2620, or 200 cells more.

Group 2. Differential Counts in Tourniquet Shock. In a group of 33 rats, 11 died within 4 hours following removal of the tourniquets, 4 died within 6 hours, one died at 21 hours, and 4 died at some undetermined time during the night. Thirteen of the animals lived. The differential counts of the 20 that died showed a decrease in the percent of lymphocytes, the greatest decrease being 46%, the least decrease 4%, and the average 18.8%. Of the 13 animals that lived, the

greatest lymphocyte decrease was 27%, the least decrease 5%, and the average 13.3% (see Fig. 1).

Group 3. Dibenamine Treated Animals. In the group of 11 rats injected intraperitoneally with 2 mg dibenamine hydrochloride all of the anmals died within 3 hours. These animals showed a considerably shorter period of life following removal of the tourniquet and they also showed a greater lymphocyte decrease than untreated rats (Table I). The greatest lymphocyte decrease was 40%, the least 17%, and the average decrease was 24.4%.

Group 4. Differential Counts on Blood in Leg Prior to Release of Tourniquets. In order to determine whether the decrease in lymphocyte count was due to a loss of cells into the traumatized limb, smears were done in 9 rats with blood taken below the tourniquet, just before its removal. These showed an average increase of 11% in lymphocytes. In contrast the decrease in lymphocyte count averaged 24% in the blood taken from the tail vein following the release of the leg tourniquets and the development of shock which resulted in death for all of the animals (Table II).

Discussion. Mann[4] has reported a general increase in the number of leucocytes in the initial stages of shock followed by a leucopenia as the animal approaches death. The magnitude of the change which occurred depended on the stage and intensity of the condition.

The group of preliminary experimental rats reported here showed different stages of shock. In the present experiments the initial changes in gross leucocyte counts were inconstant whereas a leucopenia invariably developed within 2 hours, consisting for the most part of a decrease in the percent of lymphocytes. The fact that, as the gross count decreased, the actual number of lymphocytes decreased more than the neutrophils increased, would seem to indicate a definite effect on the individual types of cells. During this general decrease, it would have been less significant, if the lymphocytes and neutrophils decreased proportionately, maintaining the original ratio.

The lymphocyte decrease would appear to be a result of a reduction in the actual number of cells, caused by decreased production or increased destruction. It has been suggested that lymphocytes may furnish antibodies or inactivate toxins for the body's defense.[5] Dougherty and White[3] have shown that an increase in pituitary-adrenal cortical secretion is paralleled by an increase in lymphocytic dissolution accompanied by an increase in antibody titer in the blood of immunized animals. Selye[6] has also shown that the pituitary-adrenal system is stimulated during the development of shock. The possibility, therefore, exists that this factor may be related to the decrease in the number of lymphocytes.

Munro and Noble[7] found that normal rats, exposed to trauma in the Noble-Collip drum showed a marked fall in lymphocytes. The decrease in number was directly related to the amount of trauma. When the trauma was great enough to cause death the lympholysis was both rapid and severe.

It is possible that the vaso-depressor substance (VDM) described by Shorr, Zweifach and Furchgott[8] as being liberated during the course of shock may be related to the change in lymphocyte count which occurs when the tourniquets have been released.

The early deaths observed in the animals injected with dibenamine hydrochloride may be due to the fact that the compensatory mechanism for combatting the presence of hypotension is made less effective by the action of this drug on the sympathetic nervous system. The secondary changes in the blood picture are similarly enhanced.

Summary. 1. A lymphocyte decrease

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[6] Selye, H., *J. Clin. Endocrinol.*, 1946, **6**, 117.

[7] Munro, D. D., and Noble, R. L., *Fed. Proc.*, 1947, **6**, 168.

[8] Shorr, E., Zweifach, B. W., and Furchgott, R. F., *Science*, 1945, **102**, 489.

occurs within 2 hours following the removal of the tourniquet in fatal shock induced by leg tourniquets in albino rats. 2. There is no decrease in the percent of lymphocytes in blood smears made below the tourniquet at the end of a 5-hour period. 3. Dibenamine hydrochloride increases the onset of death in tourniquet-shock and increases the lymphocyte decline.

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17227. Protecting Effect of Heparin on the Inactivation of Thrombin by Heat.*

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Thrombin and thrombokinase, unlike prothrombin, are oxidizable and become inactive in their oxidized form, as reported previously.[1] Further investigations proved that γ-quantities of heparin inhibit completely the inactivation of thrombin by oxidation. This property of heparin, unknown till now, showed itself to be specific; it can be concluded, therefore, that heparin possesses not only an antagonistic action on thrombin, but, under certain circumstances, has also qualities protecting thrombin.[2] The inactivation of the coagulation factors by means of oxidation supports our previous theory on the role of oxidation in blood coagulation. Our experiments show that increased oxidation diminishes coagulability of blood, whereas oxygen deficiency accelerates coagulation. Therefore breathing itself must be the essential regulator in blood coagulation *in vivo* as has been shown.[3] The thrombin-protecting effect of heparin, which contrasts with its inhibitory effect on coagulation, is not clarified in our earlier work. It is supposed that this question could be answered by clearing up the nature of the plasma factor (co-factor) of heparin. This supposition is supported by the fact that the unknown plasma factor can modify the heparin effect, as may be seen by the different reactions of heparin against plasma and purified fibrinogen. Heparin has no coagulation-inhibitory effect on purified fibrinogen.[4]

In the present work we investigated whether heparin has an inhibiting effect on heat inactivation of thrombin, similar to its protecting effect toward oxidation. We used for our investigations thrombin and heparin (Liquemin, Hoffmann-La Roche). The activity of thrombin was measured with a solution of fibrinogen, containing 15 mg/ml of dry substance; the time of coagulation was noted in seconds. The solutions used for heat inactivation contained thrombin mixed with varied quantities of heparin. In each experiment the mixtures of thrombin and heparin were kept exactly 10 minutes in the water bath. A control series was similarly treated without heparin. The activity of thrombin was measured 1 hour after the samples were removed from the water bath.

It can be seen from Table I that the inactivation of thrombin by heat is inhibited most efficiently by an equal quantity of heparin. Smaller amounts of heparin inhibit less; however, a larger amount does not essentially increase the inhibitory effect. The heat inactivation of thrombin in the presence of an equal amount of heparin is demonstrated in a curve which is essentially similar to the inactivation curve of thrombin following oxidation.

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2 Palos, L. A., *Experientia*, 1949, **5**, 207.

3 Palos, L. A., *Acta Medica Scandinavica*, in print.

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